Order Number 9315506

The design and application of two versatile computer-based electrochemical instruments for static and flow chemical analysis

Sukenick, George D., Ph.D.

City University of New York, 1993

U·M·I
300 N. Zeeb Rd.
Ann Arbor, MI 48106

The Design and Application of Two Versatile Computer Based Electrochemical Instruments for Static and Flow Chemical Analysis

by

George D. Sukenick

A dissertation submitted to the Graduate Faculty in Chemistry in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The City University of New York.

1993

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Thesis Approval

This manuscript has been read and accepted for the Graduate Faculty in Chemistry in satisfaction of the dissertation requirement for the degree of Doctor of Philosophy.

1/12/93
Date

Ronald L Birke
Chairman of Examining Committee

1/26/93
Date

Executive Officer

Supervisory Committee

The City University of New York

Abstract

The Design and Application of Two Versatile Computer Based Electrochemical Instruments for Static and Flow Chemical Analysis

by

George D. Sukenick

Advisor: Professor Ronald Birke

The design, development and applications of two electrochemical instrumentation projects are presented. Each project involved the original design and construction of an electrochemical instrument, along with applications to analysis and detection of chemical species and components in mixtures for both static and flowing systems. The first project consisted of an automatic titration instrument which automates the calibration, data acquisition and reagent delivery process. This instrument was applied to study carbonate and bicarbonate mixtures, which traditionally are analyzed via a multiple step wet chemistry method. Results obtained with the instrument were similar to or better than the wet chemistry method. In the second project, a versatile multi-functional electrochemical instrument for voltammetry, polarography and electrochemical flow analysis detection was developed. The instrument was designed with an automatic static drop mercury electrode which was used as part of a flow cell detection system. The instrument was applied to the detection and examination of the differential catalytic current of B vitamins eluting from a High Pressure Liquid Chromatography (HPLC) column. The instrument was demonstrated in several of its modes, with successful separation and analysis of the vitamins.

Acknowledgments

There are many people that I would like to dedicate this thesis to. I cannot name everyone, so I will try to name some of the most significant people in my life, even though I know that I will miss someone.

I would like to thank Dr. Birke for his help and guidance through the years. He gave me the freedom to expand my interests and the confidence to attempt tasks that I never though that I would be able to accomplish. He was always there when I needed his wisdom. I would like to thank my thesis committee, including Dr. Birke, Dr. Locke, and Dr. Gosser for their advice, encouragement, and help with my dissertation.

I would also like to acknowledge other people who have given me support and understanding, now and in the past. Other teachers that I had include Mr. Tarendash who taught physics and chemistry in high school and gave me the motivation to learn about these fields in college. Dr. Rubin who taught me computer interfacing and had the confidence in me to let me independently develop my own and my first computer interfacing project. George Kliener whose casual discussions with me gave me in-depth understandings of computers.

There are many teachers in one's life who influence you, but perhaps the ones who are most important are those that love and care for you and keep you going in life. By this I mean family. I would like to dedicate this thesis to them. My late father Martin, who I was very close to, taught me his optimism about life and his love and fascination of nature and science. My mother Esther taught and tutored me early in life and gave me a head start. My sister Marlene gave me an interesting view of the world and shared her college textbooks with me when I was in grade school, giving me a boost. My wife Dian, patient and loving and gave me support, understanding, and kindness when I needed it most. Her family, who helped us when we need it. Most of all, I would like to dedicate this thesis to my children, Leah and Martina, who give me hope for the future and never cease to amaze me.

Table of Contents

List of Tables

Section 1 Tables

Section 2 Tables

Appendix A Tables

Appendix B Tables

List of Figures

Section 1 Figures

Section 2 Figures

Appendix A Figures

Appendix B Figures

Introduction

The topic of this thesis is comprised of two instrumentation projects, their development and applications. Each project involved the original design and building of an electrochemical instrument. Each design used a computer that was interfaced to custom electronics and standard instruments to create a final instrument that provided versatility as well as data acquisition, control and manipulation available only in computerized instrumentation.

One project involved the research, design and construction of an automatic titration instrument to automate the calibration and the titration process. This instrument dispenses reagent which reacts with analyte in the reaction vessel. It then measures the pH and records these values of volume and pH. The aliquot volume to be delivered to the analyte is determined by the instrument and depends upon the pH response of the analyte to the reagent. The goal of the instrument, in its most common mode of operation, is to add reagent to the reaction vessel in an amount such that the change in pH is a constant, even when the pH is in a rapidly changing region. This provides for maximum resolution in regions where detail is important, and analytic speed in regions where resolution is not important. This instrument was applied to study composition and other properties of carbonate and bicarbonate mixtures. Besides the calibration of the instrument, use of this instrument for this analysis involved only one step. Carbonate and bicarbonate is usually analyzed using a more involved multiple step wet chemistry analysis. This project is discussed in Section 1 and Appendix A. An overview of the titration method, automatic titrations and automatic

computerized titrations is given. Then the instrument which was developed, its features and modifications required to make this instrument are discussed.

Carbonate titration methods are then discussed. The application of the instrument to the analysis of carbonate, bicarbonate, and sesquicarbonate mixtures and comparison to traditional methods of this type of titration follows. Finally, another application is discussed, calculation of equilibrium constants from the data obtained. Appendix section A goes into the detail of the instrument construction and operation as well as derivations of equations and fitting procedures.

In the second project, discussed in section 2, the instrument developed and constructed was a versatile multifunctional electrochemical instrument for voltammetry, polarography and electrochemical flow analysis detection. The instrument was designed with an automatic static drop mercury electrode which was used as part of a flow cell detection system. The instrument was applied to detect and examine the differential catalytic current of B vitamins eluting from a High Pressure Liquid Chromatography (HPLC) column. Novel aspects of this project include the instrument, which gives control of almost every aspect of the electrochemical waveform, detection of B vitamins using the catalytic current on a mercury electrode, and the use of HPLC scanning electrochemical detection (HPLC-EC) for electrochemistry to examine the current-voltage curves rather than its familiar application to resolve peaks overlapped in the time domain.

The voltammetric method is discussed, followed by a short description of High Pressure Liquid Chromatography with electrochemical detection (HPLC-EC). Following that, choices of electrode and flow cell are discussed,

as well as the instrument that was developed. This part of the thesis consists of the possibilities and capabilities of the instrument, an overview of how the instrument is constructed, how it works, and how it is used. The specifications of the instrument are then presented, and then the electrode system and flow cell which were used to demonstrate the capabilities of the instrument are discussed. Finally, the experiments which were performed are discussed. These include experimental details such as the deaeration method, catalytic current, various HPLC characteristics of the instrument, optimization of parameters and an examination of mixtures. Appendix section B gives examples, diagrams, and other details concerning the instrument construction and operation.

Section 1 The Automatic Titration Instrument

Titration Introduction and Background

A Digital Equipment Corporation 11/23 computer has been programmed and interfaced to a modified motorized buret and a modified pH meter to perform general potentiometric titrations. This instrument was applied to various mixtures of sodium sesquicarbonate, sodium carbonate, and sodium bicarbonate with dilute (ca. 0.1M) HCl as titrant. The computer controlled the experiments, changing the pH by 0.1 pH unit for each aliquot delivered through a numerical derivative prediction algorithm. The data were then fit to an empirical model and to a derived model using a simplex minimization procedure in order to examine errors, analyze percent material, and determine dissociation constants.

Titration

Titration is a powerful analytical technique. It is a chemical reaction under perfect control, since control of reacting species is maintained. The method yields information about the stoichiometry of the reaction, reaction steps, equilibrium constants and probable molecular species in solution. For methods of quantitative analysis, volumetric titration stands out for versatility, accuracy, and convenience.[1] A titration will usually yield information on the formal concentration of the solutes, as a titration is ordinarily set to progress slower than equilibrium of the reactants.

Still, a titration is simply a method that only involves adding a measured quantity of reactant to a substrate until some property of the substrate changes. In acid or base titrations, an analytical technique used

since 1897,[2] the pH of the solution is the property that changes. As titrant, hereafter referred to as reagent, is added, the pH will change slowly in the buffer region until it reaches the endpoint region. This is a transition region where the pH changes at a rapid rate. Inside the endpoint region is the equivalence point, the point when all of the substrate has reacted. Before this point, there is only a small amount of reagent (which has not reacted) in solution; after the equivalence point there is excess of reagent. If we can measure the total volume of reagent added up to the equivalence point, we can determine the quantity of substrate.

The change can be detected by adding a small quantity of a substance that changes color at the pH around the equivalence point or by measuring electrical properties of the solution using electrodes. There are many electrodes that may be used, but the glass electrode, which has been in existence since 1906[3] is used extensively for this purpose. Modern versions of this device have a linear dynamic range of up to 12 pH units (pH 2 to 14). This pH range is equivalent to measuring activities of hydrogen from 10^{-2} to 10^{-14} moles / liter. Few other sensors have this range of detection.

If the pH is plotted against the amount of reagent added, most of the curve will look relatively flat, except around the endpoint region where there is a transition, forming a sigmoidal shaped curve (Figure 1). The peak of the derivative may be examined to determine the inflection point (Figure 2), or the second derivative goes through zero at the inflection point (Figure 3). If the curve is symmetrical, the inflection point (or "endpoint"), will coincide with the equivalence point[4] The endpoint is the point where the slope is maximum; the absolute value of the derivative of the curve is at a maximum and the second derivative is zero. This method of using the derivative to find the endpoint was first proposed in 1919 by Hostetter & Roberts[5]

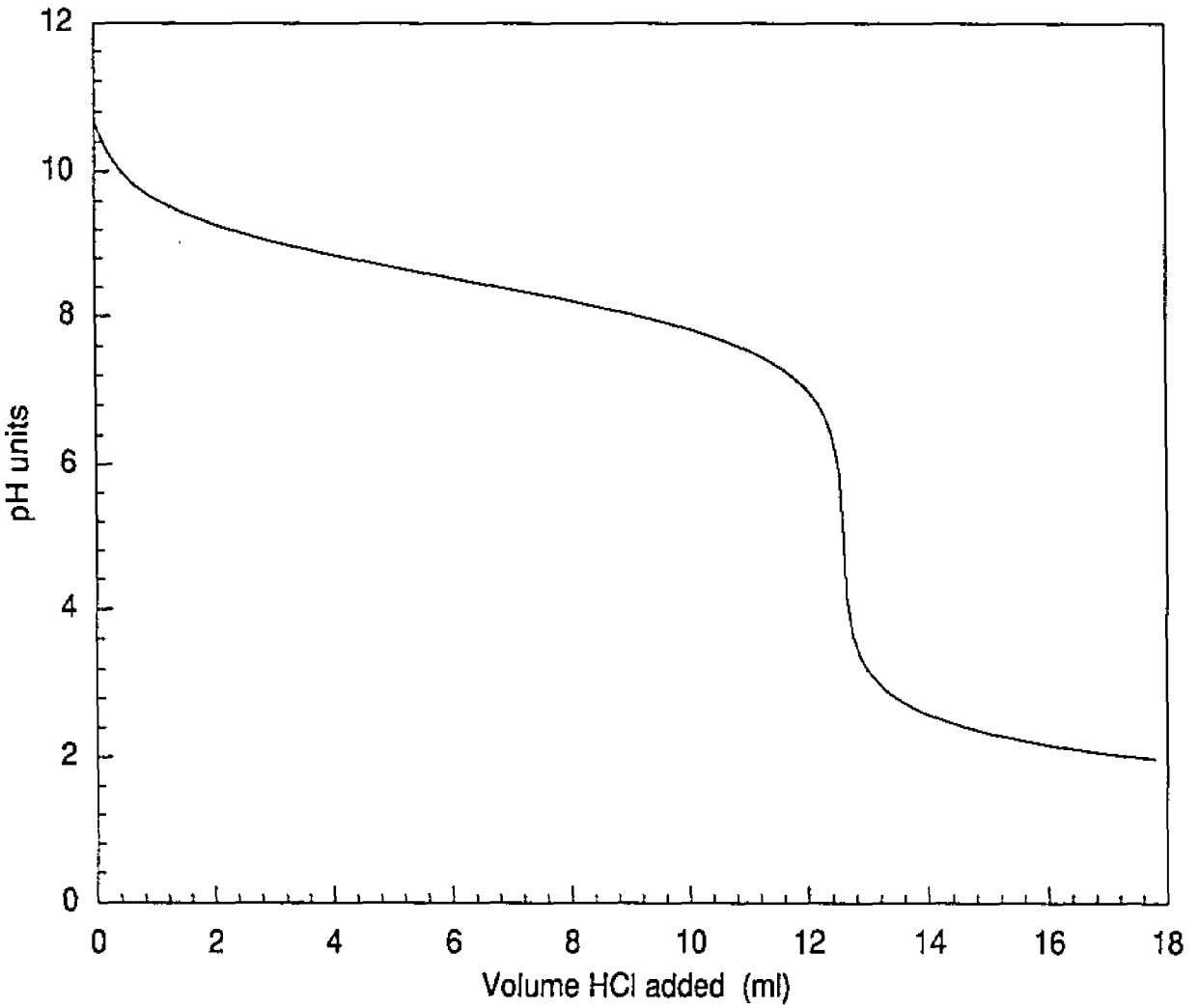

Figure 1 Titration of TRIS with ca. 0.12 M HCl

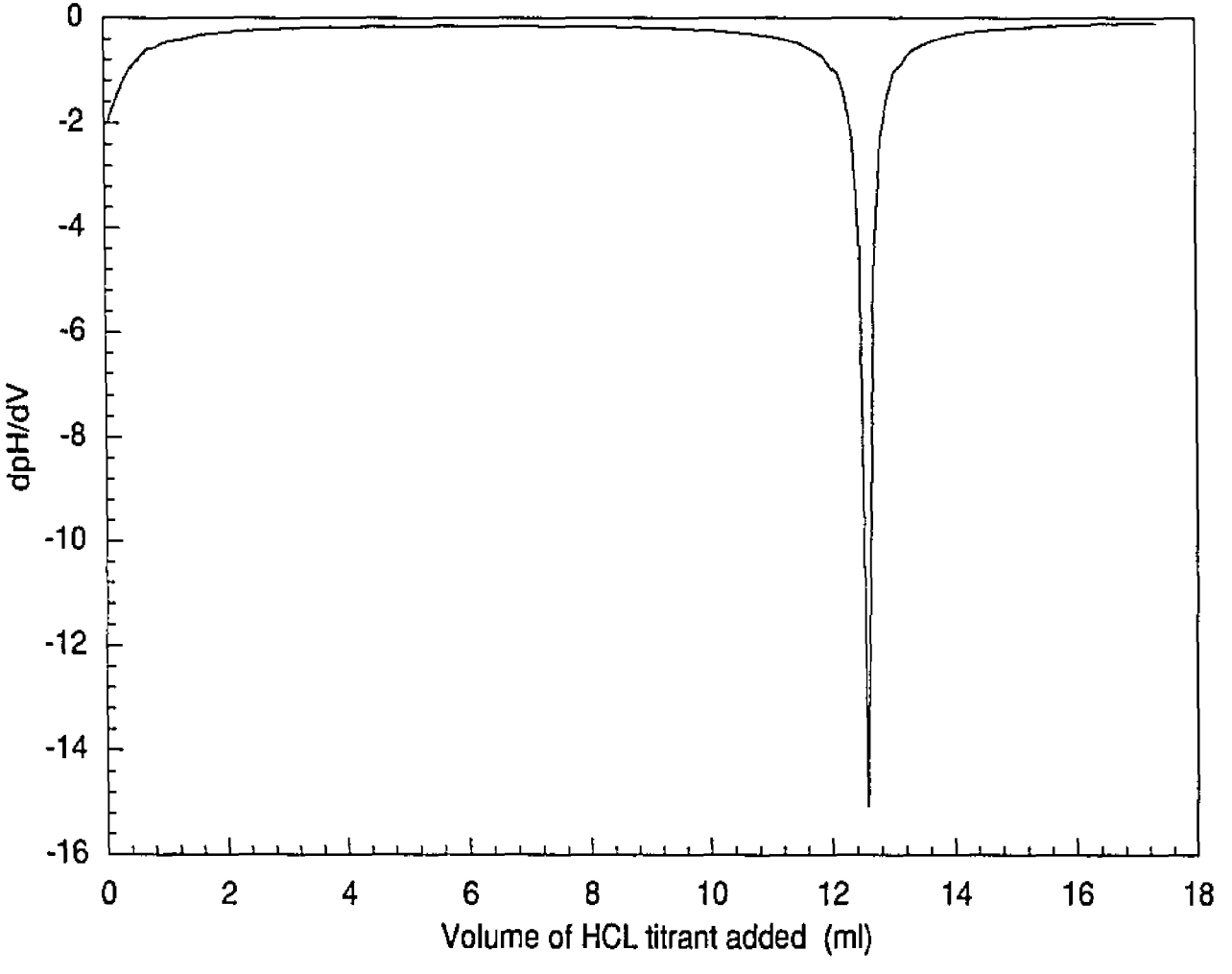

Figure 2 First Derivative of TRIS Titration

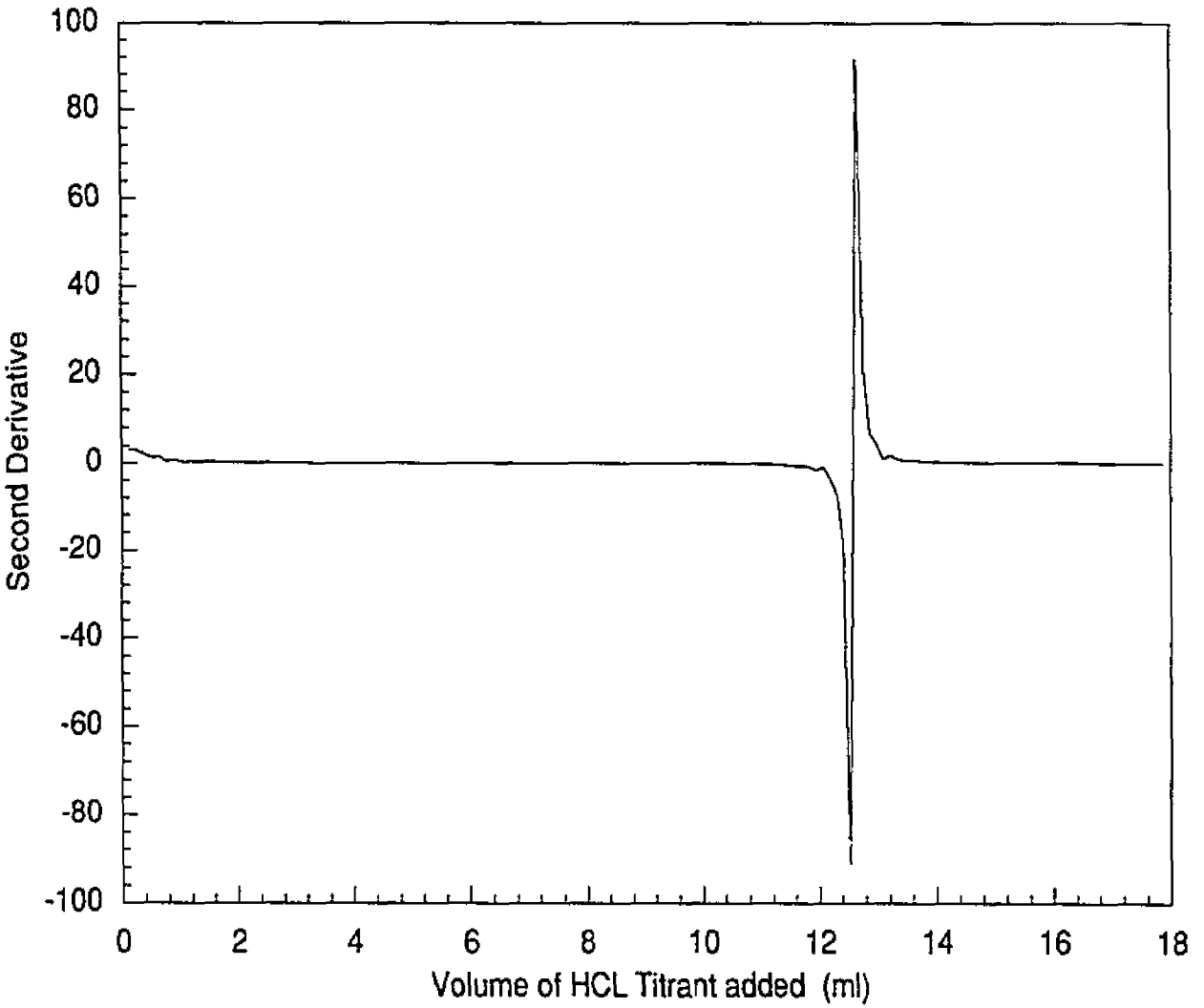

Figure 3 TRIS Titration, Second Derivative

Automatic Titration

The titration process can be tedious, and so methods have been devised to automate the process, even as far back as 1914[6]. Use of automation can eliminate personal bias to an extent or at least better define the bias; many of the titration parameters can be set up by using a particular criteria[7]. The criteria may include items such as a mathematical determination of the endpoint, deciding when a pH value is stable, and when to add more or less reagent to the substrate being titrated. Automation of the titration process can be performed by many different means, from clever design of the experiment to using analog and/or digital electronics. In the titration process, there are several aspects, some or all of which can be automated: data treatment, control of reactant introduction (dispenser), data acquisition, and endpoint detection method. In 1925, Cox[8] developed the first method which automatically calculates the derivative. Two equal aliquots of sample solution were placed into two separate vessels. Before the start of the titration, one vessel had a small known amount of reagent added to it. Thereafter, both were titrated with constant increments of reagent. The difference voltage was recorded, which gave the derivative curve. In 1939, Barker & Mükker performed differential titrations using mechanical devices[6]. In 1947 an electrical differential method was published[9] by Delahay. He used a method for constant flow titration measuring current flowing into a capacitor from the cell. Lingane[10] in 1948 developed a device that would deliver reagent at a constant rate with a motor driven syringe and then slow down near the endpoint and then stop. The endpoint was not directly detected. The pH of the endpoint is previously determined and used as a set point criteria. The motor would slow when the value of the pH compared to the set point was small. This

apparatus was connected to a recording potentiometer in order to synchronize volume to pH readings. In 1954, Malmstadt[11] took this design one step further and automated the endpoint recognition. He added a circuit that calculates the second derivative of the signal from the sensor; when the current in the calculation circuitry goes through a maximum and then reverses, a relay shuts off the motor. Since the electrodes were placed near the reagent delivery tube, the sensor would detect a large change in pH in the adjacent solution when the experiment was near the endpoint. When the material mixed, the titration would resume and this cycle would repeat until the endpoint was reached or passed. Positioning of electrodes was very important; if they were not positioned properly, this hysteresis effect would be lost or diminished, and the instrument might not slow down for the endpoint. This method does not work well for chemical systems with small slope about the endpoint.

All of the designs that involved circuitry used analog devices, whose disadvantages will be discussed later. They also were meant for constant flow titration, which is not well suited for slow equilibrium systems. Set points for pH as a criteria for the endpoint requires knowledge of the exact pH of the endpoint, which can vary depending upon temperature, calibration and concentration. Therefore, a method that does not depend upon the an exact pH value has some advantage.

Automatic Computerized Titration

Around the 1970's, affordable digital circuits and minicomputers came about, and automatic titrators were built around them. Computer control instrumentation is usually more advantageous than direct analog feedback and control. While analog systems can be of very high quality, the computer controlled instrument can be more versatile than the analog system. The computer can perform the function required, and when not being used for the experiment, can be used for other purposes such as calculations or other experiments. An inexpensive computer (with quality I/O devices) can usually control an experiment as well as or better than an analog system which must have a delicate balance of high quality parts. In addition, the computer can be easier to configure if an unusual requirement arises, while the analog equipment is usually more difficult to alter.

Not all digital systems have a computer or microprocessor. Digital control, which essentially is what the computer does, can also be performed by digital circuits. These systems do not have the versatility or programmability of computer control, and shares some of the design complexity and inflexibility of analog devices. They usually does not have the delicate adjustments associated with analog circuitry. However, in 1980, Warner[12] designed a pH stat/titrator system with digital circuits. It used a stepping motor coupled to a 2.5 ml syringe. Like Lingane's design, this system employed a set point to determine where to slow addition of the reagent. It does slow the addition of reagent in a much finer and more precise manner than the older method. The difference voltage between the pH and the set point is converted to a frequency which drives the motor.

A major advantage of a computerized system is in the handling of data. Titration data can consist of hundreds of points, and several runs are usually performed for a single analysis. The digital computer can save data for later recall, manipulate the data to display the derivatives of the data and compute a value for the endpoint from the derivatives. In 1979, Hänisch [13] had an automatic spectrometric and pH titrator built around a computer. In 1983, Chipperfield[14] made an autotitrator with the then popular PET computer. This design used a stepper motor connected to a 100ml gas syringe with automatic refill capability using a micro-switch activated valve. The pH was measured by an operational amplifier connected to an analog to digital converter. The rate of pH change was used to determine size of additions. In 1990, Ren[15] made an autotitrator with similar parts. Ren's paper discussed some important features of such an instrument. The criteria for the point that the pH is measured at the electrode is important; Ren used a method which involved comparing two readings temporally five minutes apart. If the difference in these readings agreed to within his criterion, then the pH was assumed to be stable. Another factor is the quantity of each addition of reagent. Ren used a reagent delivery volume spacing scheme which did not take delta pH into account but instead used the derivative. Volumetric spacing of aliquot deliveries depended upon the first two reagent delivery volumes and the volume of each subsequent delivery varied as a parabolic dependence on the numerical derivative of the data and the first two points.

Automatic Titrator Instrumentation and Program

This section will discuss the automatic titration instrument, along with the methodology and the features of the instrument. Then, the construction of the instrument, the devices and parts used, and the operation of the instrument are discussed.

Overview of the Instrument

The instrument consists of a computer which controls various devices to perform the automatic titration. The computer program which performs the automatic titration is called **TIGHTC**. The computer acquires the pH through an analog to digital converter (A/D) which is connected to a pH meter which itself is connected to an electrode immersed in the solution of interest. The computer is programmed with criteria to determine when a pH value is stable. Reagent is delivered by a motorized buret with a DC motor. The buret is controlled by the computer through a digital line connected to a relay which turns the motor on or off. The amount of reagent delivered is measured as a count by a tachometer attached to the motor in the buret.

The instrument gives prompts, and when there are many choices, a menu of functions. When the instrument is first started, the electrode is calibrated by immersion into different pH buffers, as directed by the instructions on the display terminal. Other parameters are then asked for, such as reagent molarity. The user is asked to set the electrode, delivery capillary and device controls into position to start the titration. Then the computer takes control of the experiment. It adds reagent to the reaction vessel and records both the reagent volume delivered and the pH of the solution. This information goes into the computer memory, is listed digitally on the monitor and is plotted on the display monitor. The first and second

numerical derivative of pH vs. volume of reagent delivered is calculated and their numerical value is displayed as well. The quantity of reagent to be delivered is calculated from the first numerical derivative, with bounds placed upon the value that is calculated. The goal of the delivery is to make each reagent delivery cause a constant change in the pH of the substrate (default setting is 0.1 pH unit). To protect the experiment from aberrant values, the quantity of reagent has bounds. There is a minimum and a maximum quantity. In addition, a delivery volume cannot be more than four times the previous value. These parameters are adjustable at any time and are listed in Table 1.1. The titration continually acquires data and adds reagent as described until the user manually tells it to stop or a set pH value has been reached. Then, the user can store, plot and otherwise manipulate the data.

Features of The Titrator

The instrument developed for the automatic titration has many features. The instrument gives prompts and descriptions for each step of the experiment, and lists of possible functions, called "menus", when applicable. Most prompts have reasonable defaults in order to simplify and speed operation of the program. When an experimental step has several possible directions, a menu with each possibility is given. Warnings are given when errors or problems are detected. Graphics are presented to the user as the experiment proceeds to display the experiment's progress. Under the plot, as new data are acquired, the numerical listing of the data is displayed along with first and second derivatives.

The pH electrodes may be calibrated with two or more buffers. This calibration is automatic, requiring only entry of the buffer pH value. The

instrument calculates the conversion factors for converting the voltage of the electrode to pH using least squares regression.

Many of the experimental parameters are adjustable, even as the titration is proceeds (see Table 1.1). These parameters include control of the experiment. There is a variable reagent delivery based upon the derivative of previous values of pH and volume of reagent delivered. It attempts to make the change in pH constant, resulting in good experimental time and resolution. There are factors which prevent unreasonable reagent delivery amounts. It is possible to change the criterion which determine when the pH electrode has stabilized after an addition of reagent to prevent false readings. Each pH reading in a titration is actually many readings averaged together to assist in eliminating random noise.

When the titration is completed, the instrument displays menus for manipulating and displaying data. It is possible to obtain a display on the monitor screen and a graph on paper from a Hewlett-Packard pen plotter of volume vs. pH, first derivative and/or second derivative. Data may be stored or retrieved. The data are stored in a universally readable text or "ASCII" form. The numerical data may be listed on the printer or the terminal screen. Along with the raw data, a list of points where the second derivative changed sign is given, along with volume, pH and molarity (of reagent) interpolated to a point where the numerical second derivative equals zero. There are common combinations of functions, so that only a few keystrokes are required for a function such as storing, displaying, and plotting data. The data may be edited to remove aberrant points; or examine specific points on the data graph.

TIGHTC Titration Program Description

The program written for the automatic titrator is named **TIGHTC**. It calibrates, controls, acquires data and displays, plots, stores and manipulates data for potentiometric titrations. This program consists of several parts, listed in the Appendix Table 1.2A. Whenever possible, the program guides the chemist through procedures such as calibrations and input of common parameters by using prompts and listing default values. The default value is used if no entry is given (if the **return** key is pressed without a value), providing convenience. If a query does not have a default, the prompt will repeat itself if no value is given.

The first part of the program is used for calibrations and entry of initial values. The system of prompts is used in this section. At the end of this section, one may change internal parameters. When all calibrations and value entry have been completed, the computer dedicates itself to performing the experiment. During this time the user sees a graph of the data being acquired. There are several possible actions for the user. They are, "pause titration", "stop experiment", "speed up titration", or "change internal parameters". Once paused, the experiment may be redone, aborted, parameters changed or completed. A list of the experimental parameters are in Table 1.1. These parameters are listed with each data report.

Once the experiment has been completed, another section of the program is called into memory. The style of user interface in this section is a menu from which the experimenter may choose to plot, store, read in new data, print data, manipulate data and print a data report. An example of the screens and prompts are given in Appendix 3.3.1A. An example of a data report is given in Appendix 3.3.2A.

#	Parameter Name	Input Method	Default Value	Description of Parameter
1	Flow Conversion	calibrate	$\frac{7.4 \cdot 10^{-3}}{4}$	conversion factor; for reagent flow: tachometer pulses. Units ml/count
2	K	calibrate	(-5, typical)	From E = K + C * pH ; conversion of pH value to voltage of electrode
3	C	calibrate	(0.7,typical)	From E = K + C * pH ; conversion of pH value to voltage of electrode
4	Reagent Molarity	entered	0.1	Molarity of Reagent.
5	pH Noise	internal	$4 \cdot 10^{-3}$	Maximum allowable noise in a valid pH measurement; Units are volts
6	pH Max. Dev. Slope	internal	$4 \cdot 10^{-3}$	Maximum slope criteria when pH reading is stable
7	pH Change	internal	0.1	goal of instrument for pH change of each reagent delivery
8	Minimum Aliquot	internal	$7.5 \cdot 10^{-3}$	minimum amount of reagent which may be delivered, Units ml
9	Maximum Aliquot	internal	2.0	Maximum amount of reagent which may be delivered, Units ml
10	Spin Down	internal	2%	Amount of slop in counts allowed for spin down of motor
11	Mix Time	internal	2.0	Minimum mixing time in seconds.
12	pH Change	internal	0.4	Volumetric correction to aid in keeping pH change goal (parameter #7)
13	Program Version	int fixed	(5.75)	Version of current program
15	Buffer #	det int	2	equal to the number of buffers used in calibration of the pH electrode
16	Calibration	det int		Mean pH of calibration buffers
17	Calibration	det int		voltage of first solution or average V
18	Calibration	det int		voltage of last solution
19	Calibration	det int		pH of first solution
20	Calibration	det int		pH of last calibrate solution

Table 1.1 Internal Parameters for the Titration Program, TIGHTC.

Meaning of the "Input Method" column:

calibrate	The parameter value is calibrated (or entered) using the instrument
entered	The parameter value is entered when prompted by the instrument
internal	The parameter value used is default; not prompted for
det int	The parameter value is calculated & determined internally
int fixed	The parameter value is internal and fixed (may not be changed)

How a Titration is Performed by TIGHTC

The size of the aliquot is important for determining the endpoint of the titration. Ideally we would want to have the best resolution or smallest possible aliquots around the endpoint so that it may be determined in the most accurate manner. If all aliquots were the same small size, we would have the desired resolution, but at a great cost of time. Even though the computer would work automatically, the throughput of experiments accomplished would be very low. Taking a long time for a particular experiment would also introduce errors due to evaporation, drifting of the pH electronics, and other errors. The goal would be for the computer to work as well or better than a chemist performing the experiment by hand and to have significant advantages.

Typically, a chemist performing a potentiometric titration by traditional means would first calibrate the pH meter using two buffers in the region that the titration would proceed. Then, with the solution being stirred, pH and reference electrodes are inserted into the solution and with a paper in front and pencil in one hand and buret control deftly held in the other, the chemist will add amounts of reagent and make notes of the pH, and volume reading from the buret. The chemist will wait before recording each reading until the reading on the pH meter has stabilized. The quantity of each addition will depend upon the chemist's perception on how the experiment is proceeding. Where there is little change in the pH change, the chemist will add large quantities of reagent. When the pH starts to change quickly, either from intuition by noting the speed that pH meter's needle transverses across the dial, or by more sophisticated means, by looking at the numerical derivative and observing it increase or perhaps by

plotting the data by hand as the experiment progresses and noting when the curve starts to become steep, the chemist will start to limit the amount of reagent that is added in order to provide better resolution at this important part of the experiment.

The problem is that this procedure is tedious and prone to errors. The chemist might not obtain enough points around the endpoint for good resolution. Due to impatience, error, or lack of skill, too much reagent might be added and the endpoint is passed. If the region of the endpoint has a low slope, the process can take a long time. Errors might also creep in from transcription or calculation errors.

Automating this process is advantageous as transcription errors are eliminated, and for the most part, calculations are reliable. The tedium is efficiently performed by the computer, which does not get impatient. If there is a good criteria for determining the quantity of reagent to deliver, the experiment need not take long and enough data about the endpoint will be taken.

TIGHTC is meant as an aid for the chemist. It performs some of its functions with the aid of the chemist, and the actual titration itself is performed with minimal interaction required. The titration procedure is performed in a manner similar to a classical potentiometric titration. First, parameters of the experiment are recorded and the pH is calibrated by either a two point or, with the computer determining the result using linear least squares, a multiple point calibration. Then the chemist is prompted to start the mixing motor and place the electrodes into the solution. When the titration has started, the computer controls the experiment until the target pH is reached or all reagent has been delivered from the buret.

In the automatic titration, there are programmed features which are similar to a chemist doing the experiment by hand. One feature is the determination of a stable pH reading. A pH measurement cannot be considered correct unless made when the pH of the solution is or is near equilibration. The chemist would not record the reading immediately after addition, and neither does **TIGHTC**, which waits for a set time, "Mix time". The chemist may then see, if after some time the pH or electrode hasn't settled, a needle moving about or a number changing up and down until it reaches a final value. There are two periods that the pH is not stable. One period occurs when the pH is changing relatively fast. This happens whenever reagent is added and the solution hasn't completely mixed. This is easy for the computer to detect. The computer will take many readings, calculate numerical derivatives of the pH with respect to reading number, and if they are high (after being processed to eliminate some noise and false readings), wait until the values are smaller than a specific criteria. Another region occurs when the pH seems to have stabilized to a particular reading, but due to incomplete mixing, reaction or noise, the pH continues to change or drift (sometimes even in a reverse direction). This shows up as a set of pH readings which have noise higher than normal (normal defined as the noise of an electrode in a solution at equilibrium). This region may be detected by looking at the standard deviation of a large set of pH measurements.

TIGHTC uses three parameters, listed in Table 1.1 as parameters #5, #6 and #11. The parameters are "pH noise", "pH maximum deviation slope" and "Mix time". "Mix time" is the time allowed for mixing. It defines the amount of time between the reagent delivery and the first measurement. The other two parameters are used in the measurement itself. When it is time to take

a measurement, the "pH maximum deviation slope" is used to determine when the pH is rapidly changing, and the "pH noise" is used to determine if incomplete mixing has occurred. The latter parameter may cause problems if a poor quality electrode is used. The details of this procedure may be found in Appendix 2.1.1A.

Another feature the determination of the quantity of reagent that is delivered. A chemist would determine how previous aliquots changed the pH (by examining the derivative, in other words) and then try to predict from intuition and experience how much more to add next. The computer calculates the amount of reagent to deliver from several parameters; from Table 1.1, #7, #8, #9 and #12 which correspond to: "pH change", "minimum aliquot", "maximum aliquot" and "pH change correction". The parameter "pH change" is the goal for each aliquot: each quantity added should change the pH by 0.1 pH units (default value). The aliquot is determined by the numerical derivative of the last two points, and the change in pH of these points. The size of the derivative determines the increase or decrease in the aliquot size. To prevent overshoot, "minimum aliquot" and "maximum aliquot" are used to prevent too little or too much reagent from being delivered. In addition, the size of an aliquot is restrained from increasing more than four times from its previous value. This helps keep changes reasonable, even when noise is present. The parameter "pH change correction" modifies the aliquot size so that when the derivative is very large, the calculated aliquot will be smaller. The details of the process is described in Appendix 2.1.2A.

Automatic Titrator Hardware Overview

The instrument is depicted in Figure 4. This diagram portrays details and adaptations which are contained inside each instrument. Each device and its contents are depicted on the diagram by a dotted box. A modified Thomas Scientific Model 258 motorized buret fitted with a two-way Thomas Scientific #9690C15 valve is used to deliver reagent through a thick walled 1 mm ID capillary into the sample beaker under the control of a digital output line (bit #9) from a Digital Equipment Corporation LSI-11/23 (MINC-11) computer. The motor of the buret is monitored both by a counter in the computer and a Light Emitting Diode (LED) which is visible to the chemist. Feedback and data from the experiment is obtained through a pH electrode which is connected to a modified Corning model 125 pH meter which provides a continuous visual display and offset, gain and buffering of the electrical signal. The output from the pH meter is amplified and goes to a 12 bit (2.44 mV resolution) Analog to Digital converter (channel #5) in the computer. Display of the data and control of the instrument is through a DEC VT105 Graphics terminal connected through the secondary output of a Hewlett-Packard 7470A Pen plotter. The pen plotter allows for plots of the data and is connected to the console 9600 baud serial port of the computer. The computer is programmed in Basic. The program provides prompts and menus for the chemist. It is used to calibrate the electrodes, and it controls and acquires data from the aforementioned devices and provides real time display of data as well as plots and a data report.

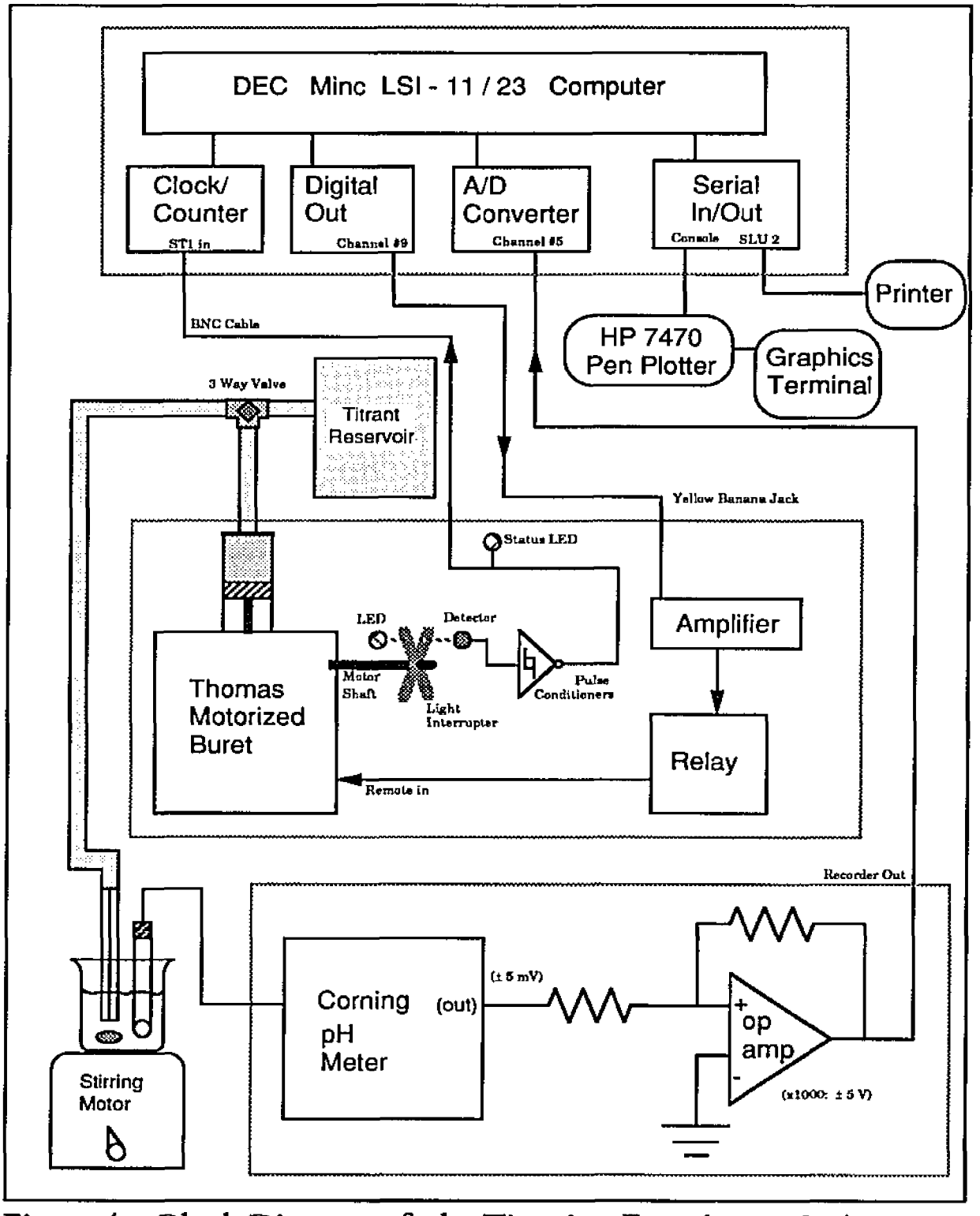

Figure 4 Block Diagram of the Titration Experiment & Apparatus

Modifications to Standard Instruments:

Modifications to the pH Meter

A pH meter was used to buffer the signal from the pH electrode. The modification was performed internally for convenience and for extra shielding of the low level data signal. The output of the pH meter was adapted to connect to the Analog to Digital converter on the computer. The pH meter output was originally meant to be connected via banana plugs to a millivolt strip chart recorder, with a full range scale of ± 5 mV. The computer's A/D converter input is designed for an input range of ± 5.115 volts. The output of the pH meter was disconnected from the banana plugs internally and then connected to a LF13741 (JFET) operational amplifier with a gain of 1000 in order to obtain a ± 5 volt full range output. This operational amplifier is on its own circuit board which was mounted inside the pH meter. The power required (± 15 volt) is obtained from the pH meter as well.

Modifications to the Buret

The motorized buret consists of a precision constant bore glass tube with a Teflon plunger. The plunger is connected to a screw which is geared to a DC motor. The buret has controls to vary motor speed (potentiometer) and direction (up = deliver reagent, down = fill buret and remote = external deliver/off), as well as a mechanical counter which is geared to the motor and calibrated to display volume dispensed to 0.01 ml. In order to interface the buret to the computer, two adaptations were required: one to control the "remote up" and the other to determine the quantity of reagent delivered. The first modification that will be discussed is to the "remote up" control of the buret.

Remote Control, Relay Modification

In the original design of the buret, the "remote control" was controlled by an external switch. Closing the switch would turn the buret on until the switch was released. The switch closure would connect approximately 15 volts DC of poorly filtered power to the motor. There is a surge when the switch is initially closed due to the induction of the motor. This voltage is out of range of most direct computer controls, and a voltage surge on a line could cause problems if not adequately shielded in the computer. Therefore, a standard TTL level output board was used in the computer and additional electronics to perform the switching was placed inside the body of the buret. In order to automate this control with the computer, a relay circuit was added to replace the switch as well as the finger of the chemist which would push the button. The power required to drive the relay (100mA) is larger than the output current capability of the computer's output line (30mA), so an amplifier is used to bring levels to their proper value. The circuit schematics and their operation is described in more detail in Appendix 1.1.1A and 1.1.3A.

Aliquot Gauge, Tachometer Modification

The other modification to the buret was to add a tachometer to the motor in the buret in order to determine the numbers of turns of the motor (Figure 5). There are many methods by which the computer may measure the aliquot delivered by the buret. A list of some practical methods are listed in Table 1.2. The most straightforward approach might be to turn the motor on and off for an exact time expecting that the amount delivered is proportional to the time. This did not work because the speed of the Thomas

buret motor modulated at 0.5 Hz. Adding large capacitors to the power supply did not solve this problem. Another approach could be by putting an electric eye across the delivery tube outlet and counting drops that break that light path. This method can be too slow, as reagent must be added dropwise, and the drops may have some variability in size. A paddle wheel, where reagent flow moves a wheel is another possibility. Replacing the internal motor with a stepper is a good choice, but expensive, and there have been designs of automatic titrators with stepping motors. The least expensive choice which retains good precision is the tachometer. The cost is about one tenth of the stepper motor and one fourth of the wheel. In fact, the BNC connector was the most expensive part of this solution. Therefore, the tachometer was the solution which was chosen.

To construct the tachometer, a chopper was attached to the shaft of the motor. An LED light source and Photo transistor detector was placed in the path of the chopper. The output of the detector was then connected to a Schmitt trigger pulse shaper to condition the output to be compatible to the ST1 input of the counter in the computer, as well as to provide a visual indication to the chemist that the tachometer is working. Each turn of the buret's motor would interrupt the light beam four times resulting in a resolution of 0.001875 ml/pulse. An in-depth description of the tachometer and the electronics may be found in Appendix 1.1.2A and 1.1.3A.

Method	Description	Comments
Time	turn motor on for exact time	Buret must have a constant speed motor It will have problems at short values of time due to inertia of the motor ($0)
Drop count	count drops	Assumes drops are constant size ($10)
Paddle	flow turns paddle wheel	Wheel is in contact with reagent; must be immune to acid ($40)
Stepper motor	replace DC with step motor	Precise; count motor turns, the buret bore must be precise($100)
Tachometer	count motor turns	The bore must be precise. Sends n counts: error is 1/n turn. ($10)

Table 1.2 Possible Methods of Determining Aliquot Size, Approximate prices.

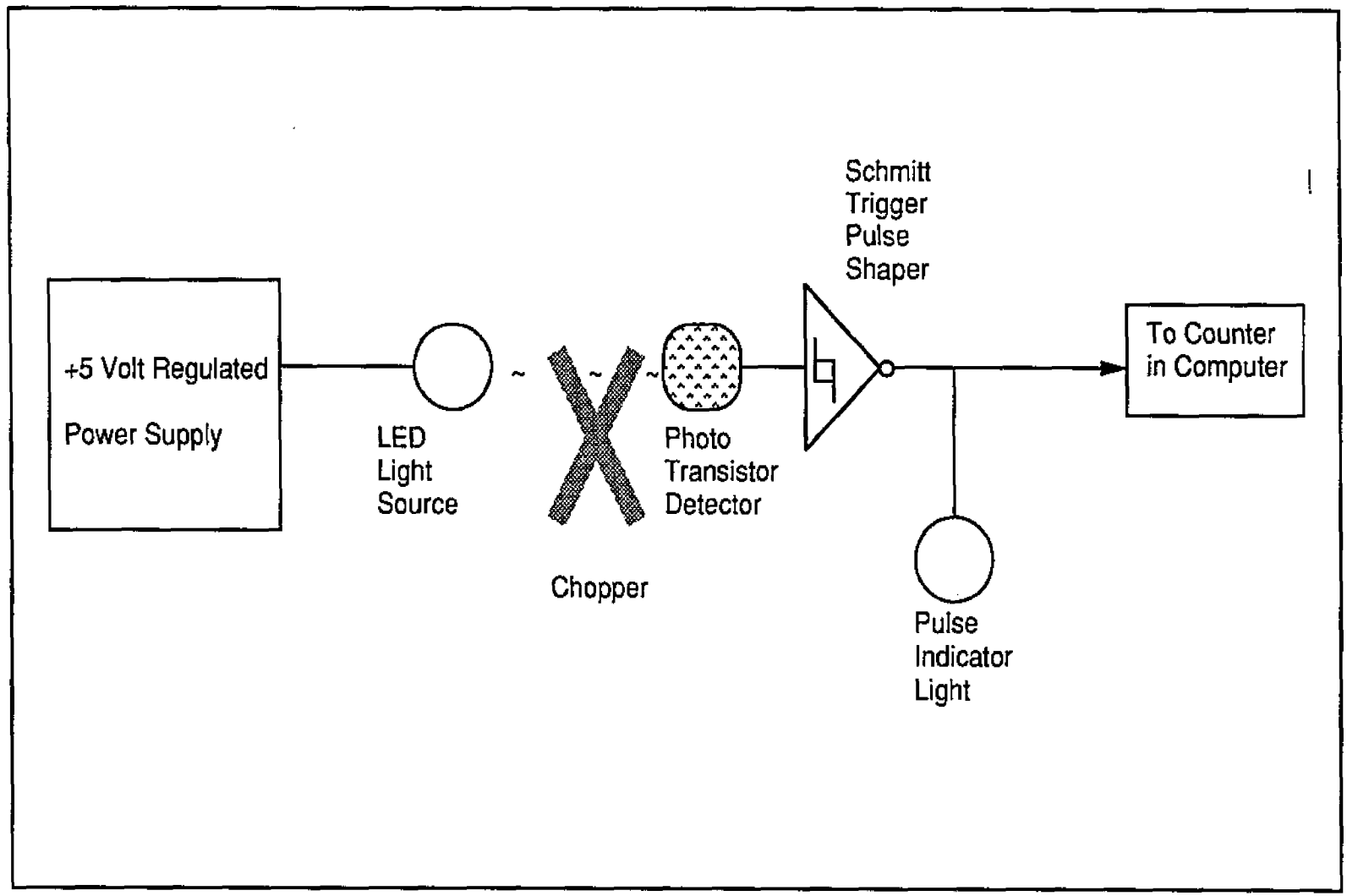

Figure 5 Block Diagram of the Buret Tachometer

Carbonate Titration and Classical Methods

Titrations of mixtures of sodium carbonate, bicarbonate and sesquicarbonate were performed to demonstrate the automatic titrator. First, other methods of performing this titration will be discussed.

The standard titration technique used to quantitate carbonate, bicarbonate and mixtures was developed in 1888 by Winkler.[2,16] In this procedure, a solution of the compound(s) is neutralized with excess of standard base, such as sodium hydroxide, and then barium chloride added to precipitate the carbonate quantitatively. The excess of base is then determined by back titration with a standard acid in presence of the barium carbonate. Since this method was developed, only a few changes were made. Variations entail the use of blanks, and detection using indicators or potentiometric endpoints. For instance, Regier[16] suggested a similar method, but used a potentiometric backtitration, with a blank containing exact same amount base with $BaCl_2$ for correction. Textbooks suggest this latter procedure as well for general carbonate/bicarbonate mixture analysis.[17] Harned[18] reported errors in the Winkler titration of 0.5% precision with 0.2% reproducibility.

Another method uses the pH of the solution.[19] This method requires great precision in the standard pH buffers, standardization of the meter and making standard solutions. A set of standard mixtures of carbonate and bicarbonate are made, and the pH of each solution is measured to generate a calibration chart. Pure carbonate has a pH of 11.6; bicarbonate is 8.4 pH units. A mixture of similar total molarity will have a pH between these values; for example, sesquicarbonate (44% [HCO_3]) is 10.1 pH units.[20] By measuring the pH of the sample solution and interpolating on the calibration

chart, the percent of carbonate and bicarbonate may be found. However, the overall shape of the calibration curve (pH vs. % [HCO_3^-]) is similar to a decaying exponential. This method is good only when HCO_3^- concentration is small, as higher concentrations will have larger errors associated with it. For example, for 0-1.25% [HCO_3^-], the calibration slope is 0.33 pH / % [HCO_3^-] or 3% change in HCO_3^- concentration for each pH unit. For 1.25% to 2% [HCO_3^-], the change is 6.7%; for 4% to 5%, the change is 8.3%. With sodium sesquicarbonate, the change is approximately 30% for each pH unit. If we assume an error in pH reading of ± 0.01 unit, and a standardization error of ± 0.04 pH units (total error in pH is ± 0.05 units), then the error in the above would be respectively, 0.15 %, 0.34 %, 0.45 % and 1.5%.

Titration using an automatic titrator involves initial calibrations of the pH meter, but a two or three point measurement suffices and does not require the multiple measurements described above. The measurement of the quantity of material is dependent upon the position of the inflection point in the curve. The inflection point is the first derivative of the curve. This is estimated by calculating the numerical derivative, equation 1.1, from pairs of experimental data:

$$\text{Numerical derivative} = \left(\frac{\Delta pH}{\Delta V}\right)_{(n,n+1)} = \frac{pH_{n+1} - pH_n}{V_{n+1} - V_n} \qquad (1.1)$$

The pair of data around where this numerical derivative is maximum is determined, and in that region, the point at which the second derivative passes through zero is determined. This involves three points around the endpoint as seen in equation 1.2.

$$V_{\left(\frac{\Delta^2 pH}{\Delta V^2}\right)_{(n+2,n+1,n)}} = 0.25 * (V_{n+2} + 2 \cdot V_{n+1} + V_n) \qquad (1.2)$$

The derivative equations are described in more detail in Appendix 4.1A . Determining the equivalence point from the derivative does not require knowledge of the *actual* value of the pH. If an error was made in the initial calibration of the meter, there would be no error in the location of the endpoint, unlike the previous method. Also, this method does not rely on only one precise measurement as in the previous case and the Winkler titration. Using points around the endpoint, and having information leading up to and after the event provides a check for reasonableness of the measurement. Another advantage of automatic titration over the classical Winkler titration is the speed, about five minutes for one titration, and a procedurally less complicated method, as only one reagent is required. Using base as a reagent is also a disadvantage, as extra care must be taken in storage, since a base such as NaOH slowly but easily picks up carbonic acid.

Experimental Section

Samples and experiments

Five samples were titrated with the automatic titrator previously discussed. The substrates are listed in Table 1.3. The primary standard for standardizing the HCl reagent was *Tris* -(hydroxymethyl) aminomethane, $(HOCH_2)_3CNH_2$, commonly called TRIS. Titration of this material gives a very sharp endpoint (Figure 1), making it an excellent standard?[1] The TRIS was triply purified in our laboratory using a standard recrystallization method. The pure compounds were titrated: Fisher certified A.C.S. alkalimetric standard grade anhydrous sodium carbonate (Na_2CO_3), and analyzed (FMC 100%) sodium sesquicarbonate ($Na_3HCO_3 \cdot CO_3 \cdot 2H_2O$). Two mixtures of sodium bicarbonate and sesquicarbonate were titrated as well. One is a commercial sodium bicarbonate / sesquicarbonate mixture sold by Kerr-McGee labeled as "sodium bicarbonate" for use in animal feed and the other was prepared by carefully weighing each component to 0.01 mg and mixing the FMC sodium sesquicarbonate with MC/B reagent A.C.S. grade sodium bicarbonate ($NaHCO_3$). Each material was titrated with ca. 0.1 M HCl (diluted from Fisher A.C.S. reagent 37% HCl) using from 4 to 12 runs, with each titration employing a new sample of material weighed to 0.1 mg. The sample sizes and molarities of the acid titrant are listed in Table 1.4.

Experiment name	Description
Ses/Bic mix	Controlled mixture of pure bicarbonate and sesquicarbonate
Sample #5	Kerr-McGee mixture of bicarbonate and sesquicarbonate
Carbonate	Heat and vacuum dried sodium carbonate
TRIS run set 3	TRIS reagent standardization for some experiments
Sesquicarbonate	Sodium sesquicarbonate

Table 1.3 Names for each experimental set and the chemicals involved.

Run ID #	Ses/Bic mix	Sample #5	Sodium Carbonate	Sodium Sesquicarbonate	TRIS (for Carbonate)
A	0.17703	0.29834		0.2759	0.34212
B	0.28070	0.32500	0.09840		0.24801
C	0.19966	0.34481	0.11450		0.17463
D	0.14800	0.21921	0.19108	0.2363	0.11839
E	0.24094	0.19299	0.17108	0.2098	0.22732
F	0.32164		0.12139	0.2483	0.23038
G			0.20383	0.2112	0.11794
H			0.15204	0.1775	0.27377
I			0.10355		0.18651
J			0.09436		
K			0.11525		
L			0.12757		
M			0.05501		
Molarity of HCl used in titration:					
	0.12554	0.12554	0.12246	0.11426, 0.11406*	
*The last two runs , G and H, of sesquicarbonate used 0.11406 M HCl					
Ses/Bic mixture consists of 2.84054 g sesquicarbonate dry mixed with 9.37764 g bicarbonate; 23.2485% sesquicarbonate and 76.7515% bicarbonate					
Sample#5 contains 20.8% sesquicarbonate and 79.1% bicarbonate					

Table 1.4 Mass in grams of each sample and molarity of acid.

Solutions, Sample Preparation, Procedures

Sample Storage, Weighing, and Standardization

All samples were kept in a desiccator filled with indicator anhydrous $CaSO_4$ (Drierite). All samples were placed in a glass container (either in a 5 ml beaker or a weighing jar) and weighed using the subtractive technique on a Mettler balance to a precision of 0.1 mg. A small amount of desiccant was kept in a vessel inside the balance chamber to minimize error due to absorption of water by the samples. Most samples were weighed out in an amount such as to require between 10 ml and 30 ml of reagent to reach the end of the titration. Each sample was dissolved in ca. 50 ml of fresh distilled deionized water. The reagent used was (ca. 0.12 M) HCl prepared by diluting concentrated 37% HCl. TRIS was used to standardize the HCl.

Drying, Mixing, Sampling Procedures

The sodium carbonate was baked in an oven at 270°C to drive off water as well as to convert any bicarbonate present into carbonate.[1] Sodium sesquicarbonate and bicarbonate were dried by storage in a desiccator for at least two days. This method was used since heating and vacuum methods were found to cause these compounds to decompose.

The Ses/Bic mix was prepared by weighing and mixing dried 9.37764g of sodium bicarbonate with 2.84054 g sodium sesquicarbonate. The mixture was mixed by a stirring bar for 4 hours then shaken by hand intermittently for an hour. The result was kept in a desiccator for at least two days to dry.

Sample#5 was mixed dry in order to assure a representative sample by manually mixing on polyethylene sheets by an operator wearing polyethylene laboratory gloves. It was then passed six times through a slotted sample divider to produce a one pound batch from which the samples were taken.

Experimental Procedure

The pH meter was calibrated once each day using Fisher standard pH 10.0 and 7.0 buffers so that the meter display would reflect the values recorded in the computer. The factors to convert the voltage output of the pH meter voltage to pH values in the computer were calibrated *before each experiment* using the **TIGHTC** least squares regression multiple point calibration procedure with pH 4.0, 7.0 and 10.0 buffers.

The capacity of the buret is ca. 30 ml. It is undesirable to stop the experiment to refill the buret as an error may result due to removal of substrate or addition of unmeasured reagent. Therefore whenever possible, samples were weighed in an amount such as to require between 10 ml and 30 ml of reagent to reach the end of the titration. Sample weights are given in Table 1.4. The sodium sesquicarbonate samples were dissolved in fresh distilled deionized water, 50 ml measured by volumetric flask; all other samples were dissolved in ca. 50 to 200 ml water. When a titration used more than 30 ml of reagent, the delivery capillary was slowly removed and washed down, taking care not to compress the plastic tubing. The buret was refilled and the capillary held above a waste beaker while backlash in the gearing of the buret was removed, and the capillary rinsed again before reinserting into the sample beaker.

Once the instrument was calibrated, the sample solution was stirred with a magnetic stirrer. The solution was stirred at the fastest rate for which mixing is smooth. The delivery capillary is held just touching the solution. The computer then automatically delivers reagent and reads pH. The computer calculates the derivative and attempts to deliver the next aliquot such that the change in pH is maintained at 0.1 unit. When the experiment is over, the titration is stopped and the computer was commanded to print the data (pH, Volume, first derivative, second derivative and possible endpoints) and plot the titration curves.

Results and Discussion

Analysis

Several analyses were performed on the titration data. Percentages of carbonate and bicarbonate in the material were determined by examining the volume of reagent added at interpolated maximum derivatives. The data were then fit using a non-linear simplex minimization procedure with an empirical ("local") model to perform smoothing and the endpoints were derived. The fitting procedure is described in Appendix 5.0A . Values of acid dissociation constants K_1 and K_2 were determined from some of the runs by analyzing several points and by fitting with a theoretically derived equation.

Determination of Inflection Points

The endpoints were determined by two methods. One method was to take a numerical derivative of the data (equation 1.1), determine the point at which the derivative was at a maximum and in that region, linearly interpolate the second derivative to zero and find the volume at that point. This volume is then considered to be the endpoint for the titration.

Another method used was to fit the data about the endpoint region to the equation 1.3:

$$F(V) = pH = \frac{A}{1 + e^{(B+CV)}} + D\,V + E \qquad (1.3)$$

Where:

V is volume

A, B, C, D, E are the parameters of the equation

This model describes a single sigmoid; therefore it was performed locally for points where the derivative of the data becomes a significant percentage of the maximum derivative; i.e. it cannot and was not used to describe the entire curve. The inflection point is determined by the equation 1.4:

$$V = -\frac{B}{C} \qquad (1.4)$$

Details of this model and derivations are given in Appendix 5.3A. This model worked very well for the region about the endpoint which was fitted, as per the statistics shown in Table 1.5. The meaning and definition of these statistics may be found in Appendix section 5.10A. This model represented the data very well in the region that the data was fit. Figure 6 through Figure 8 show Sample#5 along with the model. In the regions that data were fit, delimited by arrows, the model represented the data, the first and the second derivatives very well. The model did not describe the curve outside these regions, which is evident by examining the points on the figures extrapolated outside the fitting region. One significance of the model fit goodness is that the equivalence point could be considered to occur at the same point as the inflection point of the curve, since the model about the inflection point is symmetrical.

Experiment	R^2 value	Residual about model	Model Correlation	RMV
carbonate 1st endpoint	0.99993	0.0335	5.284	0.99993
carbonate 2nd endpoint	0.9997	0.7256	21.136	0.99965
TRIS run set 2	0.9986	0.8108	12.369	0.99928
TRIS run set 3	0.9994	1.204	29.548	0.99915
Ses/Bic mix 1st endpoint	0.99998	0.00954	3.8445	0.99998
Ses/Bic mix 2nd endpoint	0.9994	0.8057	12.841	0.99864
Sample #5 1st endpoint	0.99994	0.02704	4.4672	0.99994
Sample #5 2nd endpoint	0.9995	0.5708	11.668	0.99948

Table 1.5 Mean statistics of modeling titration experiments with equation 1.3. The residual is variation of the fit about the model; Model correlation is variance due to model; RMV is Relative Model Variance.

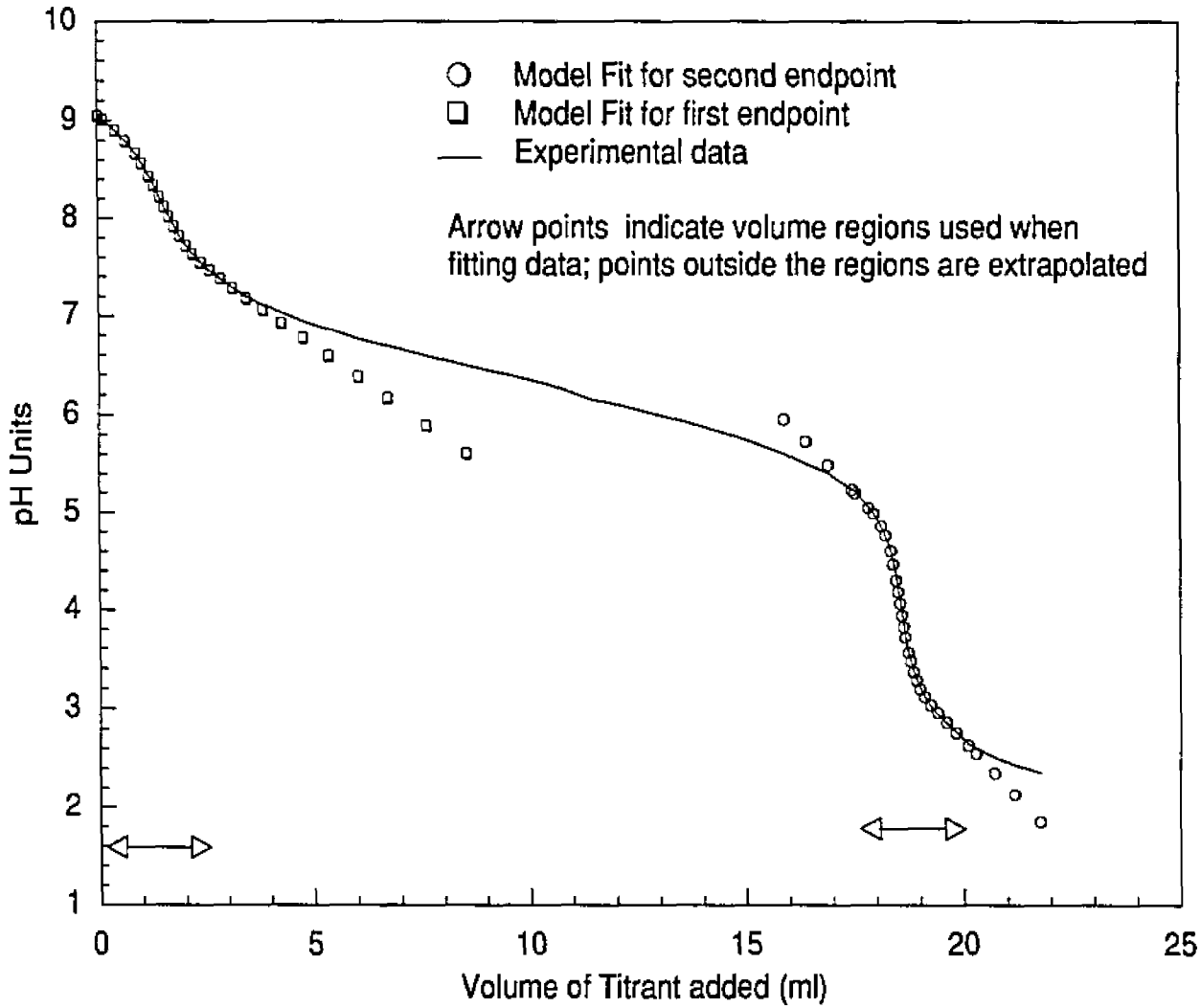

Figure 6 Sample #5 Experimental Data and Data from Local Model

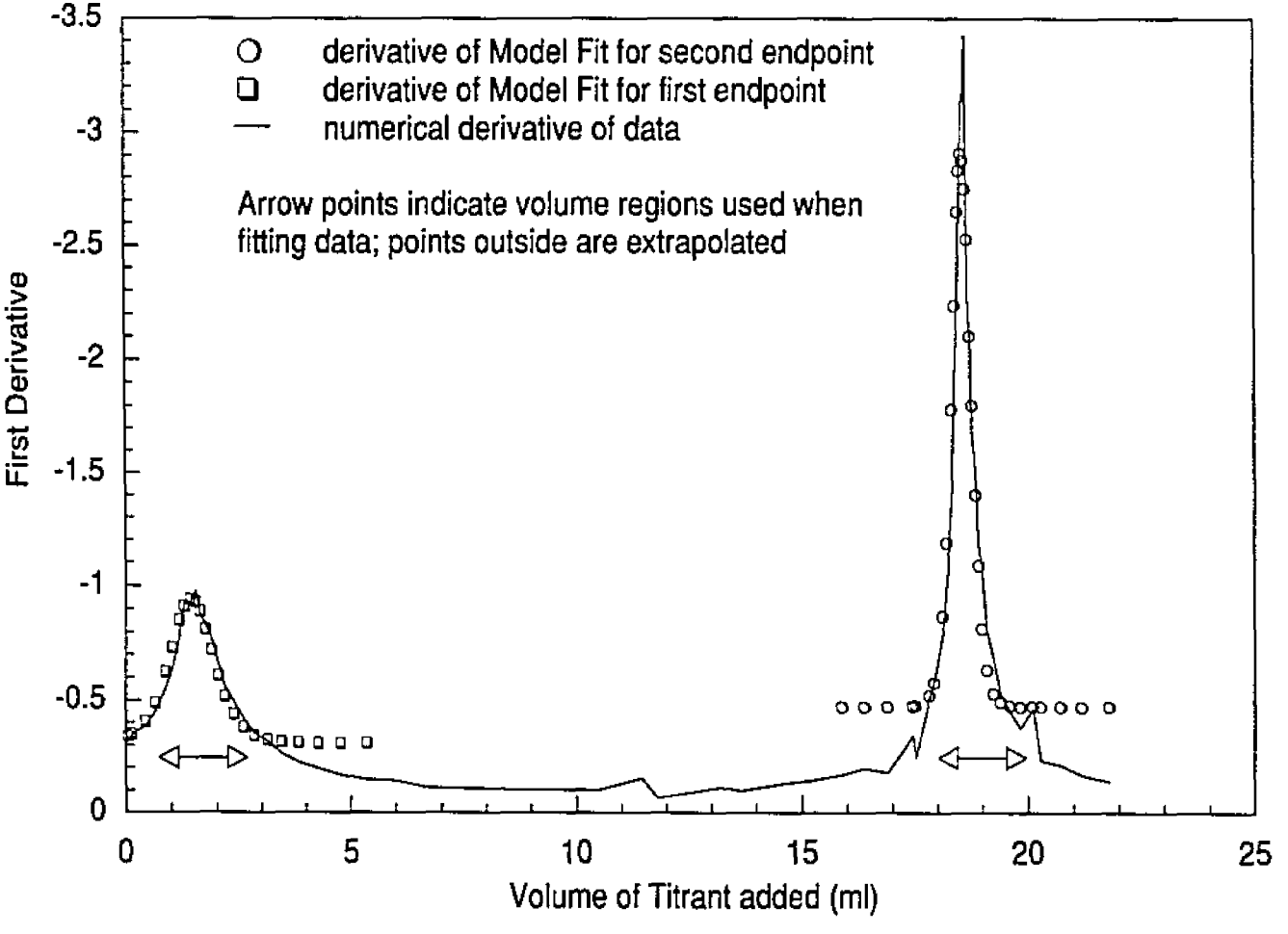

Figure 7 First Derivative of Both Sample #5 Experimental Data and Model

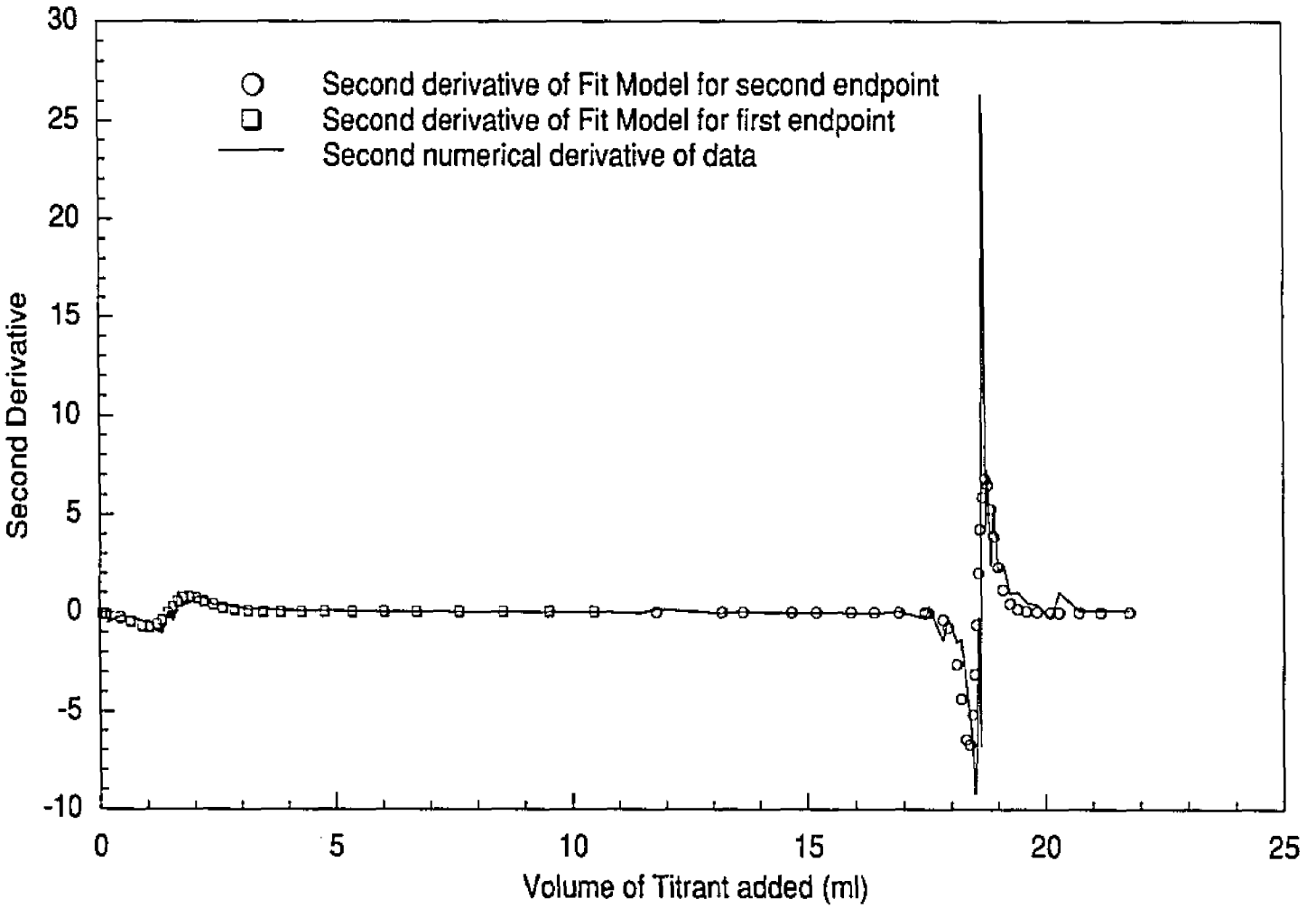

Figure 8 Second Derivative of Both Sample #5 Experimental Data and Model

Equivalence Point Results and Calculations

The equivalence point results from both the model fit and the calculation from the derivative are given in Table 1.6. The expected value in the second column is the value which should be measured, barring errors in weighing, unknown sample absorption of water or CO_2 , etc. The expected value of sesquicarbonate comes from calculation from the formula weights. Ses/Bic mixture assumes no error in weighing and mixing of the two components, and Sample#5 value is from a thermal gravimetric analysis of a sample from the lot. The next columns, three and five, show the mean error between the measured quantity and the expected quantity, when the endpoint is determined using the numerical derivative from equations 1.1 and 1.2. The relative error is given, calculated by equation 1.5:

$$\text{relative error \%} = 100 * \frac{\text{result - expected value}}{\text{expected value}} \qquad (1.5)$$

The absolute error (abs) is given in parenthesis, where applicable. The next columns, four and six, have the mean of the data along with standard deviation, showing the spread of values. Each set of results are discussed, and errors are calculated and discussed in more detail.

First, the ca. 0.1 M HCl solution was standardized with TRIS. The molarity of the acid was calculated by the average of runs using equation 1.6:

$$M_{HCl} = \frac{\text{TRIS weight}}{\text{TRIS Molecular Weight} * \text{Endpoint Volume}} \qquad (1.6)$$

Where:

TRIS Molecular weight is 121.14 g

Whenever a new batch of HCl was prepared, it was restandardized. A list of values for the molarity of the HCl used for each set of experiments is at the bottom of Table 1.4. As an example of the quality of a TRIS titration, a typical standardization result is shown in Table 1.6. Precision is very good for this titration, with the error in the fourth significant digit; or a relative standard deviation of 0.23%. Figure 1 shows a typical titration curve of TRIS with ca. 0.12 M HCl, with first and second derivative curves shown in Figure 2 and Figure 3. At the beginning of the titration, buffer capacity is low and thus the derivative is relatively large. As more HCl is added, the solution is in the buffer region, with a change of pH from 9.8 to 7.6 with the addition of ca. 12 ml acid. The endpoint is very sharp, with the change in pH almost vertical. The endpoint occurred at a mean pH of 4.54 ± 0.18 (fit 4.79 ± 0.11).

Pure dried sodium carbonate was titrated. A sample of one of the runs in depicted in Figure 9. The curve consists of two sigmoids. The point of the first inflection is less well defined than the second inflection (Figure 10 and Figure 11). The first buffer region occurs before pH 9.4, after which the first endpoint occurred at a mean experimental pH of 8.45 ± 0.11 (fit 8.43 ± 0.087) The first endpoint signifies the point where most of the carbonate has changed to bicarbonate. The second buffer region occurs between pH 7 and 5.3. In this region, bicarbonate is being changed to carbonic acid and solvated carbon dioxide. At approximately pH 5.4, with the concentrations used in these experiments, there is saturation of CO_2 indicated by formation of gas bubbles in the solution. The experimental pH at the second endpoint is 4.11 ± 0.11 (fit is the same, 4.11 ± 0.11). The second endpoint is twice as steep as the first, with mean derivatives 4.54 ± 1.54 (the fit is: 3.99 ± 1.35) 2.11 ± 1.01 (the fit is: 1.99 ± 0.88). The second endpoint is expected to occur at twice the volume of the first endpoint.

Percent carbonate is expected to be 100%. It was calculated separately from each endpoint by the following equation 1.7:

$$\% \text{ carbonate} = \frac{\text{Reagent Molarity} * \text{Endpoint} * \text{Formula Weight}}{\text{Sample Weight} * \text{endpoint \#}} * 100 \quad (1.7)$$

Where:

The Molarity of the reagent was 0.12246 M
Formula Weight of Sodium carbonate is 105.9887 g/M
endpoint # is 1 for the CO_3 to HCO_3 point and
endpoint # is 2 for CO_3 to CO_2/H_2CO_3 point

The equation 1.7 is divided by two (the endpoint #) at the second endpoint because the volume should be offset by the amount required to titrate the carbonate to bicarbonate, which should be equal. Calculation of percent carbonate shows that the spread of values is much greater if calculated from the first endpoint with a standard deviation of 3.8 times that of the second endpoint. This spread is significantly decreased by using the model fit; while the values obtained from the second endpoint changed little, the first endpoint spread decreased by half. This was the only data set whose precision improved significantly (F-test) by fitting with the local model. The error relative to the expected value decreased by about 18% but remained the same for the second endpoint values, with the result being that after the fit, the second endpoint gave a result twice as accurate as the first.

Sesquicarbonate samples were examined. This material is sodium bicarbonate and sodium carbonate bonded together with two waters. A sample titration is given in Figures 12, 13 and 14. Pure sesquicarbonate should consist of 37.17% bicarbonate, 46.89% carbonate and the rest water by mass. The data were noisy enough that fitting to the local model in equation 1.3 was not possible, as the model did not converge to reasonable

values. The sesquicarbonate was examined in several ways: as pure material where % sesquicarbonate is determined individually from each endpoint and looked at as a mixture of bicarbonate and carbonate. To determine percent from each endpoint value, equation 1.8 was used:

$$\%\ \text{Sesquicarbonate} = \frac{\text{Reagent Molarity} * \text{Endpoint} * \text{Formula Weight}}{\text{Sample Weight} * \text{endpoint\#}} \quad (1.8)$$

Where:

Reagent Molarity was 0.12554
Formula Weight of sesquicarbonate is 226.02601 g/M
endpoint # is 1 for the first e.p., and 3 for the second.

The divisor, endpoint #, is 3 because first X moles of material (carbonate) is titrated, translating the endpoint once, and after the first endpoint, since the compound is equimolar in carbonate and bicarbonate, $2 \cdot X$ moles of bicarbonate remain, making the factor 3. The experimental results show an order of magnitude difference in accuracy between the first and second endpoint, but the precision was only slightly better for the second endpoint. To consider percent carbonate equation 1.7 was used at endpoint 1, and for bicarbonate, equation 1.9 was used.

$$\%\ \text{bicarbonate} = 100 * \frac{\text{Reagent Molarity} * (\text{Endpoint2} - 2 * \text{Endpoint1}) * \text{Formula Weight}}{\text{Sample Weight}} \quad (1.9)$$

Where:

Reagent Molarity was 0.12554
Formula Weight of bicarbonate is 84.00687 g/M

This equation assumes no proportionality between the first endpoint and the second. Twice endpoint 1 is subtracted from endpoint 2. This volume has to

be subtracted, and this volume is also proportional to the amount of bicarbonate added to the initial concentration of bicarbonate. Here, precision for the carbonate declines and overall error for the expected value increases. A smaller quantity of carbonate is found, and a larger value of bicarbonate. Combined, the two have a relative experimental error of 0.09%.

Two mixtures of sesquicarbonate and bicarbonate were titrated. They are depicted in Figures 6 to 8 and 15 to 20. One was prepared from the individual components, and the proportion was 23.25% sesquicarbonate and 76.75% bicarbonate. The other mixture, Sample#5, was determined to contain 20.8% sesquicarbonate and 79.1% bicarbonate by thermal gravimetric analysis. The errors here are much larger than with the pure compounds, and the accuracy of the Ses/Bic mixture benefits from performing the local fit (but the precision shows little difference).

Identities (1)	Expected (2) value	N. Rel (abs) (3) error	Numeric (4) Mean ± SD	Fit Rel (5) error	Fit (6) Mean ± SD
TRIS (Molarity)			0.12554 ± 0.00029		0.12556 ±0.00029
Carbonate					
1st endpoint %	100	0.579%	99.421 ± 0.563	0.469	99.531 ±0.272
2nd endpoint %	100	0.245%	100.245 ±0.147	0.245	100.245 ±0.142
Sesquicarbonate					
1st endpoint %	100	0.64%	100.64 ±0.28		
2nd endpoint %	100	0.06%	100.06 ±0.21		
Carbonate %	37.17	1.05%(-0.39)	36.78 ±0.38		
Bicarbonate%	46.89	0.66 %(0.31)	47.20 ±0.13		
Combined %	84.06	0.095%(-0.08)	83.98		
Ses/Bic mixture					
Sesquicarbonate %	23.249	2.97 (-0.69)	22.559 ±0.438	1.65 (-0.384)	22.865 ±0.324
Bicarbonate %	76.752	0.883(0.678)	77.429 ±0.465	0.465(0.593)	77.108 ±0.337
Combined %	100	0.012	99.988	0.027	99.973
Sample#5					
Sesquicarbonate %	20.8*	8.149(1.70)	22.495 ±1.245	8.837(1.838)	22.638 ±1.211
Bicarbonate %	79.1*	3.247(-2.57)	76.532 ±1.368	3.442(-2.72)	76.377 ±1.280
Combined %	99.9	0.87	99.027	0.886	99.015 (0.885)
NOTE: *Expected value for Sample#5 is from Thermal Gravimetric analysis of a sample from the lot. All others assume pure homogeneous mixtures or materials					

Table 1.6 Results from endpoint determinations; error calculated as: (expected-experiment) ÷ expected; ± values are experimental standard deviation. For more detail, see the text.

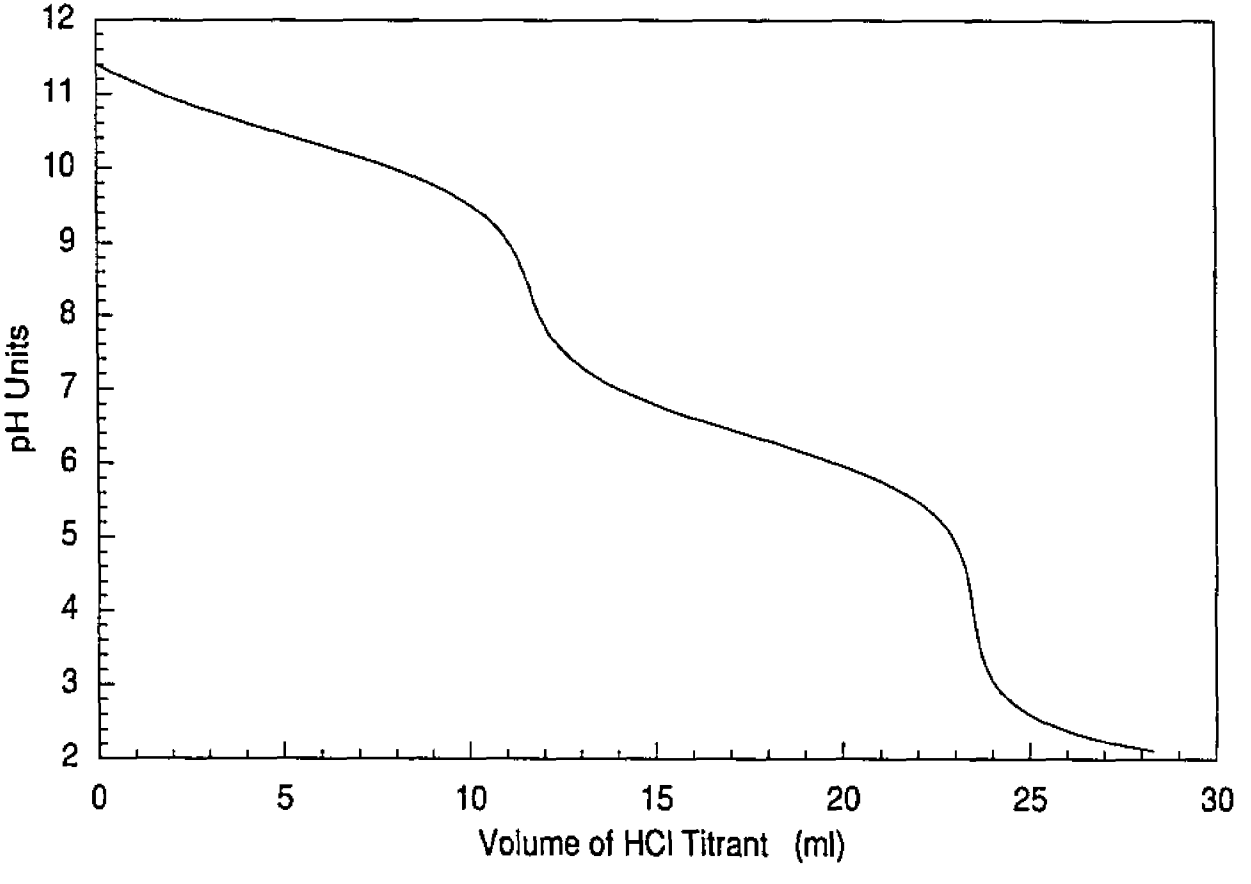

Figure 9 Titration of Sodium Carbonate with HCl

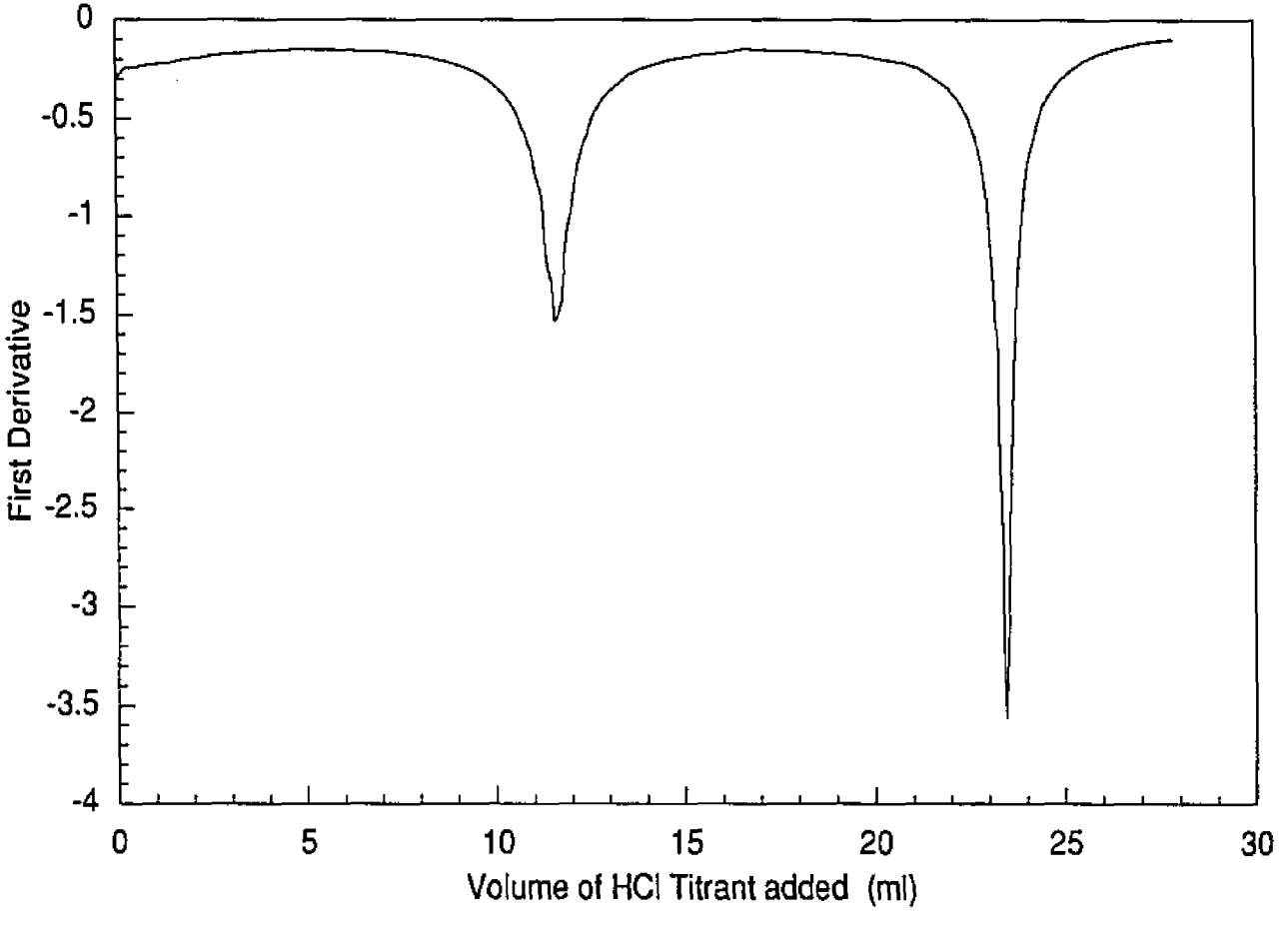

Figure 10 First Derivative of Sodium Carbonate Titration

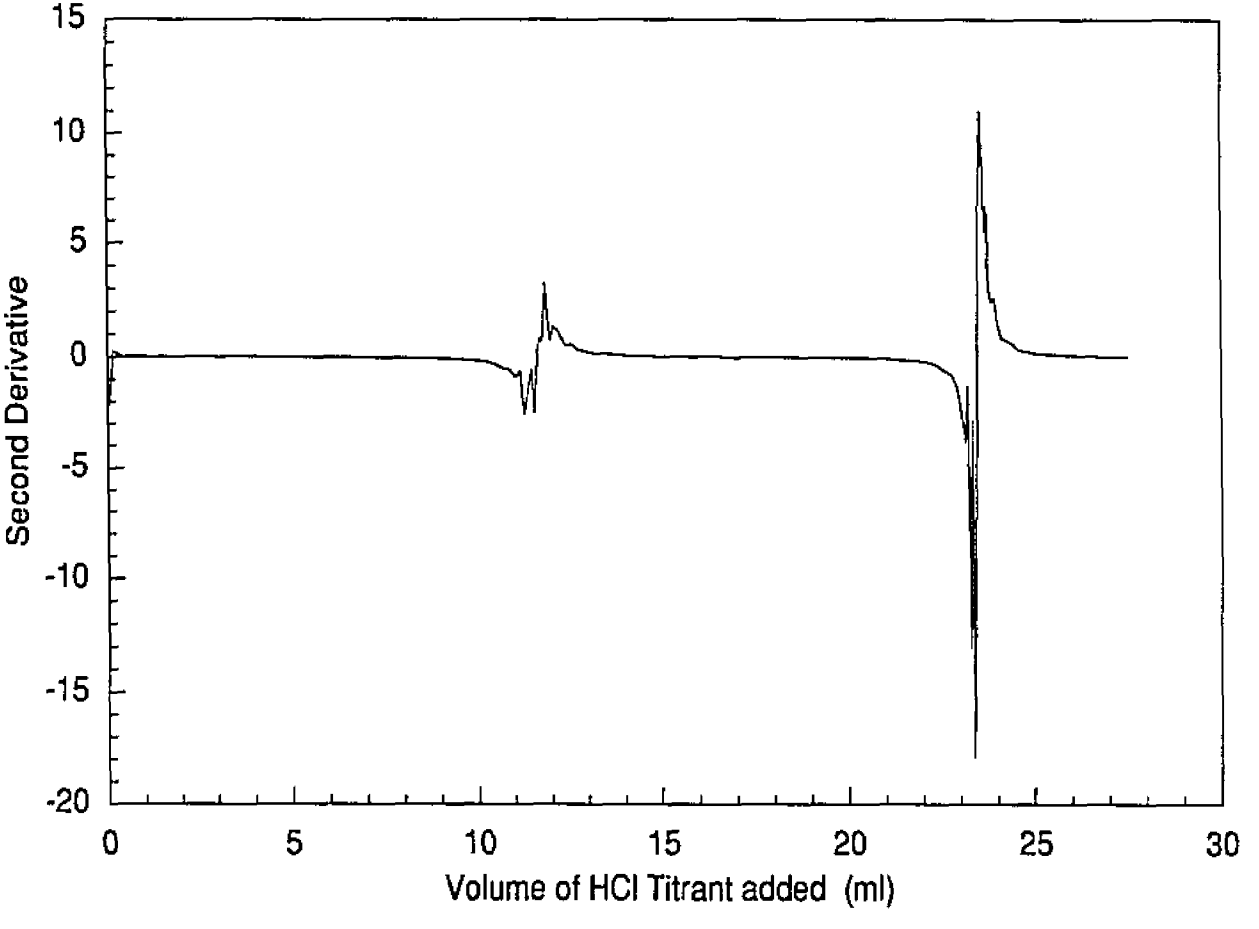

Figure 11 Second Derivative of Sodium Carbonate Titration

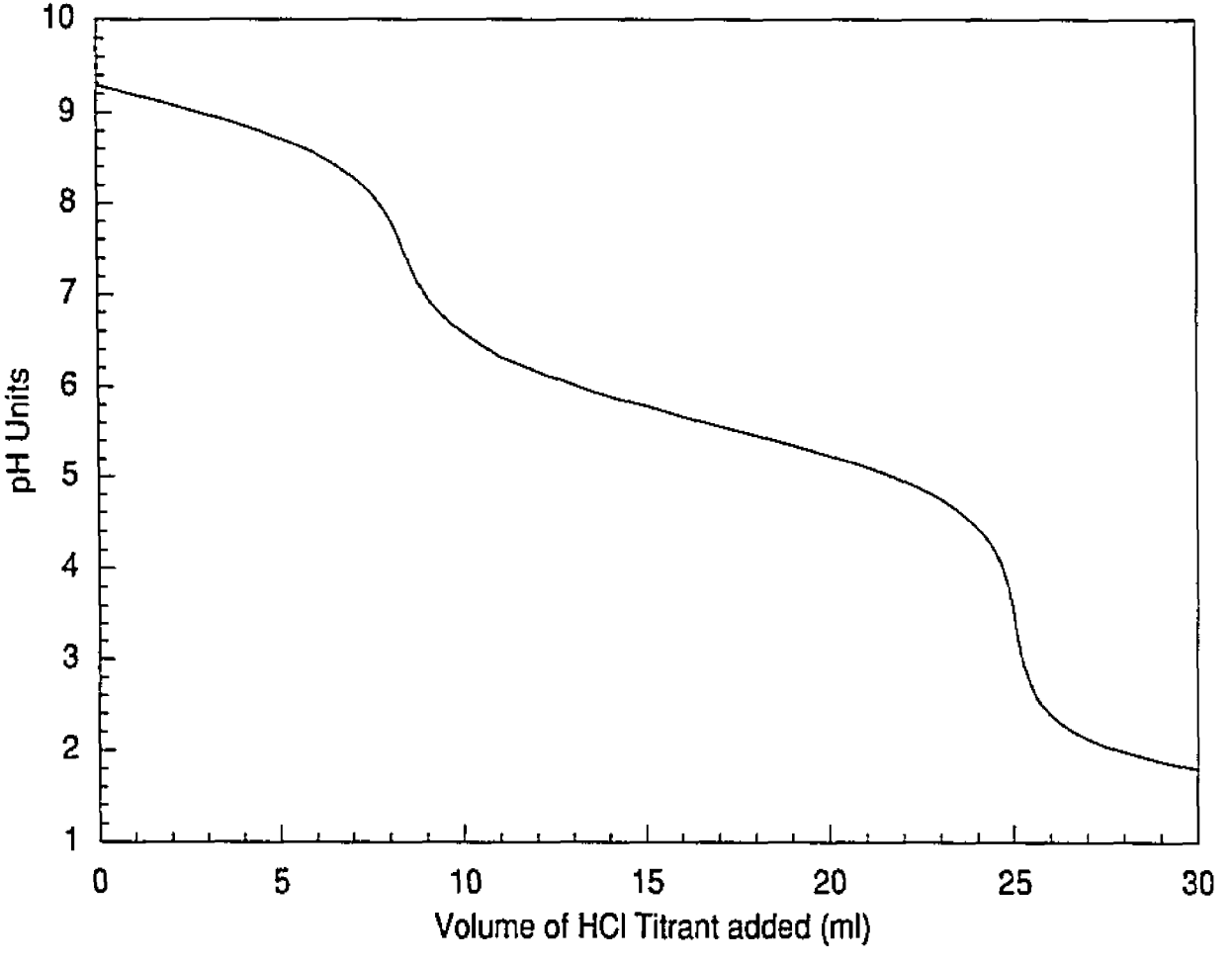

Figure 12 Titration of Sodium Sesquicarbonate with HCl

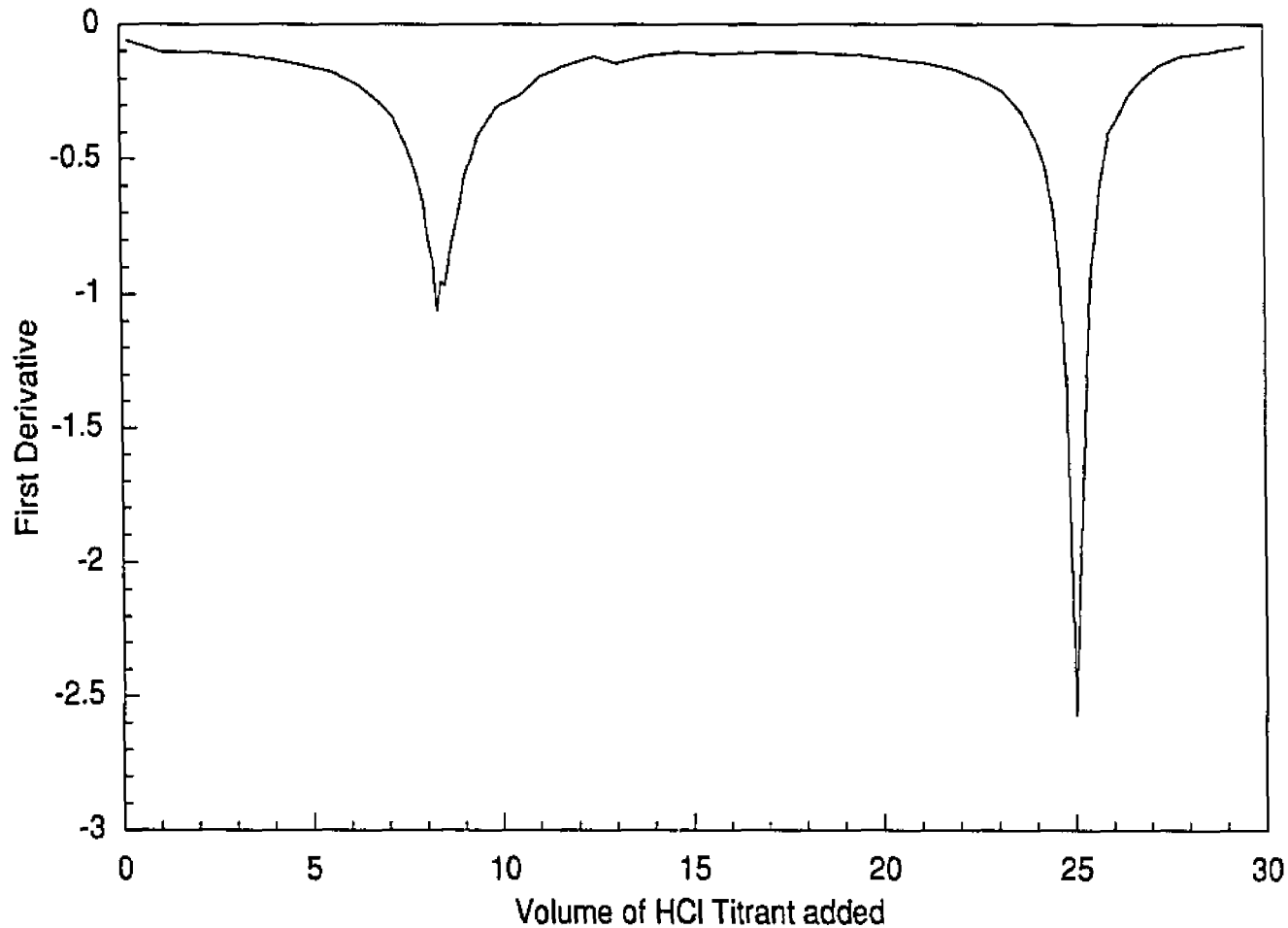

Figure 13 Sodium Sesquicarbonate Titration, First Derivative

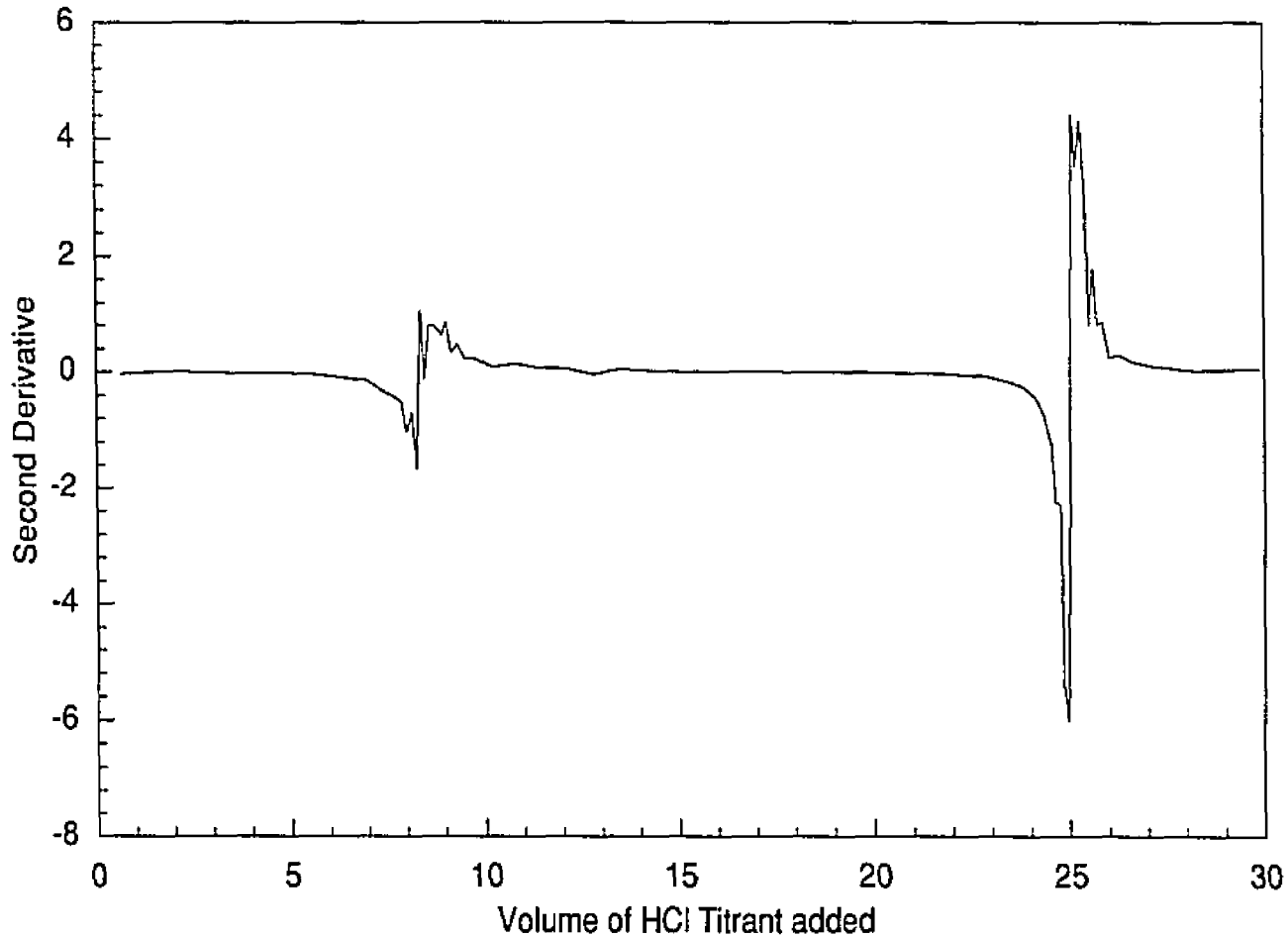

Figure 14 Sodium Sesquicarbonate Titration, Second Derivative

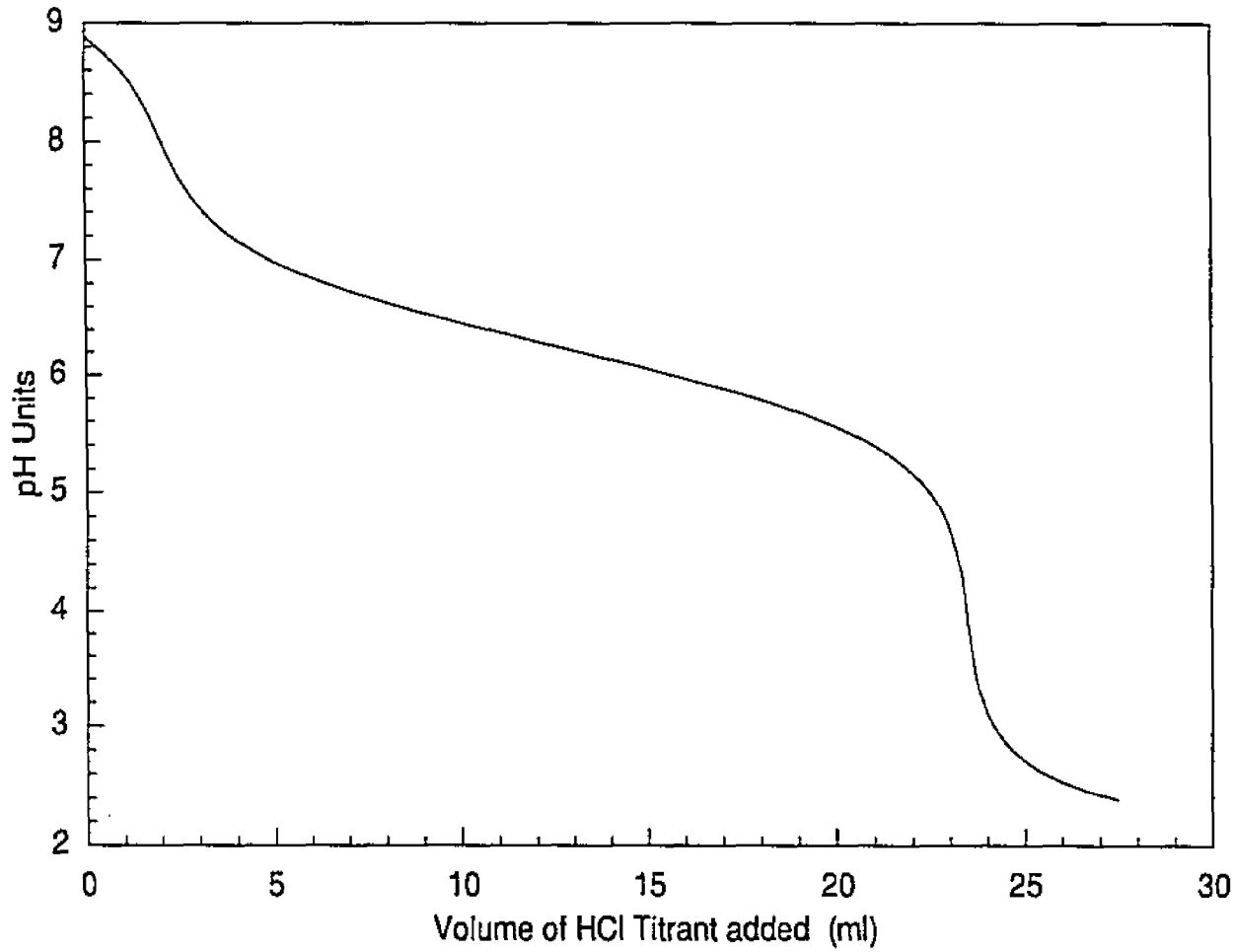

Figure 15 Titration of Sesquicarbonate/Bicarbonate Mixture

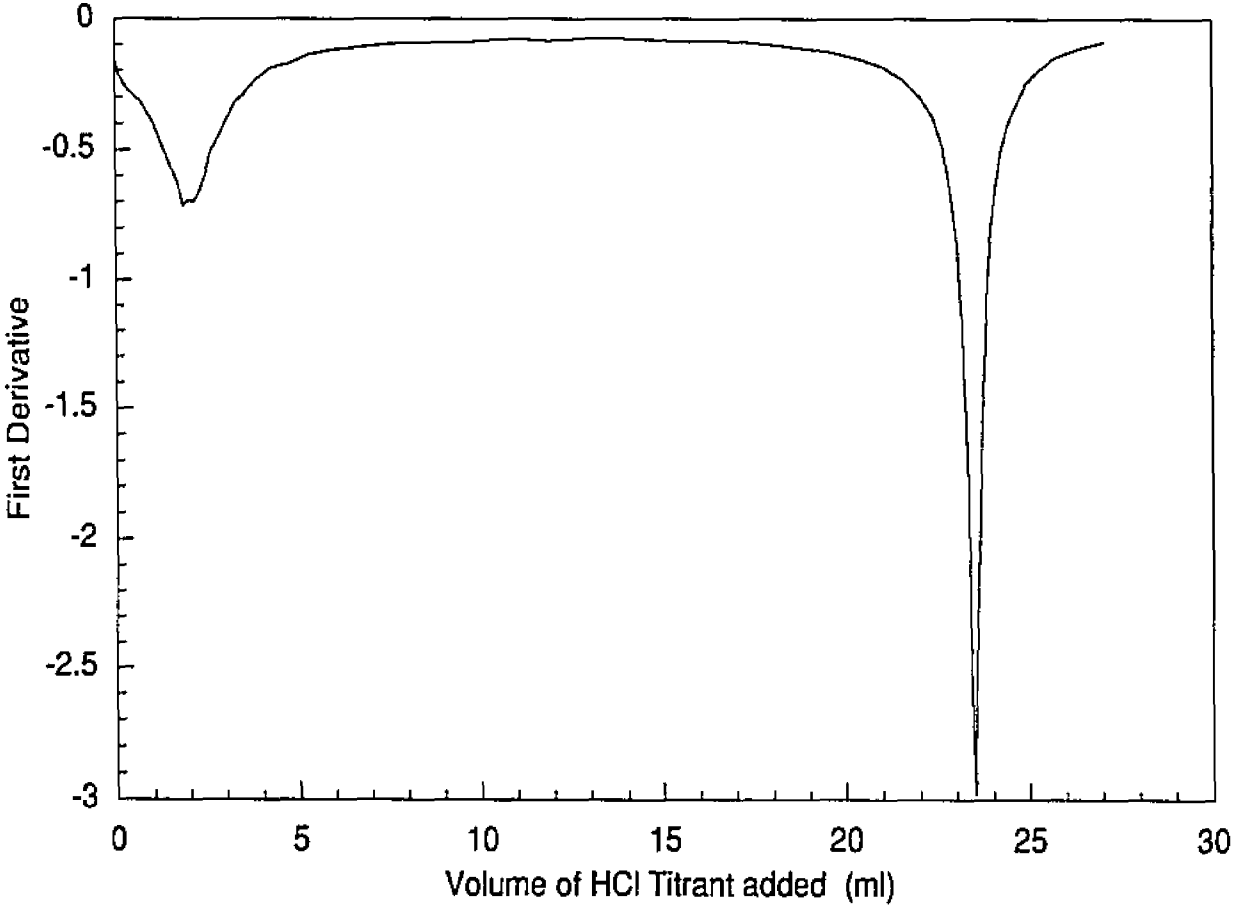

Figure 16 First Derivative Sesquicarbonate/Bicarbonate Mixture

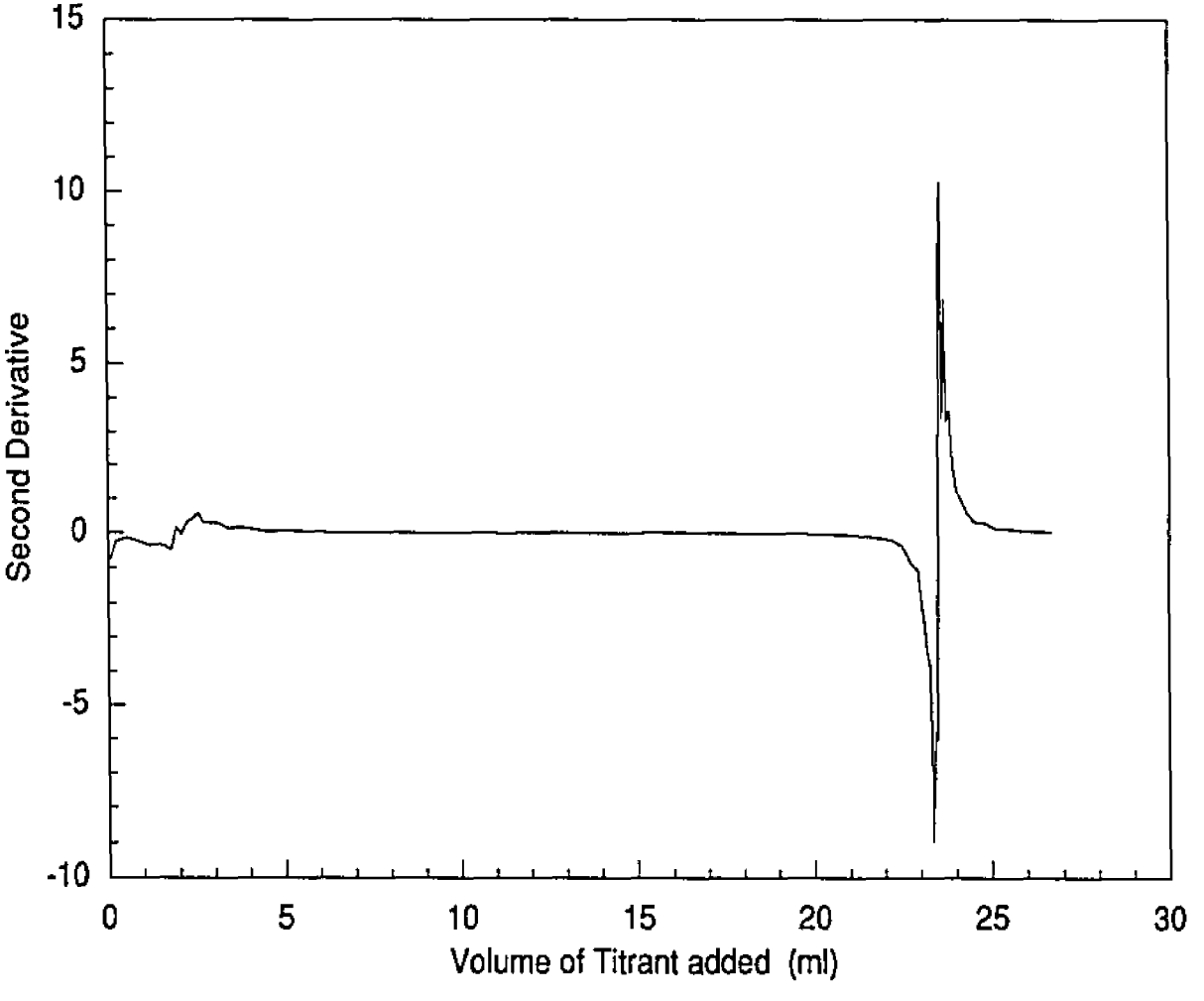

Figure 17 Second Derivative of Sesquicarbonate/Bicarbonate Mixture

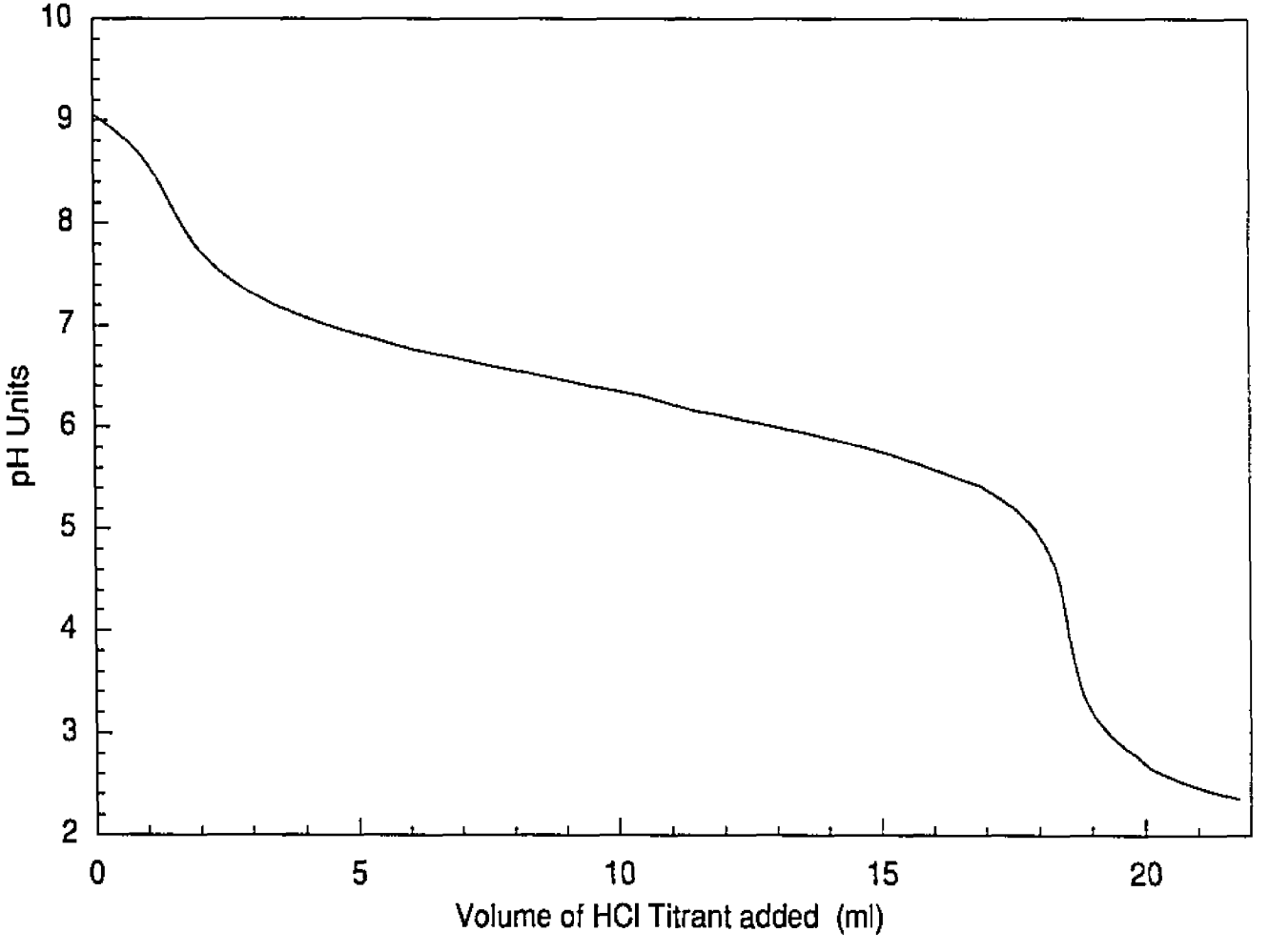

Figure 18 Titration of Sample #5 with HCl

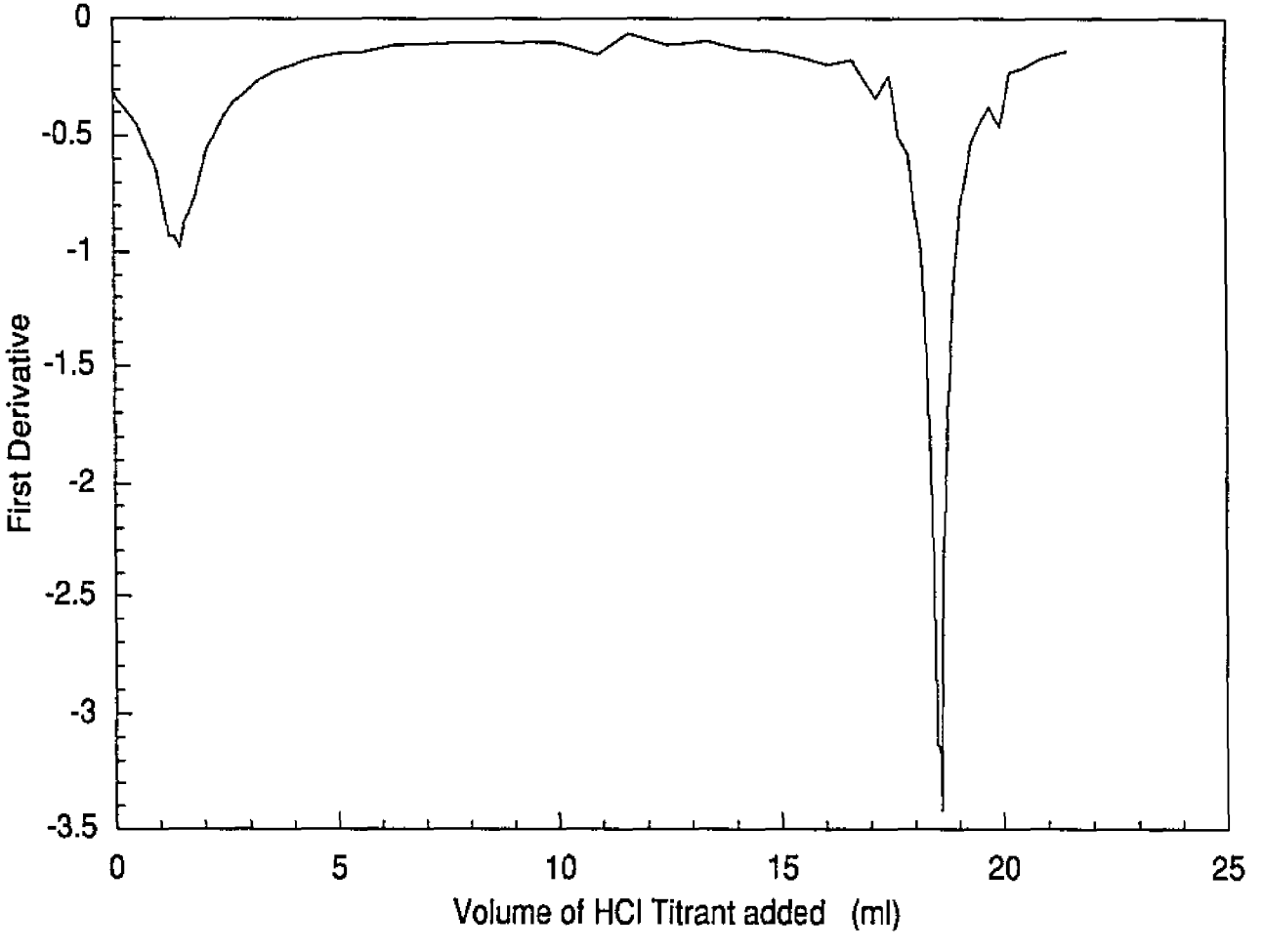

Figure 19 First Derivative of Sample #5 Experimental Data

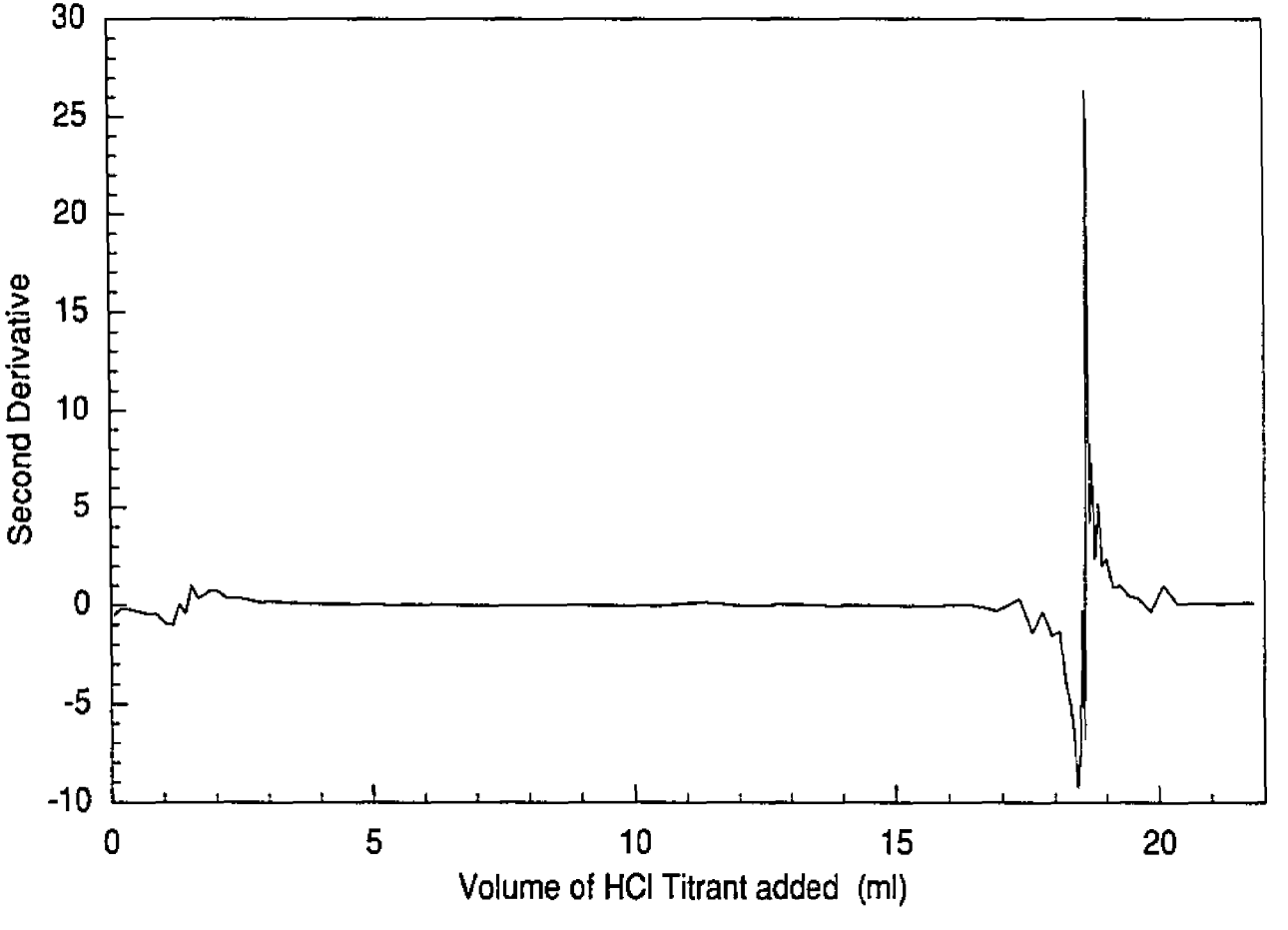

Figure 20 Second Derivative of Sample #5 Titration

Error in Titration

The basic errors in the titration come from the errors in the parts of the equations used to calculate the percentage results. Examining equations 1.6 through 1.9, we can see that the errors come from:

1) Location of endpoint(s)
2) Calibration of the reagent
3) Weight of the sample
4) Value of the formula weight, which is less than one thousandth of a percent, and thus negligible.

Errors in locating the endpoint are the most complex, and are discussed first.

Given a titration method and a measurement of a specific precision, a higher slope ($\Delta pH / \Delta V$) at the equivalence point will yield better analytic precision.[1] The slope depends upon the equilibrium constant of the material and upon the stoichiometry. Material with a larger equilibrium constant will result in a larger derivative and thus a sharper endpoint. Material with a smaller constant will have a less sharp endpoint. The derivative is broader and the top flatter, and thus is more poorly defined. In this case, a small error in the volume results in the measured endpoint being at a point with more unreacted material, resulting in a larger error. This error is independent of method. This has been called the innate titration error in Grunwald's book[1] and can be described by a sharpness index or sharpness of detection of endpoint[20] in equation 1.10.

$$\eta = \frac{d\,pH}{d\,T} \qquad (1.10)$$

Where:

pH = the pH of the equivalence point
T = the fraction of material titrated.

For the specific case of the Winkler titration, if we assume that the ability to detect the color change of the indicator is ± 0.5 pH unit; using comparison, ± 0.1 pH unit,[17] and values of $\eta = 22$ and 74 for the first and second endpoints;[22] the error in volume expected would be 5% and 0.7% respectively for ± 0.5 pH unit detection and 2% and 0.1 % respectively for ± 0.1 pH unit detection. This is the error in the volume due to the reading of the pH; other errors involved in the volume, such as reading the buret, buret calibration, etc. are not figured in.

The error in volume from the automatic titrator used in this experiment results from three factors. One error comes from reading the delivered volume, which has a precision of $\pm 1.9 \cdot 10^{-3}$ ml. This error comes from the instrumental design as described in Appendix 1.1.2A and is the error at any volume (i.e., it is not cumulative). This value cannot describe the entire system, as there are delivery errors due to motor gear variations, irregularities in the glass buret tube, small leaks, variation in delivery to the solution and flexing of plastic tubing in the system. Therefore, the variation was determined experimentally. A set of aliquots were delivered into a beaker. Each was weighed and the number of motor turns recorded. From density, mass was converted to volume and the flow rate conversion calculated in equation 1.11:

$$\text{Flow rate factor} = \frac{\text{Mass}}{\text{Density} * \text{Turns}} \qquad (1.11)$$

Where:

Mass	is mass of liquid delivered in grams
Density	is the density of water at the temperature, 23°C
Turns	is the number of pulses from the tachometer buret

The variation in ml for each delivery was found to be $\pm 2.5 \cdot 10^{-3}$ml, about thirty percent larger than the precision. This variation was calculated by determining the mean flow rate, applying it to the turns from each measurement, and finding the standard deviation between it and the experimentally determined volume.

Another error is in accuracy, due to calibration of the flow rate. The mean flow rate factor from the above experiment was found to be 1.875 ± 0.006 ml/Turn; the relative error in calibration is 0.32%. An error in this value will make a reading consistently high or low. However, if the value of the flow rate does not change between experiments, this error in the HCl solution molarity and endpoint volume will cancel. Differences in temperature can change this value since the density of water changes with temperature. This calibration was done at 23°C; from 20° to 25°, the density varies from 0.998234 g/l to 0.997075 g/l which will make the flow rate vary by ± 0.001 ml or 0.05% variation.

The third error is due to η in equation 1.10 and is derived from the error in the pH. The pH is read from the electrode via an analog to digital converter (A/D) in the computer. The A/D converter is 12 bits; it reads ± 5.115V in 4096 units or $2.498 \cdot 10^{-3}$ volts/unit. The linearity error of this device is ± 1 unit, an error of $\pm 2.498 \cdot 10^{-3}$ volts/unit. The mean of the factor to convert relative volts to pH is 0.7429 volts/pH unit (typical experimental values; from the eleven carbonate titrations). Using this value, the error from the A/D converter is $3.36 \cdot 10^{-3}$ pH unit. Another error in reading the pH is noise in the measurement. In actual practice, each pH value was the mean of eight sequential measurements. The criteria used for assuming that a pH value was stable was two fold. First, the mean change of a minimum of forty samples must be less than a fixed criteria, in these experiments, less

than $4.0 \cdot 10^{-3}$ V. Then the standard deviation of a minimum of twenty of the pH values must be less than $\pm 4.0 \cdot 10^{-3}$ volts. When both conditions hold, the reading was considered stable. These values were experimentally determined for a variety of electrodes and buffer conditions. Using the conversion value of 0.74292 volts/pH unit, the maximum accepted noise is $2.97 \cdot 10^{-3}$ pH units. With the A/D error added, the error in reading a value is 0.0063 pH units. Calibration error manifests itself in the factors which convert voltage to pH. It is in the error of the actual pH value, which does not affect the accuracy of the endpoint volume.

The mean derivatives of the experimental data is given in Table 1.6. The derivatives at the endpoint for the experimental data were calculated as $\Delta pH / \Delta V$, with the values of pH and volume straddling the actual endpoint. Derivatives calculated from the fit model are calculated from the analytic derivative of the (local) model described in equation 1.3 and Appendix 5.3A. For the sesquicarbonate data, the derivatives are numerically determined by an iterative bisection method with the theoretical model and parameters fit to that model, given in equation .12. Since the error equation 1.10 is given in terms of $\Delta pH / \Delta T$, the derivatives were also calculated in this manner. Equation 1.12 is used to convert from $\Delta pH/\Delta V$ to $\Delta pH / \Delta T$:

$$\frac{\Delta pH}{\Delta T} = \frac{\Delta pH}{\frac{\Delta V}{V_{endpoint}}} = \frac{\Delta pH * V_{endpoint}}{\Delta V} \qquad (1.12)$$

Where:

V = volume; for this paper, ml is used

T = fraction of material titrated;

T = 0 when no material has been titrated;

T = 1 at the equivalence point

The inherent error in volume due to the experiment as calculated from the derivative is given in Table 1.7. The mixtures have an error in the first endpoint volume of 0.4%, and the pure compounds are much smaller, 0.04 % to 0.08 %. The second endpoint, with larger derivative has smaller predicted error over a narrower range of values, from 0.01 % to 0.017 %.

Another error is simply the error in the mass reading. The Mettler balance used had a weighing error of ± 0.05 mg (sesquicarbonate weighing error was ± 0.1 mg).

Finally, the error in calibration is from the titration of TRIS. Since the endpoint is very sharp, the inherent error in volume is negligible. The errors in this calibration should be due to weight, fluctuation in volume and volume calibration. Here, the error due to weighing is 0.023%. The volume error is 0.017% from reading . The total error in the TRIS standardization is 0.029%.

The errors that come from the experimental conditions and the inherent errors are presented in Table 1.8. The total errors presented reflect propagation of errors from each component of the equations 1.7, 1.8 and 1.9 used to calculate the percent composition of the samples. The calculations for the pure samples have the smallest predicted errors of 0.06% for calculations using the second endpoint, and about twice the error when the first endpoint is used: 0.1% for sesquicarbonate and 0.08% for carbonate. The mixtures have much larger errors, approximately 0.3% for calculations of the bicarbonate and 0.6% for sesquicarbonate.

	Endpoint 1 ΔpH / ΔV	Endpoint 1 ΔpH / ΔT	Error in Volume	Endpoint 2 dpH / dV	Endpoint 2 dpH / dT	Error in Volume
Ses/Bic						
numeric	0.88±0.28	1.47±0.08	0.43%	3.3±0.7	63.4±8.1	0.010%
model	0.84±0.26	1.43±0.03	0.44%	2.8±0.7	53.5±6.2	0.012%
Carbonate						
numeric	2.1±1.0	18.2±1.3	0.035%	4.5±1.5	41.8±6.2	0.015%
model	2.0 ±0.9	17.3±0.8	0.037%	4.0±1.4	36.6±5.3	0.017%
Sample#5						
numeric	0.76±0.20	1.49±0.10	0.42%	2.7±0.6	59.9±3.2	0.011%
model	0.72±0.19	1.42±0.08	0.45%	2.3±0.5	51.6±5.1	0.012%
Sesquicarb.						
numeric	1.34±0.33	11.88±3.64	0.053%	3.4±0.5	59.3±10.4	0.011%
model	0.97±0.19	8.28±0.61	0.076%	2.8±0.3	48.1±5.5	0.013%
TRIS		210	$3.0 \cdot 10^{-5}$%			

Table 1.7 Average of absolute values of numerical and model derivatives with respect to volume (V) and percent titrated (T). Volume errors are from the inherent error only and assume ΔpH of 0.0063.

	Mean Relative Errors in:						
		Values #1			Values #2		
	Mass	Volume e.p. 1	from deriv.	Total # 1	Volume e.p. 2	from deriv.	Total #2
Ses/Bic	0.024	0.15	0.44	0.59	0.12	0.012	0.26
Carbonate	0.04	0.028	0.037	0.082	0.014	0.017	0.058
Sample#5	0.020	0.13	0.45	0.58	0.10	0.012	0.21
Sesquicarb.	0.045	0.029	0.076	0.12	0.0097	0.013	0.058

Table 1.8 Relative errors in titration. Values #1 are the errors in % sesquicarbonate in mixtures and % compound calculated from the first endpoint in pure samples. Values #2 are from calculation of % bicarbonate in mixtures and % compound calculated from the second endpoint in pure samples.

Equilibrium Constants

Introduction and Background

The dissociation constant values, K_1 and K_2, of carbonate and bicarbonate are of importance in biology, geology and inorganic chemistry. Any process which involves dissolved carbon dioxide or carbonic acid depends upon these values, as carbonic acid is converted to bicarbonate and carbonate depending upon the quantity of base or acid in solution. These values, given the mass action law, relate the ratio of the amount products to reactants and are constant at equilibrium. These values may be spread over many orders of magnitude so an associated quantity is used, the pK value defined in equation 7.0.

$$pK = -\log_{10}(K) \qquad (7.0)$$

Results will be discussed using the K values.

The K_2 constant corresponds to the first dissociation constant of carbonate salts or the second dissociation of carbonic acid:

$$[H^+] + [CO_3^{-}] = [HCO_3^-]$$

$$K_2 = \frac{[H^+]\,[CO_3^{-}]}{[HCO_3^-]} \cdot \frac{\gamma_{H^+}\,\gamma_{CO_3^{-}}}{\gamma_{HCO_3^-}} \qquad (7.1)$$

Where:

quantities in square brackets represent formal concentrations and γ represents the activity coefficients corresponding to the species in the subscript.

The K_1 constant is the first dissociation of carbonic acid or of bicarbonate and is usually written as:

$$[H^+] + [HCO_3^-] = [HCO_3^-]$$

$$K_1 = \frac{[H^+]\,[HCO_3^-]}{[H_2CO_3]} \cdot \frac{\gamma_{H^+}\,\gamma_{HCO_3^-}}{\gamma_{H_2CO_3}} \qquad (7.2)$$

Where:

$\gamma_{H_2CO_3}$ is assumed to be 1

However, interestingly, the "K_1" that is actually measured is only partially described by the equation (7.2).[23] When bicarbonate is reduced to carbonic acid (or for dissolved CO_2 in non basic solution), at equilibrium, the solution contains less than 1% of the dissolved carbonic acid in the form of H_2CO_3,[24] the remainder in the form of a loosely hydrated carbon dioxide.

$$CO_{2(aq)} + H_2O = H_2CO_{3\,(aq)}$$

$$K_{CO_2} = \frac{[CO_2]}{[H_2CO_3]} \qquad (7.3)$$

Where:

activities are assumed to be unity due to uncharged species.

Considering only the process described by K_1 (equation 7.2), Cotton's book[23] discusses a pK_1 value of 3.58 (or $K_{CO_2} = 2.63 \cdot 10^{-4}$), which is similar for oxo-acids of similar structure. Taking the ratio of CO_2 to carbonic acid or K_1/K_{CO_2} shows that there is approximately 600 times more carbon dioxide than carbonic acid. The quantity directly measured and reported as K_1, will be designated K_{1m}, and it consists of:

$$K_{1m} = \frac{K_1}{1 + \frac{1}{K_{CO_2}}} = \frac{[H^+]\,[HCO_3^-]}{[H_2CO_3] + [CO_2]} \cdot \gamma_{H^+}\,\gamma_{HCO_3^-} \qquad (7.4)$$

The values of these constants vary somewhat depending upon the literature source, but are in agreement as to the order of magnitude. A list of some K and pK values from various sources is found in Table 1.9. A possible explanation for the spread of values might be that the values are measurements made at various solution ionic strengths where activities were not considered or compensated for. Another possibility is just that different methods tend to produce different values. MacInnes and Blecher[24] employed a potentiometric method to determine these constants. They determined the K values by making solutions of various ionic strength (dissolving known quantities of chloride, carbonate, bicarbonate, carbon dioxide), pressure, and temperature, and measuring the potential of a glass electrode relative to a silver/silver chloride reference. This potential is linearly related to the pK value by the Nerst equation. Since the formal concentrations were used to calculate pK instead of activities, to correct, they defined a pK' value:

$$pK' = pK - \log_{10}(\gamma) \qquad (7.5)$$

Where:

pK' is the calculated value using solution potential and formal concentration

γ is the activity coefficient term in the K relations.

To determine the value of pK, they plot pK' values against activity (from ionic strength calculations). The slope for the K_2 dissociation value is much greater than that for K_1, due to the presence of the doubly charged CO_3^{--}.

The ionic strength may be calculated using the following equation 7.6:

$$\mu = 0.5 \sum_i c_i \cdot z_i^2 \qquad (7.6)$$

Where:

c_i is the concentration of the ith species

z_i is the charge on the species.

which affects the activity of each species by the extended Debye Hückel equation 7.7 for solutions under 0.1 M:

$$-\log_{10}\gamma = \frac{-0.51\, z^2 \sqrt{\mu}}{1 + \alpha \frac{\sqrt{\mu}}{305}} \qquad (7.7)$$

Where:

α is the ionic radius of the species

The effect can be seen in Figure 21, which shows a theoretical plot of ionic strength and activities of various species in a titration of 0.0018 moles (0.19108g) of sodium carbonate with 0.12246 M HCl in a starting volume of 50 ml. At the beginning of the titration, ionic strength decreases and activities increase greatly due to the conversion of the doubly charged carbonate to singly charged bicarbonate. Around the first endpoint, they level off and change only slightly, because now there is only singly charged species in solution. The ionic strength levels off again at the second endpoint. The ionic strength then starts increasing and activities decrease as the contribution from the acid take effect.

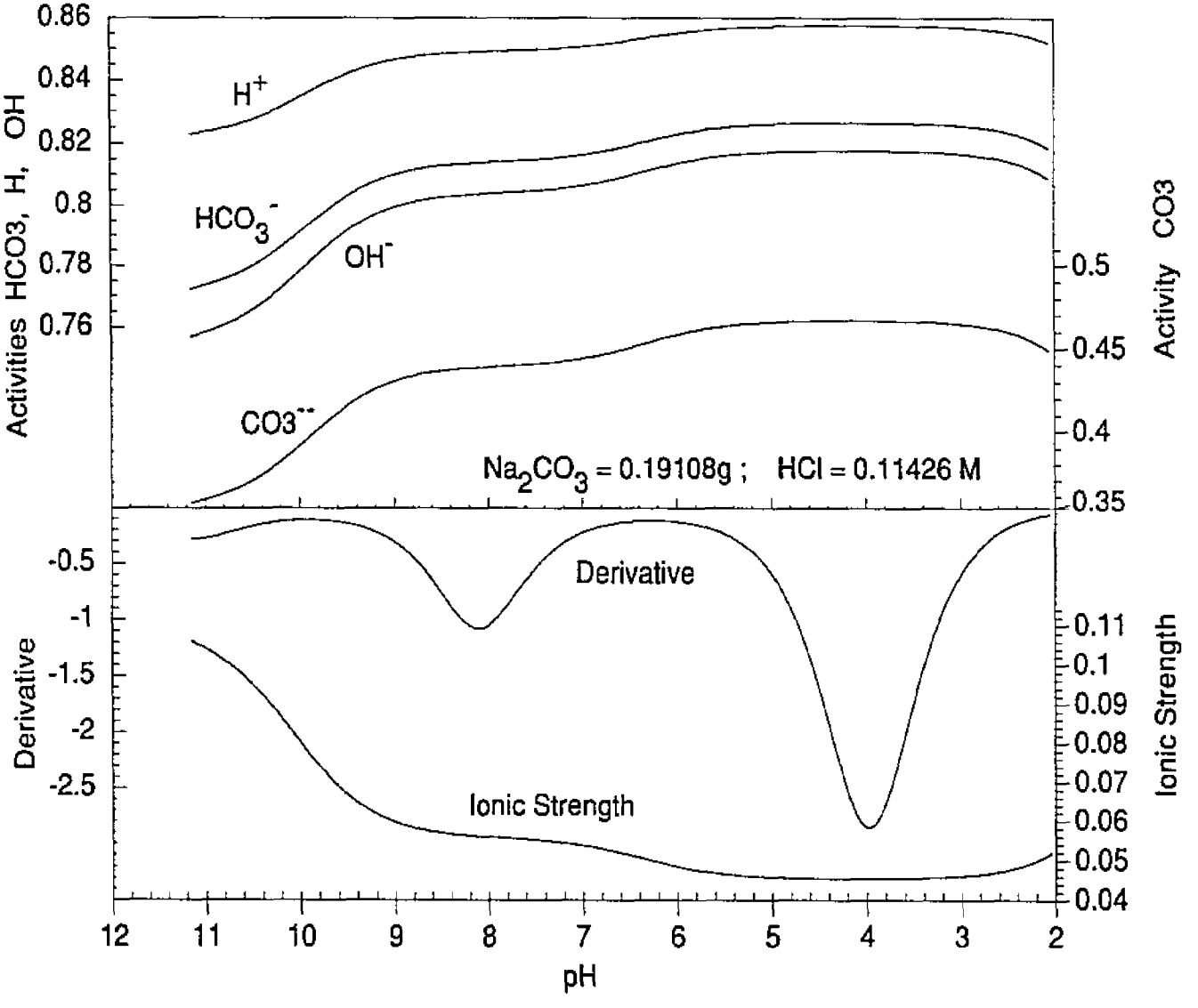

Figure 21 Calculated Activities and Ionic Strength of Sodium Carbonate

Dissociation Constant Determination

In this study, the K_1 and K_2 dissociation constants for sodium carbonate and bicarbonate were approximated from titration curves of Na_2CO_3 with HCl and for sodium sesquicarbonate, $Na_3{\cdot}HCO_3{\cdot}CO_3{\cdot}2H_2O$, with HCl, using the automatic titrator discussed in other sections. Two main methods of analysis were used for the carbonate analysis. The resultant values are presented in Tables 1.10 through 1.13. The methods will be discussed, and then the results compared and discussed.

This first method is a simply performed technique to approximate the equilibrium values. It requires knowledge of the volume used to reach the first endpoint and pH values at that endpoint and at the volume halfway to the endpoint.

To determine K_2: At the half volume,

$$[HCO_3^-] \approx [CO_3^{--}] \qquad (7.8)$$

assuming that all of the acid has reacted completely with the carbonate. Rearranging the dissociation equation, 7.1 and applying equation 7.8:

$$K_2 = \frac{\gamma_{H^+}\,\gamma_{CO_3^{--}}}{\gamma_{HCO_3^-}} \cdot [H^+]_{1/2 \text{ volume to first endpoint}} \qquad (7.9)$$

or, taking the log:

$$pK_2 = -\log_{10}\left(\frac{\gamma_{CO_3^{--}}}{\gamma_{HCO_3^-}}\right) + pH_{1/2 \text{ volume to first endpoint}} \qquad (7.10)$$

The titrations of sodium carbonate with HCl were examined using this method by finding the volume and pH data values about the half volume point from the experimental titration curve. The pH at the half point was

then determined by finding the equation of a straight line which passed through these data values and solving for pH.

The K_1 value was then determined from the equation 7.11:

$$\sqrt{K_1 \cdot K_2} \approx [H^+]_{\text{at the first endpoint}} \quad (7.11)$$

Using the value of K_2 determined previously.

Another method was used to find K_1, K_2 for both the carbonate and the sesquicarbonate titration. This method fit selective parts of the titration curve with the theoretical expression relating pH and volume. The expression was derived from the equilibria of the various components of the solution, charge balance and the carbon mass balance. The final equation derived (details in Appendix 5.4A) for the model is equation 7.12:

$$V_{HCl} = \frac{\left(- M^{\circ}_{T2} + \dfrac{M^{\circ}_{T} \cdot \left(\dfrac{2K_2}{[H^+]} + 1 \right)}{\dfrac{K_2}{[H^+]} + 1 + (1 + K_h) \cdot \dfrac{[H^+]}{K_1}} \right) \Bigg/ \left(\dfrac{-K_w}{[H^+]} + [H^+] \right) - V^{\circ}}{1 - \dfrac{C_{HCl}}{\dfrac{-K_w}{[H^+]} + [H^+]}} \quad (7.12)$$

Where:

M°_{T}	is the total initial moles of carbonate and bicarbonate.
M°_{T2}	is the total initial quantity of bicarbonate and twice that of carbonate (quantity of Na)
V_{HCl}	is the volume of HCl added at any point in titration
V°	is the initial volume in which the material is dissolved
Kw	is the auto protolysis equilibrium constant for water
C_{HCl}	is the molarity of HCl
K_1	is the equilibrium constant for bicarbonate/carbonic acid
K_2	is the equilibrium constant for carbonate/carbonate

An example of a fit may be seen in Figure 22. Input to the equation 7.12 is the initial volume of water used to dissolve the material, the initial mass of sodium carbonate, the initial mass of sesquicarbonate, the initial mass of sodium bicarbonate and the molarity of the HCl reagent. The equation was fitted to the experimental data using the same simplex minimization program as that used when fitting the local model. The parameters that were varied were K_1 and K_2. For each titration curve, the fit was performed using different parts of the titration curve, labeled A, B and T. Part A was around the first endpoint, the carbonate transition to bicarbonate ($K_1(A)$ and $K_2(A)$), part B was fit around the second endpoint, the bicarbonate transition to carbonic acid and CO_2 ($K_1(B)$ and $K_2(B)$) , and a fit of the entire titration curve ($K_1(T)$ and $K_2(T)$) was performed.

The simplex program requires initial guesses for the varied parameters. Guesses which are closer to the "correct" values will usually result in faster convergence of the fit and fewer problems with local minimums. For each part of the titration curve that was fit, several runs with different initial guesses for K_1 and K_2 were used. One guess consisted of the values from the literature[20] in Table 1.9. Another was the previous results (i.e., the simplex was run several times with the results from the previous run as the initial guess). Initial guesses were used which were several orders of magnitude different (higher and lower) than these values. In all cases, except that mentioned below, they converged to the same values with four significant digits.

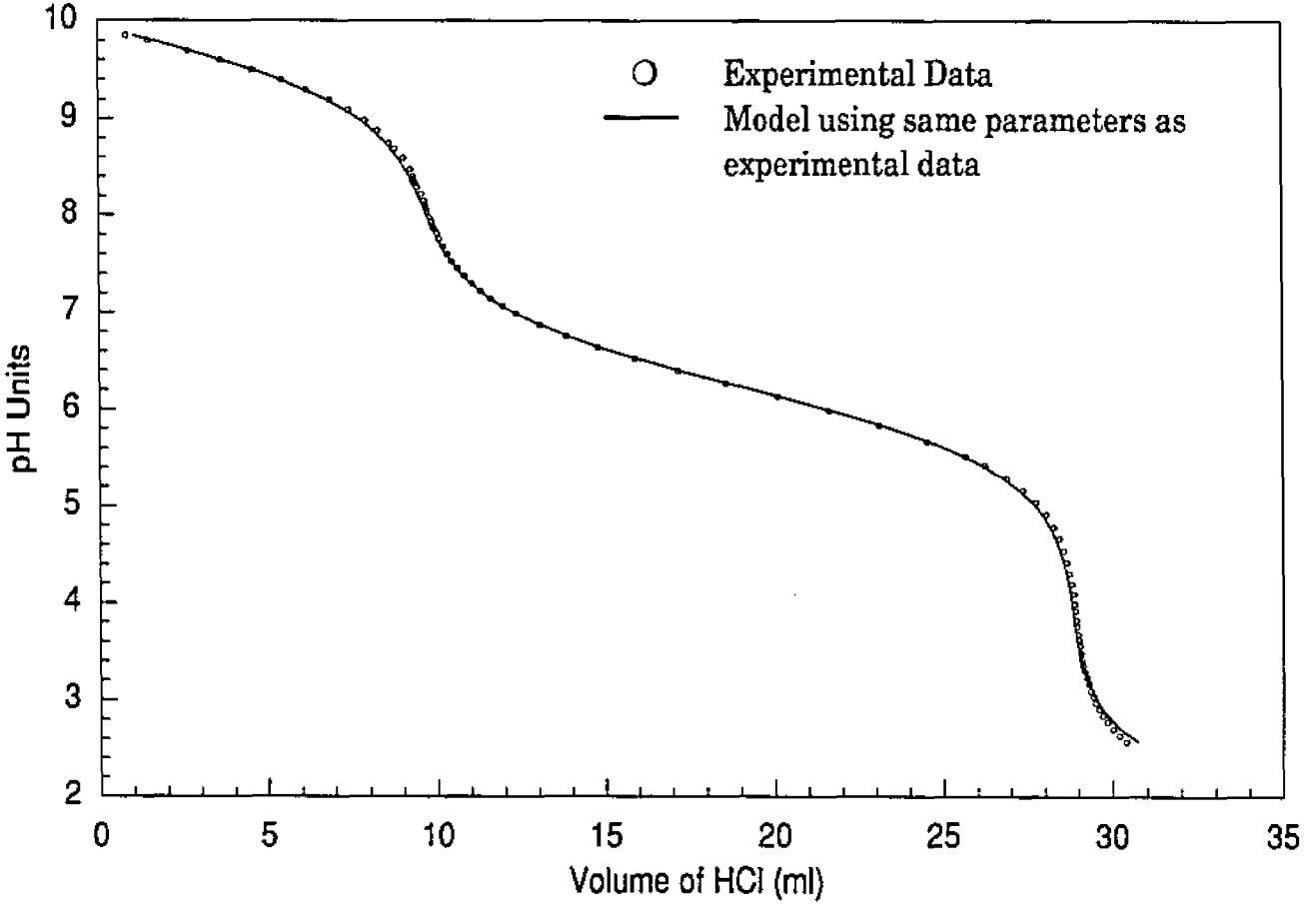

Figure 22 Sesquicarbonate Experimental Data and "Exact" Model

Dissociation Constants Results

The dissociation constants were determined by four methods described previously: (A) fit of data points around the first endpoint, (B) fit of data points around the second endpoint, (T) fit of the entire titration curve and (S) using values of data about the half volume to the first endpoint (equations 7.10 and 7.11). The results are presented in Table 1.10, 1.11 and 1.12 and discussed below. For comparison, values from the literature are given in Table 1.9.

Sodium carbonate

The K_2 value from the fit about the first endpoint (K_2(A)) gave the best result, $5.16 \cdot 10^{-11}$, with the relative error from literature values of 1.6% (mean value $5.08 \cdot 10^{-11}$). The (K_2(T)) gave ($4.22 \cdot 10^{-11}$) with and error of 17%. The spread of values for each method is similar, about $\pm\ 0.7 \cdot 10^{-11}$. The half volume method (K_2(S)) did not differ much from that of the fit of the entire curve ($4.30 \cdot 10^{-11}$). However, K_2(B) gave a large range of values orders of magnitude different than the literature values. This is probably due to the terms in equation 7.12 that involved K_2 being negligible or canceling. This is reasonable, as the value of K_2 depends on carbonate concentration which is negligible in this region of the titration. The simplex program will try any small number and finding that it satisfies the convergence criteria, return that value as the result.

The K_1 values yielded different results. Comparison of the fit values to the mean literature value ($4.39 \cdot 10^{-7}$) shows that the fit about each individual endpoint region gave errors of around 20%. Fit of the entire titration curve (K_1(T)) gave a result closer to literature, ($3.88 \cdot 10^{-7}$) within 11.7%. But the result closest to the mean literature value was that from the

pH method (K_1(S)) with an error of 5.4% ($K_1 = 4.15 \cdot 10^{-7}$), even though it relied on the previous K_1(S) calculation.

The model equation 7.12 has an incorrect assumption which occurs around the second endpoint or after approximately pH 5.3. Around this pH, bubbles were observed, indicating that the solution was saturated with CO_2. The equation uses mass balance and an equilibrium equation which assumes the product is H_2CO_3. Much of that product (600:1) actually converts to CO_2. and after ca. pH 5.3, the solution is saturated with this gas (and at lower pH, the material is lost from the system). This might account for the larger distribution of values when fit around the second endpoint. Activities, which were neglected in the fit, should influence the results less as the ionic strength of the solution is less after the first endpoint (and activities are closer to 1).

Sodium Sesquicarbonate

Sodium sesquicarbonate was analyzed for K_1 and K_2 by the same fitting process and methods as above. Similarly, the (K_2(B)) value did not give meaningful results, for the reasons stated above. However, all of the results are further off from the accepted values than the carbonate results were. The values had a 12% to 80% error and K_2 had larger errors than K_1 (Tables 1.12 and 1.13). However, K_2 is more sensitive to ionic strength of the solution than K_1. The ionic strengths and activities was estimated at points in the centers of all of the fit (K_1(A), K_2(A), K_1(B), K_2(T), K_1(T)) . The values that include activities are indicated in the tables by values in parenthesis. Considering the activities brought the values much closer to the mean of the accepted values (1.4% to 29%). For these values, a K_2 determination at K_2(A) had one third the error of that using K_2(T). The K_1(B) fit gave the closest values with an error of 1.4%.

Source	(20)	(24)	(25)	(23)	(18)	(25)	
K_1	$4.30 \cdot 10^{-7}$	$4.54 \cdot 10^{-7}$	$4.45 \cdot 10^{-7}$	$4.16 \cdot 10^{-7}$	$4.452 \cdot 10^{-7}$	$4.45 \cdot 10^{-7}$	
K_2	$5.61 \cdot 10^{-11}$	$5.61 \cdot 10^{-11}$	$4.69 \cdot 10^{-11}$	$4.79 \cdot 10^{-11}$		$4.7 \cdot 10^{-11}$	
K_1 range:	$4.16 - 4.54 \cdot 10^{-7}$		mean: $4.39 \cdot 10^{-7}$				
K_2 range:	$4.69 - 5.61 \cdot 10^{-11}$		mean: $5.08 \cdot 10^{-11}$				

Table 1.9 Values of K_1, K_2 from various sources.

Run	Fit around 1st e.p. $K_2(A)$	Fit around 2nd e.p. $K_2(B)$	Fit of entire curve $K_2(T)$	$K_2(S)$ from pH at $V_{1/2 \text{ to endpoint1}}$
B	$5.915 \cdot 10^{-11}$	$1.753 \cdot 10^{-7}$	$5.146 \cdot 10^{-11}$	$5.159 \cdot 10^{-11}$
C	$5.760 \cdot 10^{-11}$	$8.535 \cdot 10^{-8}$	$5.036 \cdot 10^{-11}$	$5.183 \cdot 10^{-11}$
D	$4.794 \cdot 10^{-11}$	$3.1072 \cdot 10^{-7}$	$4.906 \cdot 10^{-11}$	$5.026 \cdot 10^{-11}$
E	$4.435 \cdot 10^{-11}$	$1.4242 \cdot 10^{-6}$	$3.899 \cdot 10^{-11}$	$3.993 \cdot 10^{-11}$
F	$5.337 \cdot 10^{-11}$	$1.2845 \cdot 10^{-6}$	$4.432 \cdot 10^{-11}$	$4.540 \cdot 10^{-11}$
G	$5.343 \cdot 10^{-11}$	$4.7263 \cdot 10^{-6}$	$4.166 \cdot 10^{-11}$	$4.211 \cdot 10^{-11}$
H	$6.207 \cdot 10^{-11}$	$9.6449 \cdot 10^{-8}$	$4.648 \cdot 10^{-11}$	$4.614 \cdot 10^{-11}$
I	$5.762 \cdot 10^{-11}$	$6.1640 \cdot 10^{-8}$	$4.038 \cdot 10^{-11}$	$4.058 \cdot 10^{-11}$
J	$4.238 \cdot 10^{-11}$	$4.9609 \cdot 10^{-9}$	$3.331 \cdot 10^{-11}$	$3.526 \cdot 10^{-11}$
K	$4.816 \cdot 10^{-11}$	$1.1934 \cdot 10^{-8}$	$3.638 \cdot 10^{-11}$	$3.736 \cdot 10^{-11}$
L	$4.173 \cdot 10^{-11}$	$2.4644 \cdot 10^{-8}$	$3.183 \cdot 10^{-11}$	$3.212 \cdot 10^{-11}$
Mean ± standard deviation $* 10^{11}$				
B to L	5.16 ± 0.71		4.22 ± 0.68	4.30 ± 0.67
% Error as compared to average literature value of $5.08 \cdot 10^{-11}$:				
B to L	1.61 %		16.9%	15.4%

Table 1.10 K_2 values experimentally derived from titration of sodium carbonate.

Run	Fit around 1st e.p. K_1(A)	Fit around 2nd e.p. K_1(B)	Fit of entire curve K_1(T)	K_1(S) from pH at 1st e.p. and K_2
B	$4.085 \cdot 10^{-7}$	$4.192 \cdot 10^{-7}$	$4.580 \cdot 10^{-7}$	$4.737 \cdot 10^{-7}$
C	$4.253 \cdot 10^{-7}$	$4.486 \cdot 10^{-7}$	$4.487 \cdot 10^{-7}$	$4.774 \cdot 10^{-7}$
D	$3.096 \cdot 10^{-7}$	$2.867 \cdot 10^{-7}$	$3.409 \cdot 10^{-7}$	$2.978 \cdot 10^{-7}$
E	$2.569 \cdot 10^{-7}$	$1.560 \cdot 10^{-7}$	$2.814 \cdot 10^{-7}$	$2.881 \cdot 10^{-7}$
F	$3.461 \cdot 10^{-7}$	$2.343 \cdot 10^{-7}$	$3.834 \cdot 10^{-7}$	$4.112 \cdot 10^{-7}$
G	$3.467 \cdot 10^{-7}$	$2.066 \cdot 10^{-7}$	$3.882 \cdot 10^{-7}$	$3.716 \cdot 10^{-7}$
H	$4.299 \cdot 10^{-7}$	$4.292 \cdot 10^{-7}$	$4.294 \cdot 10^{-7}$	$5.827 \cdot 10^{-7}$
I	$4.341 \cdot 10^{-7}$	$5.761 \cdot 10^{-7}$	$5.261 \cdot 10^{-7}$	$6.233 \cdot 10^{-7}$
J	$2.812 \cdot 10^{-7}$	$3.202 \cdot 10^{-7}$	$2.969 \cdot 10^{-7}$	$3.436 \cdot 10^{-7}$
K	$3.350 \cdot 10^{-7}$	$4.069 \cdot 10^{-7}$	$3.715 \cdot 10^{-7}$	$4.371 \cdot 10^{-7}$
L	$3.188 \cdot 10^{-7}$	$3.666 \cdot 10^{-7}$	$3.404 \cdot 10^{-7}$	$2.616 \cdot 10^{-7}$
Mean± standard deviation $* 10^7$				
B to L	3.54 ± 0.62	3.50 ± 1.23	3.88 ± 0.734	4.15 ± 1.18
% Error as compared to average literature value of $4.39 \cdot 10^{-7}$:				
B to L	19.4 %	20.3%	11.7%	5.40%

Table 1.11 K_1 values experimentally derived from titration of sodium carbonate.

Run	$K_2(A) * 10^{11}$ Fit around 1st endpoint	(with activities)	$K_2(B)$ Fit around 2nd endpoint	$K_2(T) * 10^{11}$ Fit of entire curve	(with activities)
A	9.666	(5.114)	$3.928 \cdot 10^{-21}$	12.24	(6.476)
D	10.01	(5.454)	$3.440 \cdot 10^{-20}$	10.99	(5.988)
E	8.869	(4.943)	$1.540 \cdot 10^{-20}$	10.11	(5.635)
F	10.45	(5.640)	$1.208 \cdot 10^{-22}$	11.43	(6.169)
G	9.847	(5.482)	$7.629 \cdot 10^{-23}$	12.51	(6.964)
H	12.24	(7.041)	$1.178 \cdot 10^{-22}$	13.95	(8.025)
Mean± standard deviation $* 10^{11}$					
B to L	1.018 ± 0.113			1.187 ± 0.734	(6.543±0.854)
% Error as compared to average literature value of $5.08 \cdot 10^{-11}$:					
B to L	79.9%	(10.7%)		76.6%	(28.8%)

Table 1.12 K_2 values experimentally derived from titration of sodium sesquicarbonate. Values in parenthesis have activities taken into account.

Run	$K_1(A) * 10^7$ Fit around 1st endpoint	(with activities)	$K_1(B) * 10^7$ Fit around 2nd endpoint	(with activities)	$K_1(T) * 10^7$ Fit of entire curve	(with activities)
A	5.572	4.506)	5.940	(4.915)	5.490	(4.499)
D	4.788	(3.911)	3.321	(2.767)	5.729	(4.734)
E	5.589	(4.600)	5.761	(4.826)	5.890	(4.898)
F	4.523	(3.683)	5.413	(4.500)	6.153	(5.071)
G	6.294	(5.178)	5.337	(4.470)	6.150	(5.112)
H	7.093	(5.899)	6.186	(5.223)	6.970	(5.848)
Mean ± standard deviation: $* 10^7$						
	5.64 ± 0.95	(4.63±0.81)	5.32 ± 1.03	(4.45±0.87)	6.06 ± 0.51	(5.03±0.46)
% Error of mean as compared to mean literature value of $4.39 \cdot 10^7$:						
	28.5%	(5.47%)	21.3%	(1.37%)	38.1%	(14.5%)

Table 1.13 K_1 values experimentally derived from titration of sodium sesquicarbonate.

Conclusion

The automatic computer controlled method of titrating carbonate has advantages over a manual titration in terms of speed. Not including preparation, the automatic titrator can take from 5 to 20 minutes for a complete titration, including all calculation and printout of data. Modeling the data to improve the accuracy or precision of the endpoint location did not show a consistent advantage. Modeling the carbonate titration showed an advantage in terms of precision, but there was no apparent advantage in modeling the pure sesquicarbonate or the mixture samples. The accuracy of the sesquicarbonate / bicarbonate mixture showed an improvement in terms of precision, but the unknown mixture, Sample#5, showed no difference. Fitting the data takes at the very least, another five minutes for each experiment, and performing this function did not show a consistent advantage.

Section 2 The Voltammetric/HPLC Instrument

Voltammetric Instrument Introduction

A Digital Equipment Corporation 11/73 computer was programmed and interfaced in order to perform as a general voltammetric function instrument. This instrument was fitted with an automatic mercury electrode and a wall flow cell and applied as a voltammetric detector for B vitamins separated by High Performance Liquid Chromatography (HPLC). The computer applied various potential waveforms for the detection and acquired electrode current data. The experiments performed demonstrate the use of this instrument as a detector for HPLC, for studying the electrode characteristics, for optimization of the electrode, and for application to resolving overlapping current voltage curves with HPLC.

There have been other electrochemical instruments built around computers[1-6]. Our instrument attempts to include the capabilities of these instruments and many more.

The Voltammetric Method

The voltammetric method in general is a powerful analytical technique. It is based on a current measurement ensuing from oxidation or reduction of chemical species at an electrode surface with an applied potential.[7] Both qualitative and quantitative information can be extracted by examining the current response as a function of potential. By utilizing various techniques of potential application, information concerning reaction thermodynamics, rate of electron transfer, mechanisms and kinetics may

be discerned. The advantages of the technique include species specificity, sensitivity, simplicity, and relatively inexpensive instrumentation.[1]

Voltammetry is an excellent method for detection of material eluting from flow analysis systems such as HPLC. The simplest and least expensive form of this method of detection is with a constant potential, usually called "amperometric" detection. In this method, the potential is set to a voltage at which the eluting material is expected to be oxidized or reduced. A variation of this method is a simple differential pulse in which the electrode is held at a constant potential and then pulsed to a potential at which the material is oxidized or reduced. The current is measured before and at the end of the pulse, and the difference is calculated. The difference current discriminates against noise and provides for increased selectivity, and will be discussed later. The theory behind differential pulse is well developed.[8] Other voltammetry methods such as staircase or square wave involve quickly scanning from one potential to another.

HPLC and Detection

HPLC is one of the most rapidly developing analytical techniques. It is used primarily for separating and analyzing mixtures. Material for analysis is injected into a stream of mobile phase that takes the material under pressure, typically on the order of 10^2 to 10^3 PSI, through a column packed with the stationary phase. The stationary phase is a substrate with a bonded organic material, such as a long hydrocarbon chain in the case of reverse phase. The flow rate is usually proportional to the pressure of the mobile phase in the column. The bonded organic material has varying affinity for the components of the injected material and thus delays passage

of these substances to different degrees, causing the components to elute from the column at different time periods. The efficiency of the separation is measured by a value called the "number of theoretical plates" which is dependent upon the retention time and the half width of the peak[9] described in equation 1.

$$N = 5.54 * \left(\frac{R}{W}\right)^2 \qquad (1)$$

Where:

N	is the number of theoretical Plates
R	is the retention time of the material
W	is width of the peak at half height

The value of N is affected by many factors including the mobile phase composition, the flow rate, and the type, quality, and age of the column. The retention time is also dependent upon these factors. The retention time is inversely proportional to the flow rate. The material eluting is detected by various methods such as spectroscopic or electrochemical techniques. Spectroscopic methods are more developed than electrochemical, but spectroscopic methods generally require equipment which is usually more expensive than electrochemical methods. These two methods generally do not share the same interferences, so occasionally one can be used when the other cannot.

When the electrochemical components of a mixture are all electroactive at a given potential, and the amount of species overlap is not significant or of no interest, amperometric detection may be used for HPLC. In this case, the information acquired is a response time curve, consisting of peaks that occur when the components that are sensed by the detection system elute. However, when there is matrix interference, possibility of components

coming out at shortly spaced intervals and overlapping, or more information about the eluting species is required (such as identification of the species), then scanning the potential is desirable. In this case, the information is contained in current-potential curves which are recorded as a function of time during elution. There are many examples of applying scanning voltammetric detection to HPLC in the literature.[10-16]

While voltammetry is a specific and sensitive method, selectivity is sometimes a problem when a sample consists of a mixture of materials. This problem can be especially pronounced when half wave potentials are close in value. In this case, a separation process such as HPLC is necessary before the material can be successfully analyzed. Usually, HPLC and electrochemistry are coupled in order to provide an additional dimension to the detection output. Rapidly scanning the potential and acquiring current values yields information about components which elute at similar values of time. The material to be analyzed is injected into the HPLC apparatus and separated. When the eluent flows past the electrochemical detector, the electrochemically active components react at the electrode surface giving a current which is proportional to the concentration or mass flow. If a rapidly varying potential is applied to the electrode, then the current response versus time can be viewed at any selected potential, or for a given time slice, the current-potential response can be viewed.

The B vitamins at trace level, are well suited for this type of analysis. The Faradaic current of the B vitamins is relatively small; however, these vitamins are able to catalyze hydrogen evolution at a mercury electrode in neutral or acidic media. The catalyzed hydrogen reduction results in a current which can be several orders of magnitude larger than the direct

reduction current of the vitamin itself. While the exact mechanism of the catalytic current depends on the specific system involved[17] the catalytic current is thought to be brought about by the vitamin accepting a proton from a donor. This coupling lowers the energy required to reduce the proton, after which the vitamin releases the hydrogen[18] A simple possible mechanism is:

$$\begin{aligned} B + HA &\rightleftharpoons BH^+ + A^- \\ BH^+ + e^- &\longrightarrow BH \\ BH &\longrightarrow B + \tfrac{1}{2} H_2 \end{aligned} \qquad (1.1)$$

This catalytic current occurs at potentials much more negative than the vitamin reduction half-wave. Small concentrations of the vitamin show the development of the current wave around -1.7 volts (vs. SCE) while larger concentrations shift the potential at which the current begins to increases to a value as low as -1.2 volts.

The catalytic current waves of the B vitamins occur in the same potential region, and different concentrations of each can shift the curves on the potential axis. In addition, responses of different vitamins can cause orders of magnitude differences in current. Therefore, it is usually difficult to examine mixtures of these vitamins by electrochemical techniques alone. Coupling HPLC with voltammetric detection allows the catalytic current response to be used for chemical analysis.

The Mercury Electrode for HPLC

The list of electrodes used for HPLC-EC detection is similar to that used in voltammetry. Electrodes made of carbon or platinum have been used for detecting oxidizable materials, and gold, silver and mercury amalgam as well as liquid mercury have been used for reductive detection. Of these materials, mercury is unique in certain properties. The size of the mercury drop and thus the electrode surface area is easily controlled. The electrode surface is a clean and easily renewed surface. In contrast, solid electrodes must be manually cleaned which changes the surface characteristics. Since the surface features of any drop of mercury vary little between drops, the electrode surface is reproducible. Also one mercury drop may be replaced by electromechanical means, this electrode lends itself to automation. Mercury also has a high overpotential to hydrogen reduction. There are disadvantages to use of a mercury electrode, which include the toxicity of the mercury, set up time, and maintenance of the system which generates the drops. For example, the capillary tubes through which the mercury flows easily clog. The mercury must be stored with minimal contact with air or else solid oxides will form which clog the system. In addition, the growing drop adds complications to the study of the theoretical aspects of the system.

Traditional mercury electrode systems are cumbersome. They involve a mercury reservoir, tubing and fittings which hold mercury, and a column to provide a head of gravity to force the mercury through the capillary. The column is usually open to the atmosphere. This setup is subject to accidental spills and is not very portable. Automatic hanging drop electrodes may diminish many of the arguments against using mercury[19] and in particular, the arguments against a dropping mercury electrode. This automatic type

of electrode uses mercury contained in a small reservoir chamber which is under pressure (less than 10 PSI of inert gas) and is not exposed to the atmosphere. A commercial version of the electrode, used for my instrument, is the Metrohm Multi-Mode electrode. It is compact and completely sealed with less chance of spills or mercury exposure to the atmosphere than in a traditional mercury electrode system. When the electrode needs to be opened, it is easily transportable to a hood or area which can retain spills. The reservoir is easier to maintain, as an inert atmosphere is used to keep it under pressure, and thus oxides form more slowly. If the electrode is properly maintained, clogs can be less common. The quantity of mercury used is much less than a traditional set up as the drop size can be smaller. When used as a static electrode, the theories describing electron transfer and other processes are simpler than that for the DME since it is possible to neglect complicating terms such as that due to the continually changing area. The drop size which affects the current is much more reproducible since the analogous mercury height is controlled by pressure which is easily measured and controlled.

Wall Flow Cell for HPLC-EC Detection

Solid electrodes can use thin layer cells, which are generally impractical for mercury drop electrodes. The chamber that the mercury electrode is situated in has to be as large as the maximum drop size and there must be an exit path through which a discarded drop can be quickly removed. The mercury amalgam electrode can be easily used in a thin-layer configuration, but this type of electrode forgoes most of the advantages of

mercury previously stated. However, the wall cell configuration used for solid electrodes can also be used for mercury.

The wall jet (WJ) and wall tube electrode (WT) configurations are discussed in the literature and theoretical equations have been derived which describe them.[20-27] Both configurations have stationary liquid media in which a fluid jet is perpendicular to the surface of the electrode, and flows radially over the surface. The WJ differs from the WT in the relative sizes of the nozzle of the jet and the electrode size. The WJ has a larger electrode relative to the jet nozzle size; the WT has an electrode small relative to the nozzle. The WT electrode has a uniform flux of material and rate limiting processes and kinetics are the same at all points of the electrode; the form of the current expression is the same as that for the rotating disk.[20]

The WJ is the more sensitive of the two methods. The reason is rather easy to conceptualize. A small jet of electroactive material will have more opportunity to contact the WJ electrode compared to the WT where most of the material doesn't touch the electrode and diffuses into the bulk. The extra sensitivity occurs at the expense of temporal resolution. If the composition of the eluent is considered to be changing with time, the WJ will remain in contact with eluent for longer time periods than the WT. The current from the WJ will show some weighed average of the eluent from various time periods.

The wall jet is a powerful tool in continuous flow or hydrodynamic voltammetry applications, which includes HPLC. It offers ease of use, high sensitivity and low solution retention. It is attractive for use in HPLC-EC as it provides high convective mass transfer characteristics and a cell volume independent of the geometric cell volume.

The WJ detector does not need to be in a thin cell configuration; in fact, Gunasingham[23-25] has shown that it is preferable to position the electrode a distance from the nozzle. If the nozzle is inside the hydrodynamic boundary layer, electrochemical currents are reduced significantly. Data from Yamada[21] show current at various flow rates and nozzle sizes; distances smaller than ca. 0.3 mm show a marked reduction in the electrical current, while moving the nozzle up to 10 mm away results in little reduction in peak current. Gunasingham[25] concluded that the effective cell volume is almost independent of geometric cell volume and is less than the volume of the hydrodynamic boundary layer. It shows little relation to the physical size of the cell.

The voltammetric current characteristics of the flow cells are expressed by the following equations. According to Albery and Bruckenstein;[20] the wall tube is described by:

$$i_{WT} = 0.61\pi\ n\ F\ C\ D^{2/3}\ \nu^{-1/6}\ V^{1/2}\ a^{-3/2}\ R^2 \qquad (2)$$

The wall jet current is:[21,24,27]

$$i_{WJ} = 0.439\ \pi\ n\ F\ C\ D^{2/3}\ \nu^{-5/12}\ V^{3/4}\ a^{-1/2}\ R^{3/4} \qquad (3)$$

Where:

- n = number of electrons transferred
- F = Faraday constant
- D = diffusion constant
- C = bulk concentration of electroactive material in eluent
- ν = kinematic viscosity
- V = Volume flow rate
- a = radius of nozzle
- R = radius of (disk) electrode

The constant term for the WT is 30% larger than for the WJ. Increasing the electrode or the viscosity increases the current more for the WT. The WJ current will increase more with increasing flow rate or nozzle size.

The Electrochemical Instrument

A highly versatile electrochemical instrument was designed and constructed for voltammetry, polarography, and electrochemical flow analysis detection. The instrument was designed around a computer with standard parts and coupled with other instruments. The computer was programmed and interfaced to be a versatile electrochemical instrument which can perform most of the tasks required in polarography and voltammetry as well as scanning or amperometric detection for flow systems. This instrument may be used to perform a large variety of electrochemical experiments. It was developed with the following capabilities:

1) Electrochemical device for routine analysis.
2) Electrochemical research tool.
    a) Control over all temporal and electrical potential attributes of a potential waveform.
    b) Ability to perform standard and cyclic voltammetric and stripping analysis.
    c) Ability to view data as current, differential, or summed pairs.
    d) Ability to view data in multiple displays (e.g., current vs. time, current vs. potential, three dimensional, etc.).
3) Electrochemical detector for HPLC or FIA:
    a) Fixed potential.
    b) Multiple repetitive potential.
    c) Swept potential analysis.
4) Ability to use solid electrodes, mercury electrodes or static electrodes.
5) Simple control of the instrument.
6) On line help for each function, including examples and hints about use.
7) Real time display, scaling, and storage of data.
8) Real time feedback to the operator concerning the data.
9) Programmable waveform.

As an electrochemical instrument, it can perform Staircase, Normal Pulse, Differential Pulse, Square Wave, Rectangle Wave, Reverse Pulse and single and multiple programmable pulse waveforms (Figures 1, 2) on solid, liquid, hanging drop, and programmable Multi-Mode Mercury electrodes. The scanning potential waveforms may be output once, in a cycle with reversal back to the initial potential (cyclical) and/or repetitively (Figure 3). The instrument can also perform pulse time studies which automatically vary the timing of any aspect of a set of voltage potentials (bottom waveform, Figure 2). As a device for routine analysis and a research tool, parameters can be set up for a specific waveform and then repeated over and over without having to reenter the parameters. An example of a menu for setting up a waveform is presented in Figure 4. All parameters of the waveforms are variable, such as timing, potentials, repetitions, the knocker and air valve timing, etc. As a detector, it can perform constant voltage, alternating pulse voltage and multiple successive voltage detection (Figure 2). It can also perform repetitive outputs of the waveforms used for voltammetry to provide three dimensional current-potential-time curves for use in flow analysis (Figure 3). In all of these modes there is the capability of examining and manipulating the data in real time either as the experiment progresses or when the experiment has completed. The data, the detected current signal, may be displayed in a differential or summed pair mode or in a normal mode, where the display will show the current as it was sampled at each part of the potential waveform (Figure 5).

There are many features which make this instrument versatile, but the two which stand out are the ability to view data in several forms and the

ability to vary all of the parameters of the waveforms. These capabilities will be discussed and defined.

Data is collected under control of the program **HSWAVE** as a list of signal current values, at a specific time and potential according to the waveform used. These values, for the purpose of this text, are called the "normal" values. The data may be displayed as six different types, listed in Table 2.1. The two most important displays are current vs. time (used mostly for HPLC and flow systems) and current vs. potential (for voltammetric experiments). For each display type, it is possible to display the data in any one of three modes: **Norm, Diff**, or **Sum**. Only one experiment is necessary, unlike an analog instrument where the experiment would have to be repeated for each mode or display type. An example of the **Norm** and **Diff** display may be viewed in Figure 5. In this example, the waveform is differential pulse and the difference display is the difference of the current obtained when the potential is pulsed from the step potential. While a waveform may have a mode associated with it (e.g., the staircase default is "**Norm**" and differential pulse and square wave is "**Diff**"), it is easy to switch to different modes, and without affecting the experimental data.

When data is displayed as "**Norm**", the data is not processed and is displayed in its normal form. When data is displayed as "**Diff**", the difference between pairs of data are displayed. The display mode "**Sum**" will display the sum of pairs of data. To further conceptualize the display mode, Table 2.3 has a short list of numerical data with each column listing the values which are plotted in the various display modes. These display modes are set automatically whenever a waveform is run, but may be changed at any time without affecting the data by simply entering the mode desired.

Some of the waveform parameters will be discussed now. Changing parameters may change not only the scan rate, but also the type of experiment. A waveform may be set up for stripping, oxidative, reductive, voltammetric, polarographic, or other analysis. While the parameters which describe each waveform vary from one waveform to another, there are parameters which are similar enough that they may be described independent of the type of waveform. These will be described here; they are also described in more detail in the manual for the instrument.

The **Initial Potential** defines the starting potential reference for a scanning waveform. It is applied for the value of time in the parameter **Initial Time.** The **Step Potential** determines the rate of a scanning waveform. It is the amount by which the potential changes for each cycle of the waveform. The simplest scanning waveform is the staircase, which consists only of steps (Figure 1). Differential pulse in Figure 6, 7 consists of pulses superimposed upon a staircase. The **Final Potential** is the limiting value of the step potentials. It defines the upper (or lower) potential bound of a scanning waveform. A scanning waveform will start at the **Initial Potential,** and the waveform ends (or in the case of a cyclical waveform, is at the turning point) when the sum of the **Step Potentials** reach (but not exceed) the value of the **Final Potential.**

There are two parameters which may be used to control a dropping mercury electrode or the Multi-Mode Electrode. These parameters affect the waveform, and are thus important to understand even when a mercury electrode is not employed.

One of these parameters is **Knock**. This parameter determines whether the drop knocker is activated, as well as the point in the waveform that it activates. While it does not affect the waveform by itself, when it has any value except **Off**, the air valve is enabled, and the parameter **Tair** may affect the form and timing of the voltage waveform, as illustrated in Figures 7-9. The four possible values that the **Knock** parameter may have are listed in Table 2.2.

The **Tair** parameter contains the value of time for which the air valve is turned on. The air valve controls the mercury drop growth time in the Multi-Mode Electrode. The longer the air valve is on, the larger the drop will be. The only event for which the value in **Tair** will not effect the waveform is if it has the value of zero or if the parameter **Knock** has the value **Off**. **Tair** is the drop growth time, and the applied potential does not change when the timing period of **Tair** has been completed. For example, if **Tair** precedes the **Initial Potential**, then the mercury drop will grow with the **Initial Potential** applied, followed by the timing period **Initial Time**. The **Initial Potential** is actually applied for the time **Tair + Initial Time**. Another example: if staircase waveform is set with the **Knock** parameter set for **Cycle**, the drop will grow with the potential of the next step for a time **Tair**. In this case, the time that the step is applied, including the drop growth time, is **Tair + Step Time**.

Plot type	Description of Plot type
E(T)	Plot POTENTIAL as a function of TIME. (i.e., what does the potential waveform look like?). For one waveform, select channel numbers which correspond to the beginning and end of one scan.
I(E)	Plot CURRENT as a function of POTENTIAL (i.e., Voltammogram). Be aware that multiple scans will overlap. This mode is suitable for most pulse waveforms.
I(T)	Plot CURRENT as a function of TIME. This is suitable for Chromatography(for some waveforms) and less often for voltammetry. (It is useful in voltammetry when viewing the normal components of a differential measurement, as I(E) may not give a good perspective).
I(En,T)	Plot CURRENT at a SPECIFIC POTENTIAL as a function of TIME. This plot is valuable when using scanning or multiple pulse waveforms in chromatography, as well as when doing multiple scans in voltammetry. This mode gives a "slice" of data at a constant potential.
I(E,T)	Plots a 3 dimensional perspective of the data: a simple surface plot (no "hidden line" removal: if that is desired, use another program!) with lines along I(T). It is a plot, as if you selected I(T) or each scan and moved the paper for each scan.
I(T,E)	Plots a 3-dimensional perspective of the data, as above, with lines along I(E). Each line is I(En,T). Note that this may yield a better view than I(E,T), but the plot takes a much longer time.

Table 2.1 Description of plot types in HSWAVE.

Off	The drop knocker is never activated.
Once	The drop knocker is activated once only at the beginning of the scan set.
Scan	The drop knocker is activated at the beginning of each scan.
Cycle	The knocker is activated (once) before the scan set starts and then only at the beginning of each cycle (i.e., after the **Initial Potential**, then the drop knocker activates before each step). At the end of the very last step, the drop knocker is NOT activated (the drop is left hanging). If **Initial Time** is zero, then the initial activation does not occur before the scan set starts.

Table 2.2 Possible values of the parameter **Knock** which controls the drop knocker.

#	Normal values (**Norm**)	Differential values (**Diff**)	Summed values (**Sum**)
1	0.12		
2	0.14	0.02	0.16
3	0.15		
4	0.16	0.01	0.31
5	0.15		
6	0.14	-0.01	0.29
7	-0.03		
8	0.12	-0.15	0.09
9	0.10		
10	-0.04	-0.14	0.06

Table 2.3 Demonstration of how data is manipulated for the three display modes in HSWAVE. The normal values are the actual currents measured, differential values are differences of current pairs (current(2i) - current(2i-1)), summed values are sums of current pairs (current(2i) + current(2i-1)).

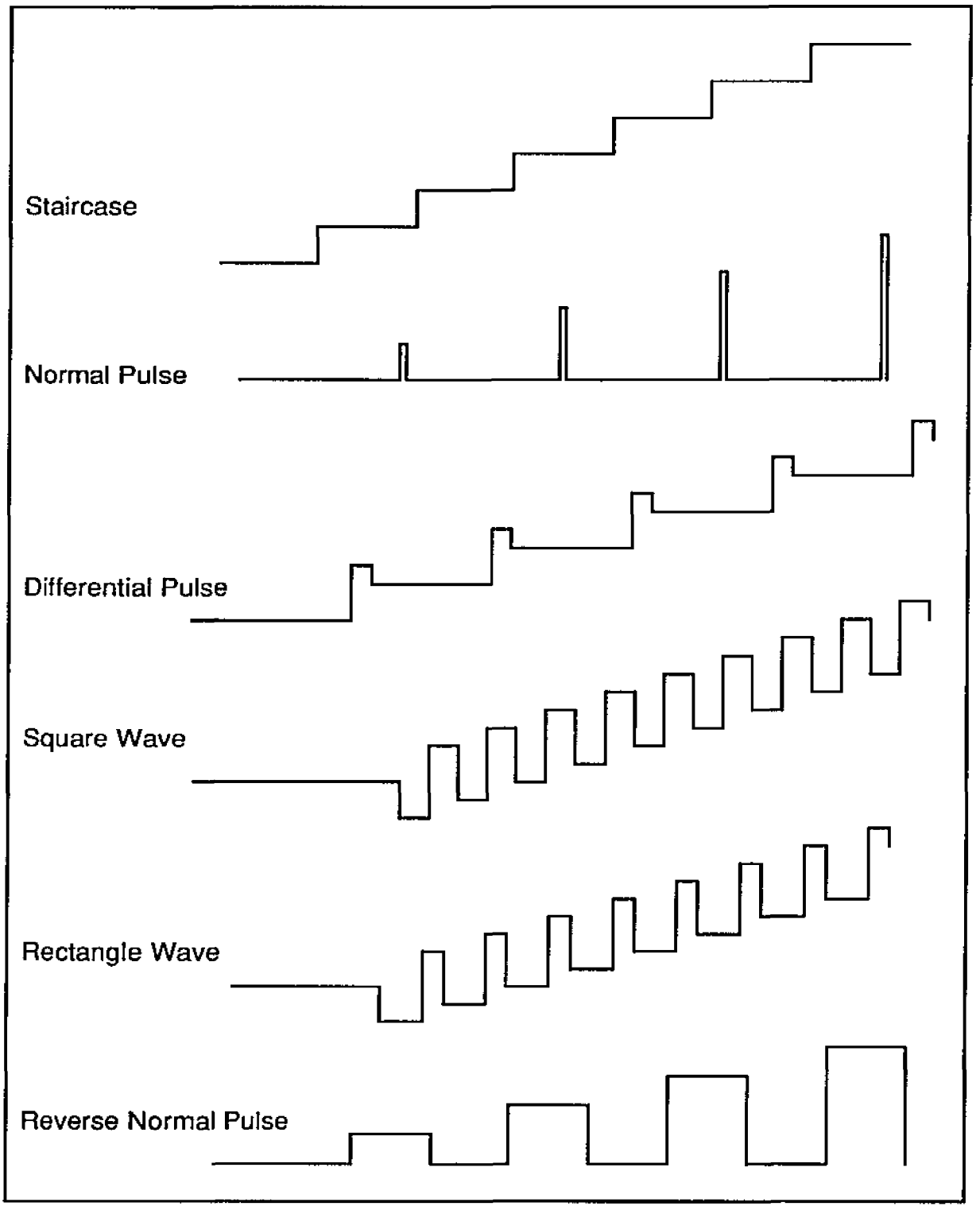

Figure 1 Voltage-Time Curves of Scanning Waveforms in HSWAVE

Waveforms illustrated use typical parameters.

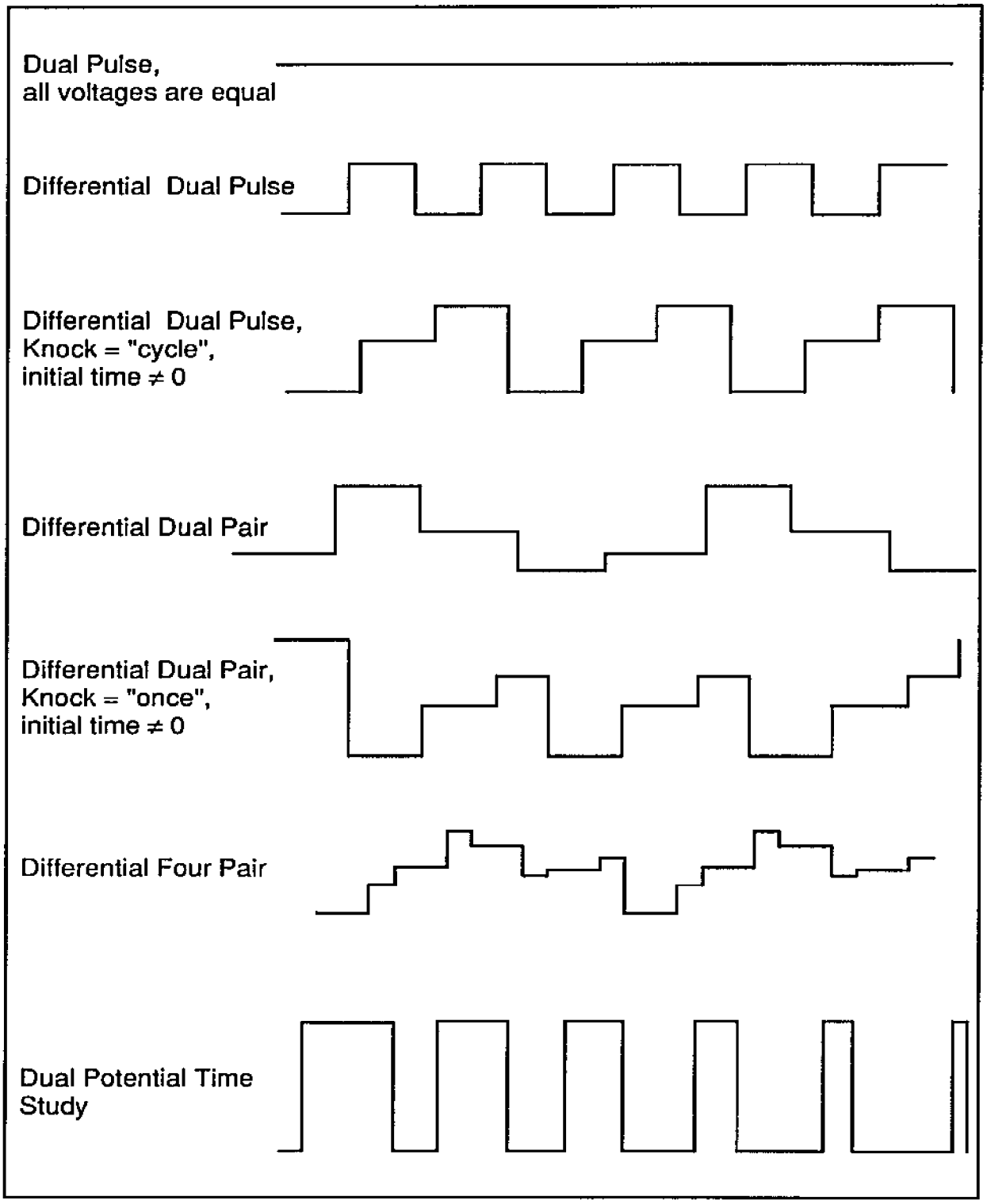

Figure 2 Examples of Some Voltage-Time Curves of Non-Scanning Waveforms in HSWAVE

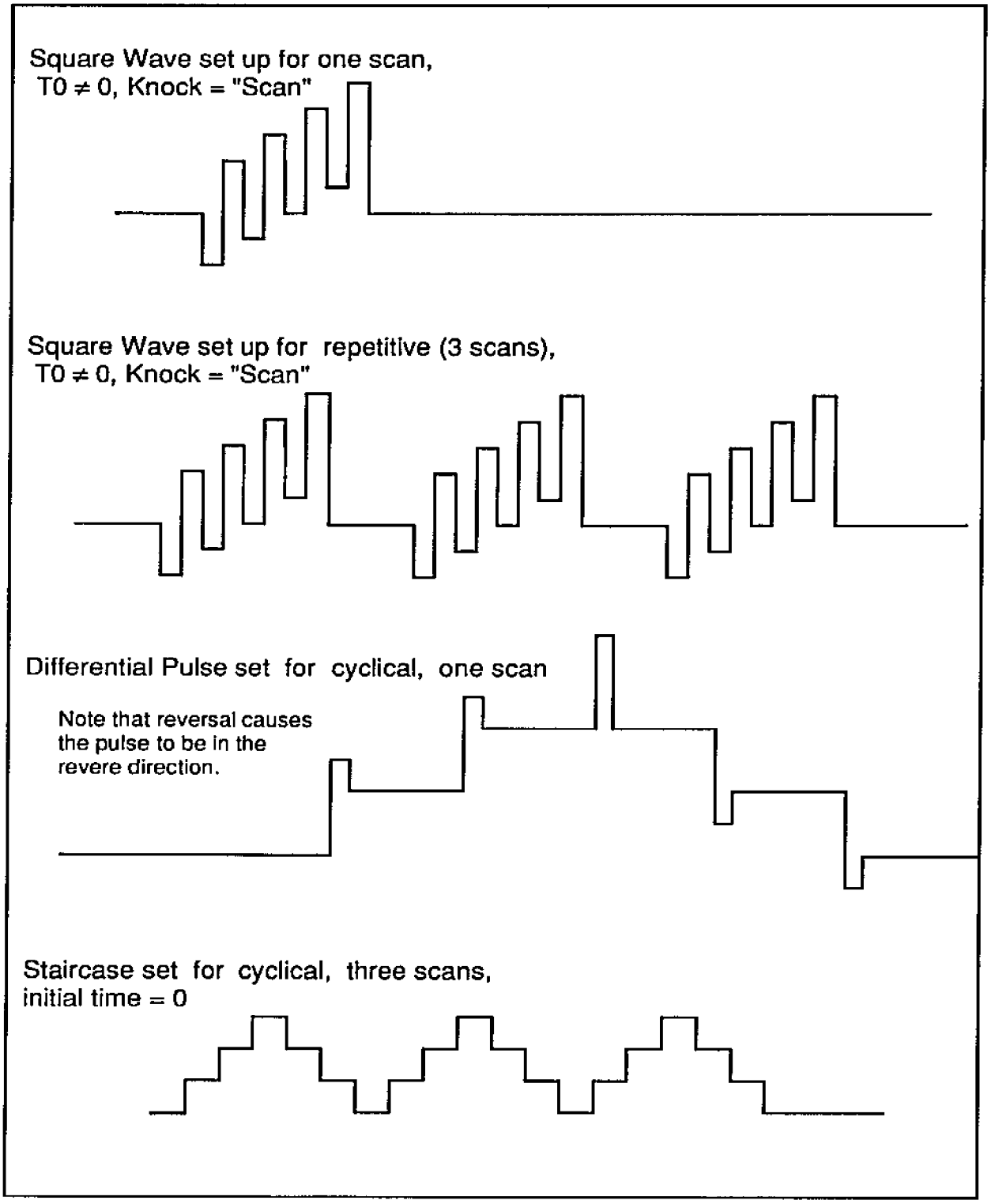

Figure 3 Voltage-Time Curves of Various Modes of Scanning Waveforms in HSWAVE

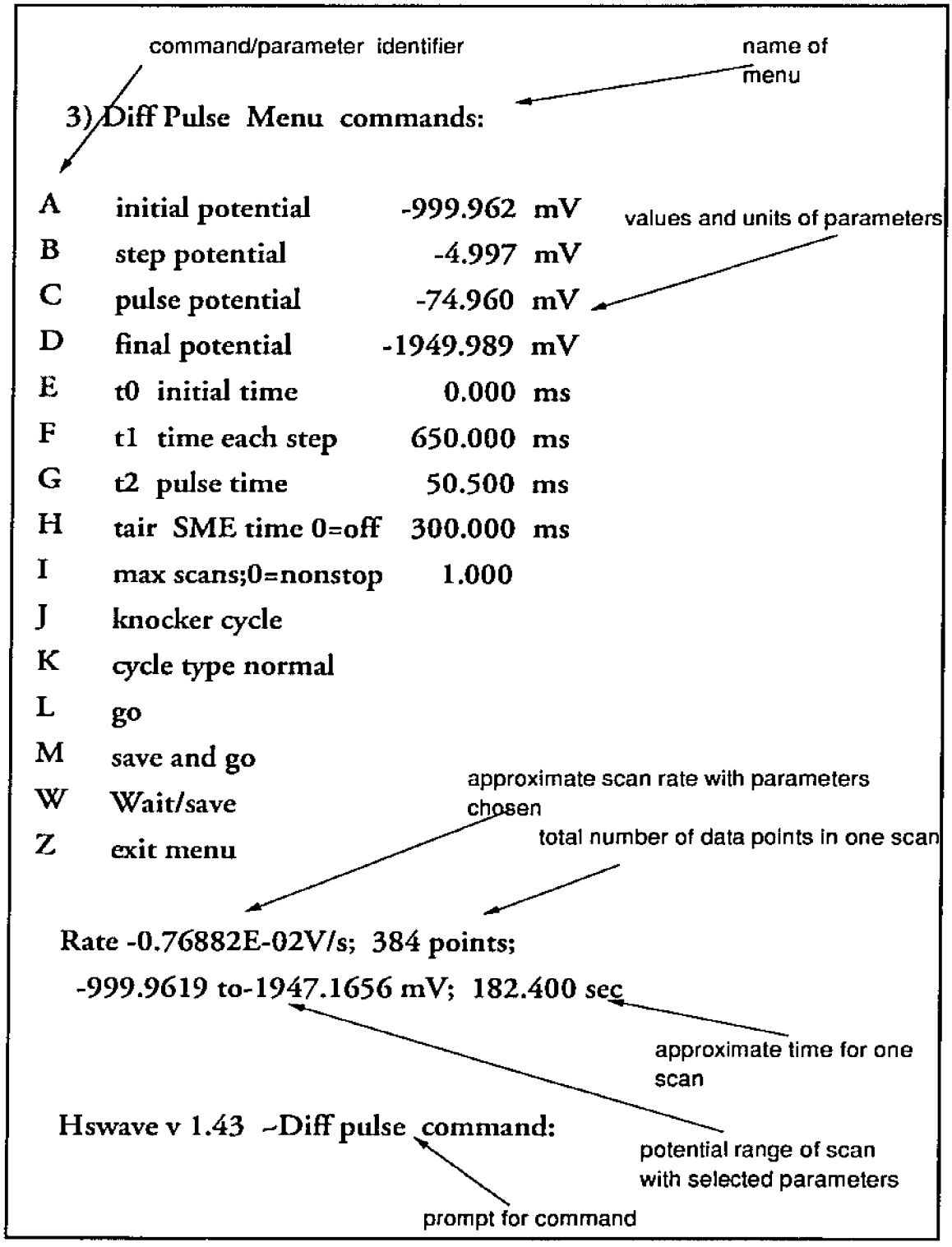

Figure 4 Differential Pulse Waveform Parameter Menu

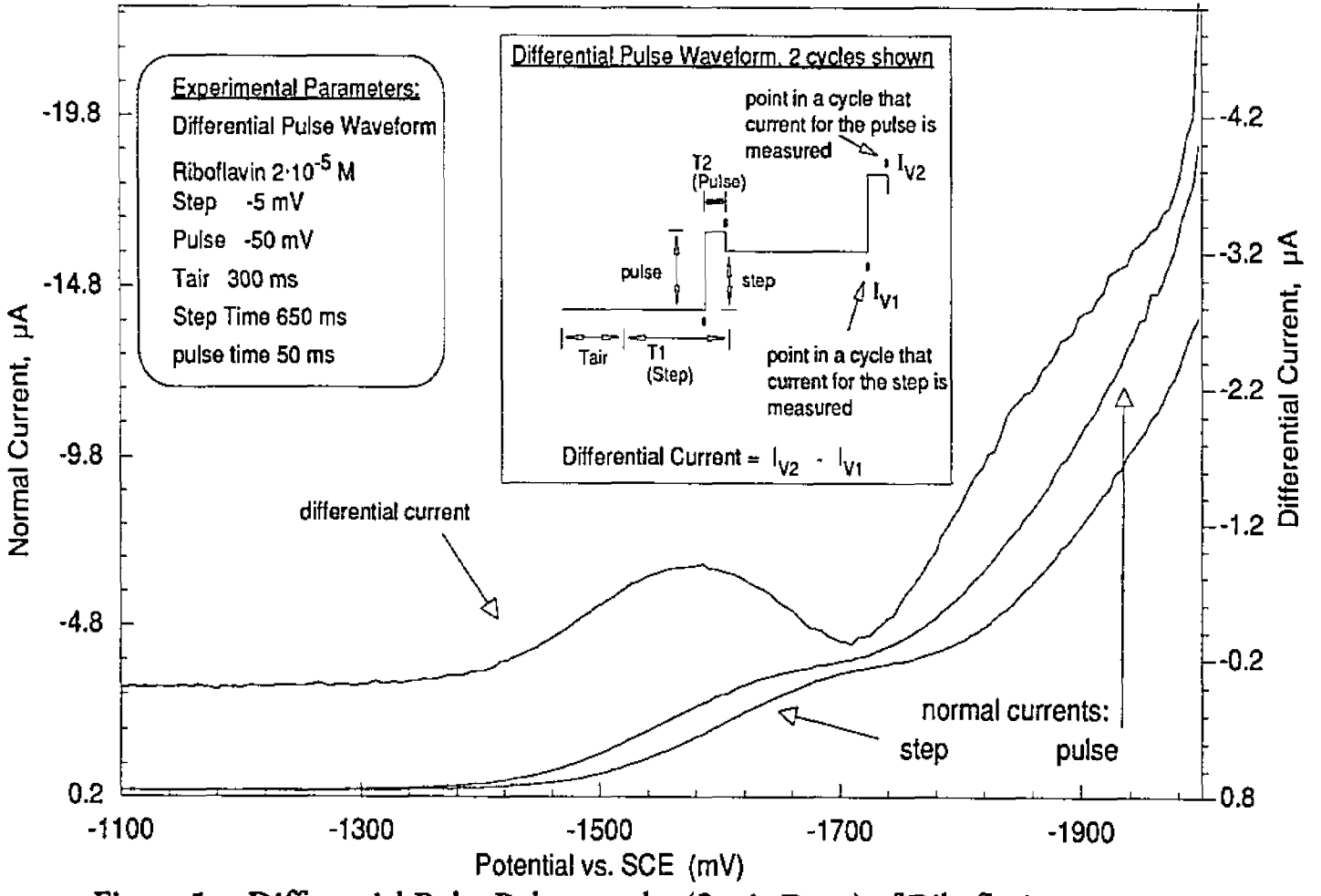

Figure 5 Differential Pulse Polarography (Static Drop) of Riboflavin, with Potential Waveform

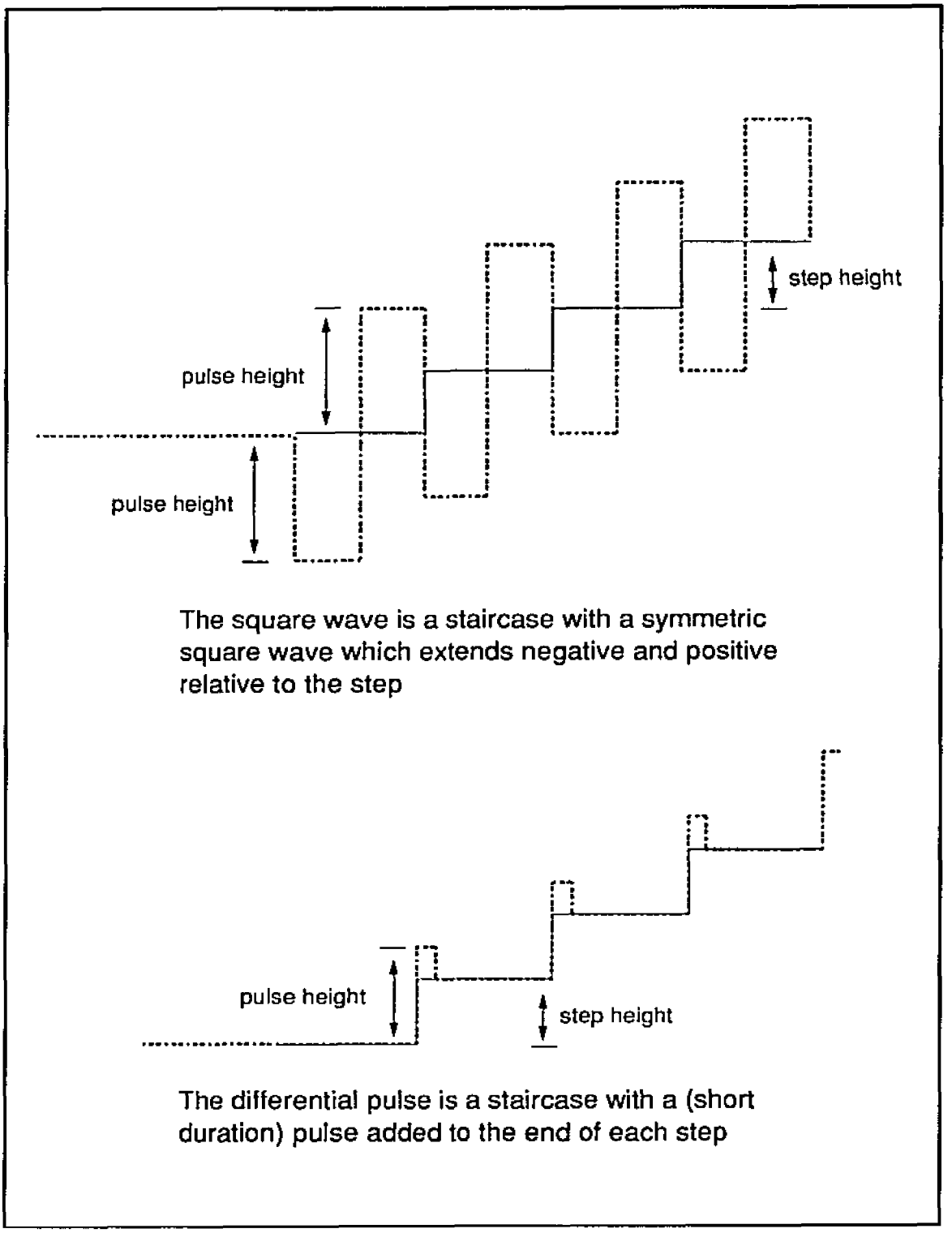

Figure 6 Square Wave and Differential Pulse Waveforms with Steps

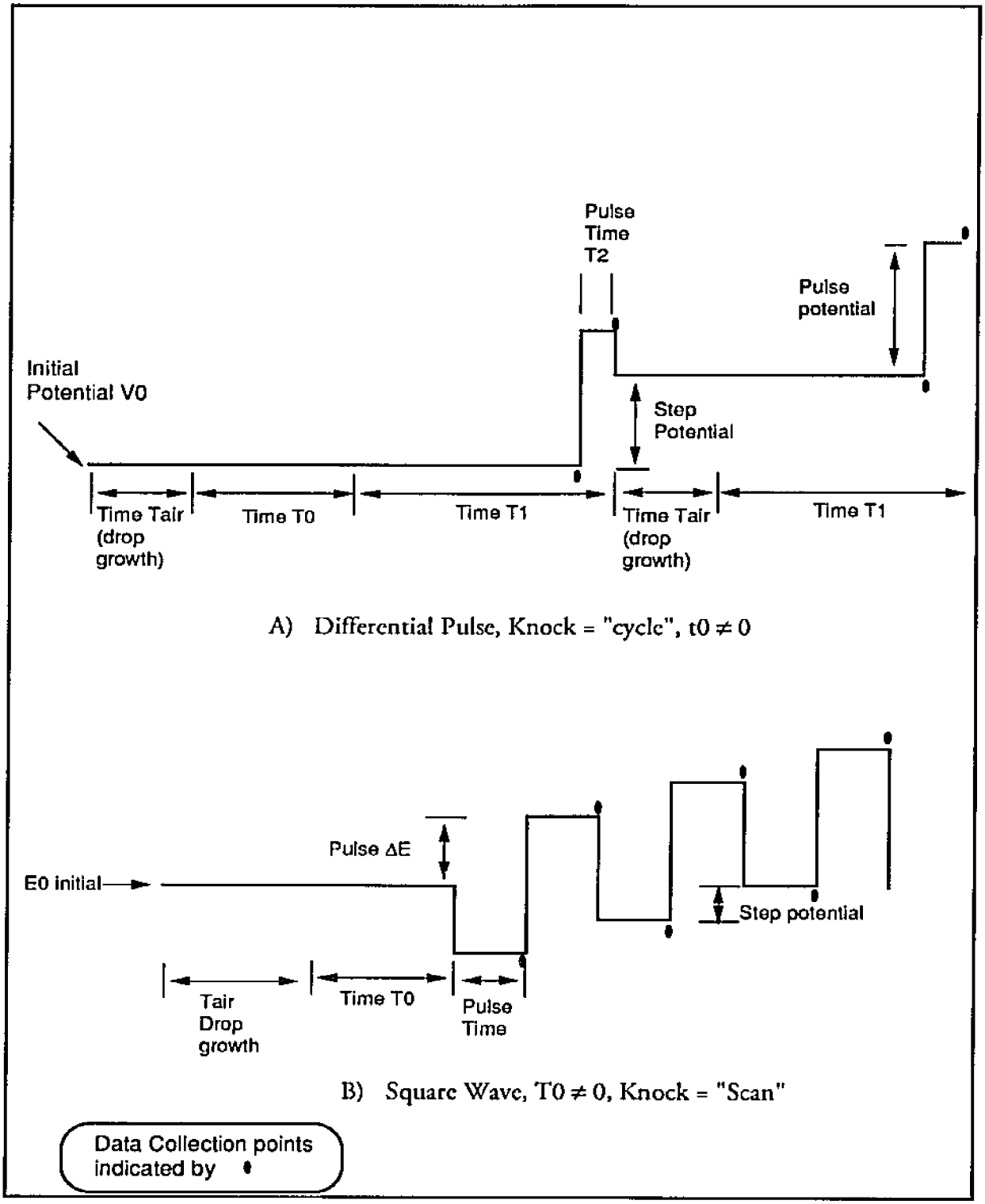

Figure 7 Differential Pulse and Square Wave Potential Time Diagrams with Parameters Labeled

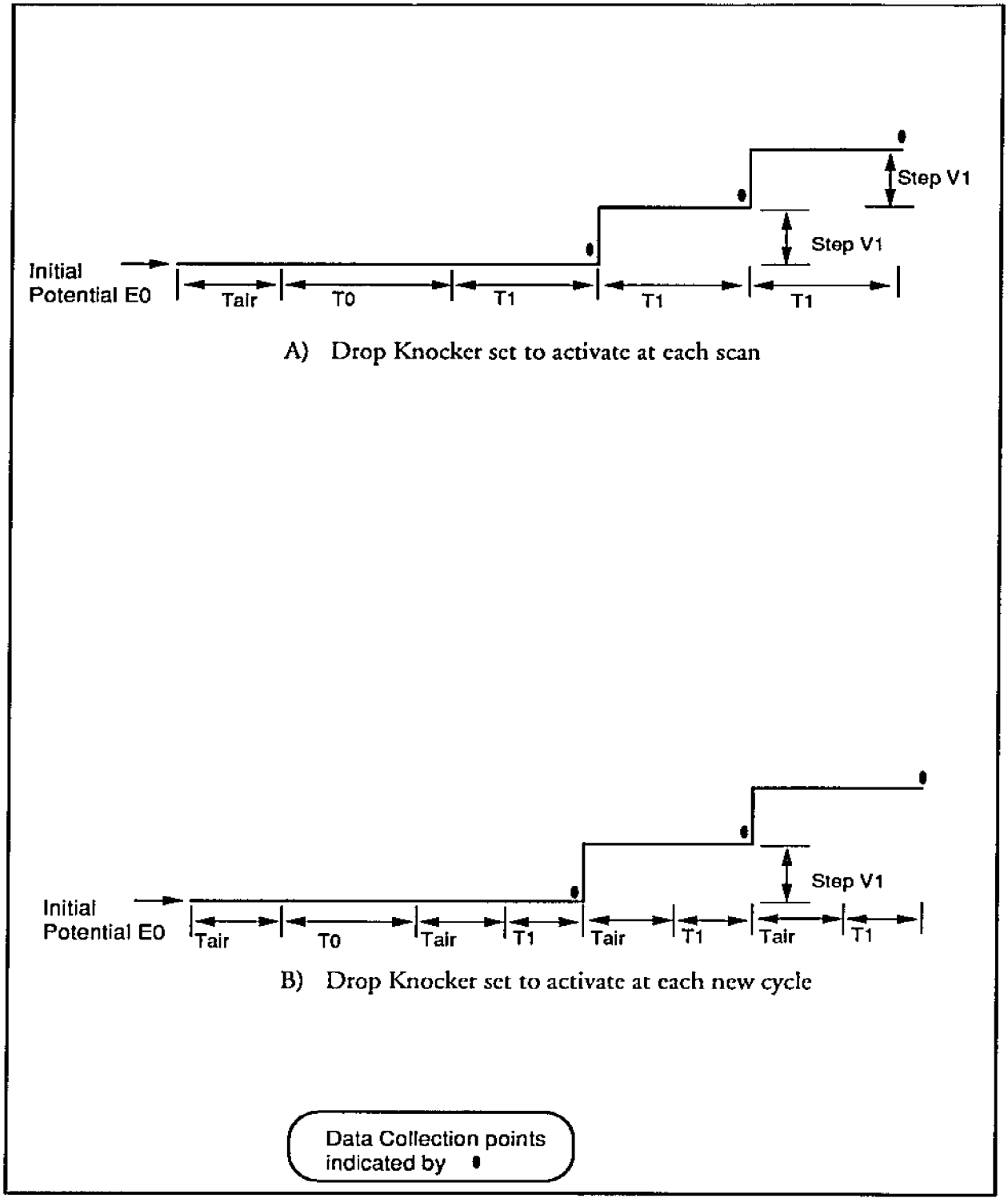

Figure 8 Staircase Potential-Time Curves for Different "Knock" Parameters

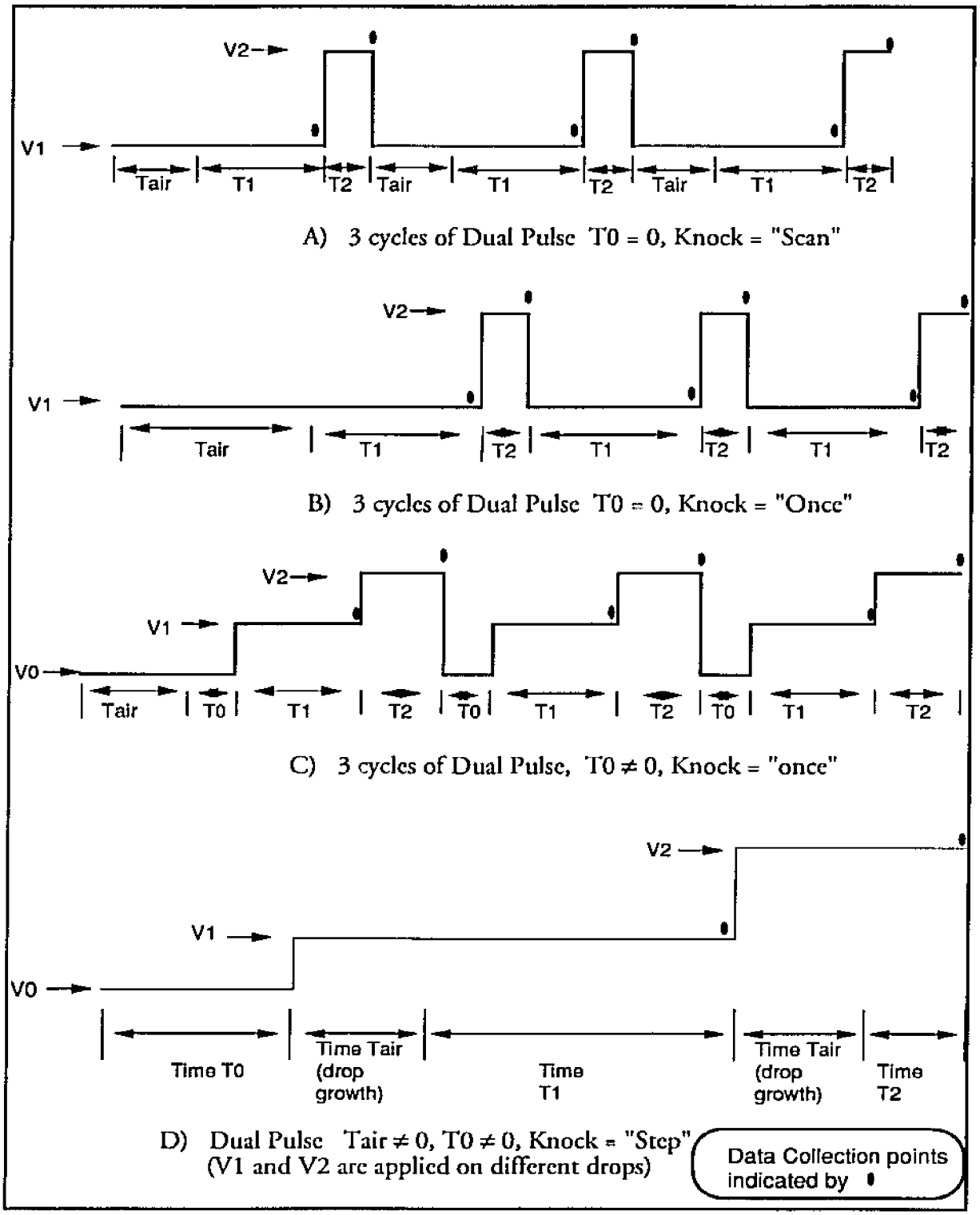

Figure 9 Differential Dual Pulse Potential-Time Curves with Various Parameter Settings

Overview of the Instrument

The instrument and its various parts are depicted in Figures 10-17 and the circuitry is described and illustrated in Appendix section 4B. The overall set-up of the instrument in one of its configurations as an HPLC detector using the Multi-Mode Electrode and the flow cell is shown in Figure 10. This configuration is described later.

The computer was programmed to control the experiment by outputting potential waveforms through a 16 bit Digital to Analog converter (D/A), acquiring current information through a 12 bit Analog to Digital converter (A/D), and controlling a Multi-Mode Mercury Electrode (MME) via an air valve and a drop knocker. The current signal information is displayed on a display monitor, output from the computer through 12 bit D/A converters to an oscilloscope, pen plotter, strip chart recorder, and as hard copy on paper or magnetic media. The program for the computer is named **HSWAVE**.

The parallel output of the Digital Equipment Corporation (DEC) 11/73 computer is connected to a Burr-Brown 729K D/A converter which is used to output a potential. This potential was applied to the control potential input of a Wenking Model 68 FR 0.5 potentiostat. The potentiostat controls the potential between a SCE reference electrode and the Metrohm MME by applying a current to the Pt counter electrode. The computer controls the MME using an Angar Scientific 339V6H8 electronic air valve to control the mercury flow and a Princeton Applied Research 174/70 drop knocker to remove the drop. Current at the MME is measured via a Keithley 614 Electrometer which serves both as a current-to-voltage (I/V) converter and as an amplifier. The current may be offset in the electrometer and by

a current offset device, controlled by a potentiometer, which injects current into the electrometer. The current data are stored in the computer in normal form. Difference or summed currents may be displayed and listed, and the original normal form is available for examination. The data are presented through a large variety of means. As the experiment progresses the current (normal, differential or summed) is displayed ("real time display") on an oscilloscope and/or strip chart recorder connected to the computers 12 bit D/A converters. It can also be displayed as current values on the monitor. When the experiment is completed, the data may be displayed on a Tektronix 4014 compatible monitor and plotted on a Hewlett-Packard 7475 Pen Plotter. An example of this display is in Figure 18. Data may be automatically or manually stored on magnetic media. Data may also be listed as values on a printer or on the display screen. Details of the parts of the instrument and connections are given in the Appendix section 4B.

The Control Program and Use of the Instrument

The computer along with the program, **HSWAVE**, is the heart of the instrument, serving as the operator's control panel for running an experiment. There are several additional utility programs for manipulating data which are listed in Appendix 5.4B. The programming for **HSWAVE** and utility programs was written using two languages, FORTRAN-77 and the assembly language specific for the DEC LSI-11 and PDP-11 computers, Macro-11. Most of the programming was done in FORTRAN, with data acquisition, device control and real time display written in Macro-11.

The program **HSWAVE** itself consists of 75 FORTRAN files and 21 Macro files. A list of the files contained in this program as well as instructions for recompilation are in Appendix section 5B.

HSWAVE is a waveform generation program for HPLC detection and general voltammetry. It is the main program used in this instrument for data acquisition, experimental control, and output of voltage waveforms. It performs the graphical display and manipulation of the data and allows the user to change experimental parameters and store and retrieve data. The interface to the experimenter uses hierarchical menus which display most of the commands and the parameters to the commands. There are help functions which provide on-line explanation of the menus as well as information concerning other features of the instrument. The **HSWAVE** program was created with the concept of ease of use, while remaining versatile. It is controlled by commands that are entered through the terminal keyboard. Some of the commands include changing parameters, running an experiment, and displaying data. Activating a command may change a parameter, a menu or start some action (such as initiate a waveform scan).

A typical experiment would involve preparation of chemicals, deaeration of buffers, and preparation of the apparatus (for example, HPLC requires running the system with the mobile phase flowing for an hour before use in order to stabilize the system). If the MME is used in the experiment, it would have to be pressurized, started, and aligned with the drop knocker. Then the desired waveform is selected from the menu and potential and time parameters are selected to fit the experiment. The Appendix section 3B shows examples of display screens and what happens during startup of the instrument. The manual has instructions concerning the initiation and operation of the instrument as well as sections for diagnostics and solving problems.

Instrument Specifications and Considerations

Specifications of the instrument system are presented in Tables 2.4-2.6. These specifications are for the instrument system as configured in Figure 10. Details pertaining to the interpretation of these specifications follow.

The timing of any potential and the air valve is performed by a DEC KWV11-A programmable clock located inside the computer. The smallest feasible time is limited by the computer response time and is conservatively set at 0.1 ms for the time of the initial potential and 0.3 ms for all other timing values. Shorter values of time are possible and may be set using **HSWAVE**, but some combinations of waveform parameters might cause potential timing to be inaccurate. The maximum time is seven orders of magnitude greater, at 2.5 hours. The computer's KWV11-A 10 MHz clock has a rated accuracy of 0.01%. Response time of the computer to the clock can vary up to 6 μs, but this error is not cumulative.

	Description	Value
Timing	Accuracy (internal clock)	0.01%
	Precision (non cumulative)	< ±6 μs
	Time of Initial Potential	0.1 ms to 2.5 hours
	Time of Any Potential	0.3 ms to 2.5 hours
	Response time of air valve	10 ms (recommended use is > 50 ms)
Data per Scan	Number of points depends upon available disk space	Approx. 500,000 points using a 1 meg floppy
Potential Output	Range	-2.5 V to +2.5 V (default)
	(The range is set by jumpers	-5.0 V to +5.0 V
	on the D/A converter; see Figure 3B)	-10 V to +10 V
	Resolution;	1 part in 65536; full range ÷ 65536
	Change in potential Δ	0.0763 mV @ range = ±2.5 volts
	D/A converter Slew rate	$2 \cdot 10^7$ V/s
	Scan Rate (see text)	50 V/s for 100 points
Current Input	(A/D converter only)	
	A/D converter Voltage Range	±10, ±1, ±0.1 ± 0.01 volt
	A/D converter Gain	1, 10, 100, 1000; set using HSWAVE
	Resolution	1 part in 4096; Range ÷ 4096
	Change in voltage	0.488 mV @ ±1 V (gain = 10)
	(A/D converter & Electrometer)	
	Range	±20,±200,±200 nA, μA, mA (electrometer)
	Change@± 1 V A/D scale	0.00488 μA @ ± 20 μA electrometer scale
	Resolution	Range / 4096 for all ranges except at A/D converter gain 1 which is Range ÷ 409.6)
Current Offset	Electrometer 20 and 200 range	± electrometer full scale, maximum
	Electrometer 2000 multiplier	± 0.1 * electrometer full scale, maximum
	Current Offset Device	± 1100 μA, maximum

Table 2.4 Specifications of the instrument as currently configured.

Electrometer Full Scale	Full scale current of instrument for each combination (Computer A/D converter Gain, Voltage in A/D converter Range)			
{nA, μA, mA}	(1 , ±10V)	(10 , ±1V)	(100 , ±0.1V)	(1000, ±0.01V)
20	20	10	0.1	0.01
200	200	100	1	0.1
2000	2000	1000	10	1

Table 2.5 Actual Full Scale of the instrument from combinations of the computer A/D converter Gain and Electrometer Full Scale. To calculate full scale when a 10kΩ resistor is across the electrometer output, multiply values in 10, 100 and 1000 columns by 2.

Electrometer Full Scale	Minimum Current Resolution of instrument for each combination (Computer A/D converter Gain, Voltage in A/D converter Range)			
{nA, μA, mA}	(1 , ±10V)	(10 , ±1V)	(100 , ±0.1V)	(1000, ±0.01V)
20	0.0488	0.00488	$4.88\ 10^{-5}$	$4.88\ 10^{-6}$
200	0.488	0.0488	$4.88\ 10^{-4}$	$4.88\ 10^{-5}$
2000	4.88	0.488	$4.88\ 10^{-3}$	$4.88\ 10^{-4}$

Table 2.6 Actual Minimum resolution of the instrument from combinations of the Computer A/D converter Gain and Electrometer Full Scale. When a 10kΩ resistor is across the electrometer output, multiply values in 10, 100 and 1000 columns by 2.

The maximum number of data points in an experiment is 4096 points if the experiment is not set to have data stored automatically. If data are set to be automatically stored, the limit of the quantity of data points is the size of available space on the storage device. In the current configuration of the instrument, an empty double sided double density disk can hold a maximum of 0.5 million points.

The range of the control potential may be changed from ± 2.5 volts to ±5 volts or ±10 volts by changing jumpers on the D/A circuit board. The schematics of the D/A converter along with instructions are given in the Appendix section 4B. The range is normally set to ±2.5 volts as this is the most common range of potentials used in electrochemistry.

A figure of merit quoted for electrochemical instruments is the scan rate. The scan rate is the time for a complete scan and is expressed in terms of V/s. In this instrument, the scan rate is not related to the slew rate, discussed later, as in an analog instrument. The scan rate is a value which is meaningful only when viewed together with data acquisition resolution or the number or points of data per scan. A scan rate should be defined by a reasonable range of values, for example, a voltage excursion of 2.0 volts and with 100 data points to define the data curve. In this case, the fastest rate for a staircase potential waveform is $\frac{2.0}{100 * 3{\cdot}10^{-4}}$ or 66.7 V/s. Obviously, if greater resolution is desired along the voltage axis, the value of scan rate will decrease; conversely, if fewer data points are required, the rate will increase. The limit of the scan rate is imposed due to the fact that the data acquisition is performed under software control. A system in which this function is performed under hardware control could be much more rapid.

The slew rate, or settling time for the applied potential, is the time required for the voltage to change and settle to a value within a specific range. The worse case occurs for a large change. The factors which limit this value are the D/A converter, the potentiostat, and the capacitance (related to the length) of the cable connected to the output of the D/A converter. From the Burr-Brown data sheet for the D/A converter, the worse case for it to settle to within ± 0.00076% of full scale range is 8 μs with a load of 2kΩ and capacitance of 100 pF . In this case, the slew rate is $2 \cdot 10^7$ V/s. The slew rate of the change of a potential should be within this order of magnitude for the instrument.

The signal measured is an electrical current, which is processed by several devices. The current of interest at the working electrode is measured by an electrometer which converts the current to a proportional value of voltage . This voltage output is then connected to a 12 bit (1 part in 4096 resolution) differential input A/D converter with programmable gain. The A/D input range may be set using the **HSWAVE** program **GAIN** command for a gain of 1, 10, 100 and 1000, or in units of voltage, ±10.0V, ±1.0V, ±0.1V or ±0.01V respectively. The equation that may be used for calculating the resolution of the converter is:

$$\text{A/D converter resolution (mV)} = \frac{\text{Voltage Range}}{2^{\#\text{A/D bits}}} * 1000 \qquad (4)$$

When the range is ± 10V,

$$\text{A/D converter resolution (mV)} = \frac{20}{2^{12}} * 1000 \qquad (5)$$

the A/D converter has a resolution value of 4.883 mV.

The signal information obtained by the A/D converter is a number proportional to the voltage applied to the A/D converter. Since the information of interest is the current, in order to convert this number to current, the combination of the A/D converter and the I/V converter must be considered. The input of the A/D converter is connected to the output of a Keithley electrometer. The electrometer has an output voltage which is proportional to the input signal. When it is set to current mode, it serves as a current-to-voltage converter. When the electrometer current input is at full scale, the output of the electrometer is 2 volts. The full scale current selection of the electrometer is ±20, ±200 or ±2000 with ranges in μA, nA or pA. To calculate the actual current resolution for data in the computer:

$$\text{A/D converter current resolution} = \text{resolution (mV)} * \frac{\text{Full scale Current}}{\text{Full scale output voltage (mV)}} \qquad (6)$$

or:

$$\text{A/D converter current resolution} = \frac{\text{Voltage Range}}{4096} * 1000 * \frac{\text{Full scale Current}}{2000\ \text{mV}} \qquad (7)$$

where the A/D converter resolution is in units of μA, nA, or pA depending upon the range selected on the electrometer. When the electrometer is set on the 20 μA scale (and thus 2 volts out means that 20μA current is measured) and the A/D converter gain in the computer is set to 10 (±1V), then

$$\text{A/D converter current resolution} = \frac{2\ \text{V}}{4096} * 1000\ \frac{\text{mV}}{\text{V}} * \frac{20\ \mu\text{A}}{2000\ \text{mV}} \qquad (8)$$

or the resolution of the current is 0.00488 μA.

For currents out of range of the current measuring devices, or to provide increased current resolution, the input current may be offset by one of two methods. The electrometer has a built-in current offset which can subtract some of the DC background and help keep the input current from going out of range, but it is limited. On the 20 and 200 multiplier scales, the offset is limited to full scale. On the 2000 (2K) multiplier range, the full scale is limited to 0.1 of full scale. For example, on the 20 μA scale, a signal up to 40 μA may be read before going off scale. On the 2000 nA scale, the signal must be lower than 2200 nA.

In order to compensate for limited offset current of the electrometer, a current injector has been placed in parallel at the input of the electrometer. The offset range of this device is ±1100 μA. The schematic for this circuit is described in the Appendix section 4B. The device is simply a potentiometer across a stable DC voltage source, a mercury battery, shielded in a box with appropriate triaxial connector and a switch that controls both polarity and power.

The actual full scale of the instrument depends upon the following factors:

1) the full scale of the electrometer
2) the selected gain of the A/D converter
3) the current offset in or applied to the electrometer

The effects of 1) and 2) on the full scale are examined in Tables 2.5 and 2.6. A gain of 1 will allow the A/D converter to read the full range output of the electrometer, but the drawback is that the resolution of the data will be low due to the electrometer output (±2) being less than the A/D full scale (±10V). In this case only one fifth of A/D will be used, or 1 part in

819 rather than 1 part in 4096. Therefore, a gain of 1 should be used only when absolutely required. The higher gain values should be avoided as well, since the signal traveling from the electrometer to the A/D converter will be subject to noise; typically in the range of several fractions of a millivolt. A preferred solution would be to use a voltage divider to scale the voltage coming from the electrometer. A 10KΩ resistor attached between the voltage output and the "com" connection located at the back of the electrometer will divide the output by 2 (the input resistance is 10KΩ), providing an output of ± 1.0 V for full scale.

The third factor, the current offset, should be used to offset the DC current such that the signal will be on scale using the smallest possible value on the electrometer. The offset obtained should be entered into the instrument (**OFF** command) so that the value of the offset is recorded with the experimental data. If an offset is provided by the electrometer, the offset is measured by pressing in the "zero check" button and recording the value on the electrometer display. Then the offset provided by the current injector should be measured by turning the electrometer "current offset" to "off", then disconnecting the connection to the working electrode and turning the current injector on. The value on the electrometer display is then the offset produced by the current injector. The addition of the values of the electrometer offset and the current injector offset is the value which should be entered into the instrument using the **OFF** command.

Working Electrodes

A working electrode is connected to the input of the I/V converter, in parallel with the current injector. The choice of the working electrode depends upon the experiment. The applied voltage output range of the instrument is between ± 2.5 volts (expandable to ± 10 volts). The electrode choice may be either reductive (e.g., silver or mercury) or oxidative (e.g., glassy carbon or gold) and it may be solid or liquid. The instrument can drive a drop knocker, described below, for liquid electrodes. The instrument has been designed to control a special liquid electrode, a pressurized mercury electrode, which is also described below.

Multi-Mode Mercury Electrode System

There have been several papers pertaining to the construction of automatic electrodes?[30,31] These designs were unsuccessfully reproduced in our laboratory. In each design, it was not possible to completely stop the growth of the mercury drop (i.e., form a completely static drop). Therefore we used the commercially available Metrohm Multi-Mode Electrode (MME) mercury electrode. The Metrohm MME can be purchased with pressure control devices, a cell and a drop knocker; however these items were added in-house. The care and use of this electrode is detailed in the manual . This is a liquid electrode which can perform several functions: it can be used as a Dropping Mercury Electrode (DME), a Hanging Mercury Drop Electrode (HMDE), or a Static Mercury Drop Electrode (SMDE). A simplified diagram of the electrode is shown in Figure 11.

The MME itself is composed of three main parts:

i) A specialized capillary tube with a fluted inlet.

ii) A main chamber which holds a mercury reservoir and is maintained at a constant pressure by Nitrogen or Argon or other inert gas applied at the main chamber's inlet.

iii) A smaller chamber which is separated from the main chamber by a diaphragm. This chamber is usually maintained at either atmospheric pressure or at the same pressure as the main chamber. This chamber has no direct contact with the mercury. Attached to the diaphragm is a stainless steel needle. The needle is tapered at the end opposite the diaphragm and seats or is near the fluted part of the capillary. The needle maintains electrical contact with the mercury in the capillary at all times.

The MME electrode requires several external parts which constitute the electrode system. This electrode system consists of the major parts:

1) The MME, which provides physical and electrical contact to the solution and contains the mercury which is forced through a capillary.

2) An air valve which controls the routing of pressure to the MME and thus controls the flow of mercury from the MME.

3) A pressure router which was built to provide for an interface to the gas connectors on the MME and the air valve to the gas cylinders.

4) An Air Valve Control unit (AVC). This electronic device was built to provide for manual control of the air valve and to provide an interface to the computer (or TTL voltage source) for automatic control.

5) A Drop Knocker to shake the capillary and knock off a mercury drop.

6) An interface to the drop knocker that was built to allows the computer (or TTL voltage pulse source with any duty cycle) to drive the PARC Drop Knocker. This interface has two controls: one which controls the strength of the knocker's strike and the other which controls the swiftness of the strike. The interface was placed within the housing of the drop knocker.

These parts are described in detail in Appendix 4B.

How the MME System Works

There are two states that the electrode system may be in when power and pressure are applied. One state is holding a drop or no flow of mercury and the other state is growing a new drop or flowing mercury. These modes are illustrated in Figures 12 and 13. The main reservoir chamber is held at constant pressure, usually less than 10 PSI. The pressure in the chamber above the diaphragm controls the mercury flow. When this chamber is kept at the same pressure as the main reservoir (Figure 12), the diaphragm is down and the needle seats upon the capillary tube, blocking any mercury flow. This is the position when the instrument is off or when a drop is being held. When the pressure in this chamber is less than the main reservoir or, in normal usage, is vented at atmospheric pressure, as shown in Figure 13, the pressure in the main reservoir pushes the diaphragm and the needle up which allows the mercury to flow.

Modes of Operation of the MME System

The MME HPLC Electrochemical Detector

In one of the modes of the instrument, the MME is used as a working electrode detector in HPLC or FIA. It was constructed of a machined Teflon™ fitting, concentrically holding the HPLC effluent tube and the capillary of the MME, forming a jet type electrode. This is immersed in a vessel containing the same buffer as the mobile phase, with Pt counter and SCE reference electrodes, as depicted in Figure 15. In actual operation, there are several modes of operation for this electrode. It can be used as a hanging drop electrode, and when response deteriorates, a new drop can be formed with the same size as the previous one. It can also be used as a dropping mercury electrode, where the drop continually grows until the drop knocker forces it off to start a new drop. Furthermore, it can be used as a static drop electrode where a new surface is created for each measurement.

Dropping Mercury Electrode Mode (DME)

As a DME, the operation of the MME is similar to a traditional DME, the difference being that the standard electrode uses the weight of a mercury column to push out a drop, while the MME uses pressurized gas. The drop time of the standard DME depends upon height of the mercury column and capillary length, while the MME drop time depends upon the pressure of the gas, as well as the capillary length. The special capillaries for the MME are only available in one length.

There are two different methods to set the MME as a DME. One is to disconnect the pressure attachment on the chamber above the diaphragm and connect the tube to the inlet labeled "DME" on the MME. The "DME" inlet is merely a convenient blocked inlet and is just a place to put the pressure tube.

The second method (which I recommend for this instrument) is to switch the Air Valve Control (AVC) to "manual". This will vent the chamber and allow the mercury to flow freely, as in a DME, until the control is turned to "off". The red indicator on the AVC will be on in this mode to indicate that mercury is enabled to flow.

In the **HSWAVE** program, for the usual polarographic DME experiment, the **Knock** selection would be set to **Cycle** to synchronize the drop knocker so that the drop size will be the same for each data point. For consistency, **Tair**, the time that the AVC is turned on and off (drop growth time) should be set to zero, even though when the AVC is on "manual", the air valve is not activated.

Static Mercury Electrode (SME)

The difference between a SME and a DME is that with the DME, mercury is flowing all the time while in the SME mode, a mercury drop is grown quickly and then held at a constant size for a period of time. The size of the drop depends upon the same parameters as in DME mode: pressure and capillary length.

This instrument is set up to work the electrode as a SME as long as the Air Valve Control not set on "manual". In the program, **Knock** should be set either to **Cycle** or **Scan**, and **Tair** to the time in which the drop grows.

Hanging Mercury Electrode (HME)

A HME is a SME which grows a drop which is fixed and is changed at regular intervals in the experiment.

Using **HSWAVE**, the **Knock** is set to **Once** (set to **Off** if a drop already exists and changing it is not required) and **Tair** is set to the desired drop formation time.

The Electrochemical Cell and the Flow Cell

The cell for flow detection (e.g., HPLC, FIA, hydrodynamic voltammetry) consists of two parts: the flow cell and the main cell. The main cell is also used for non-flow analysis (voltammetry, polarography) as well. It consisted of a Brinkmann 6.1415.250 electrochemical cell (250 ml) with a ca. 2 mm hole drilled in the glass ca. 20 mm from the top to provide for overflow of liquid.

The flow cell is illustrated in Figure 16. It is constructed of a machined cylindrical Teflon™ fitting, ca. 26 mm in length and 20 mm in diameter. Approximately halfway along the long dimension there is a 10mm hole to allow for expulsion of the drop and eluent. The hole is tapered downward so as to ease removal of mercury drops. On the top there is a hole for fitting the MME electrode. On the bottom is another passage for fitting the eluent tube from an HPLC column. Both passages are concentric with each other. The Bio-Analytical Systems CS-2 HPLC with a C_{18} packed column was used for most measurements.

The flow cell is immersed in the main cell which contains the same buffer as the mobile phase, with Pt counter and SCE reference electrodes, depicted in Figure 15. In operation, material eluting from the column flows across the mercury drop and then diffuses into the bulk solution. This configuration forms a Wall Jet / Wall Tube electrode system. The overflow hole is loosely coupled to a pipette which draws overflow into a large collection vessel. This overflow can be used to collect excess solution for disposal or for measurement and determination of flow rate.

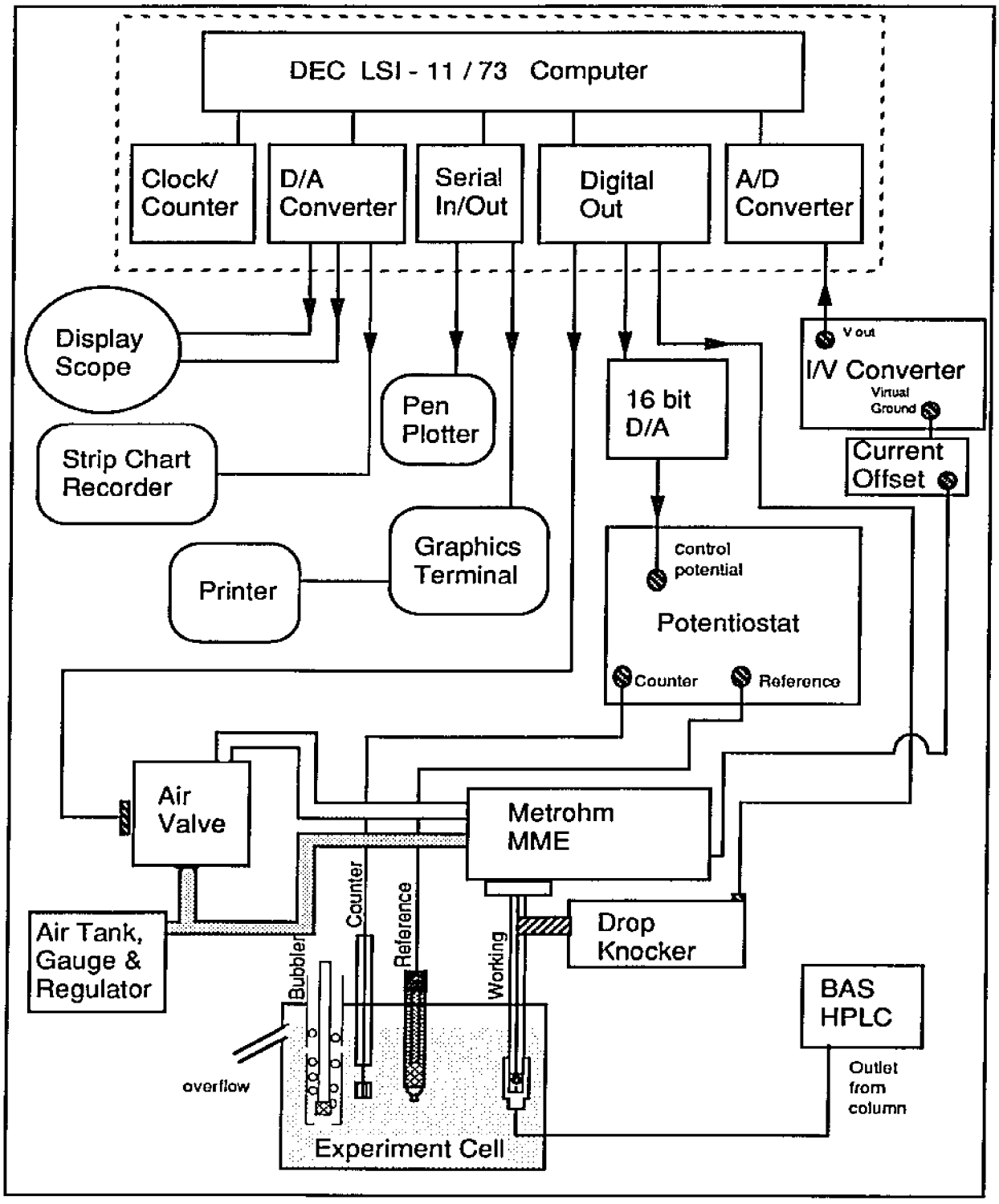

Figure 10 Block Diagram of the HPLC Instrument and Experiment

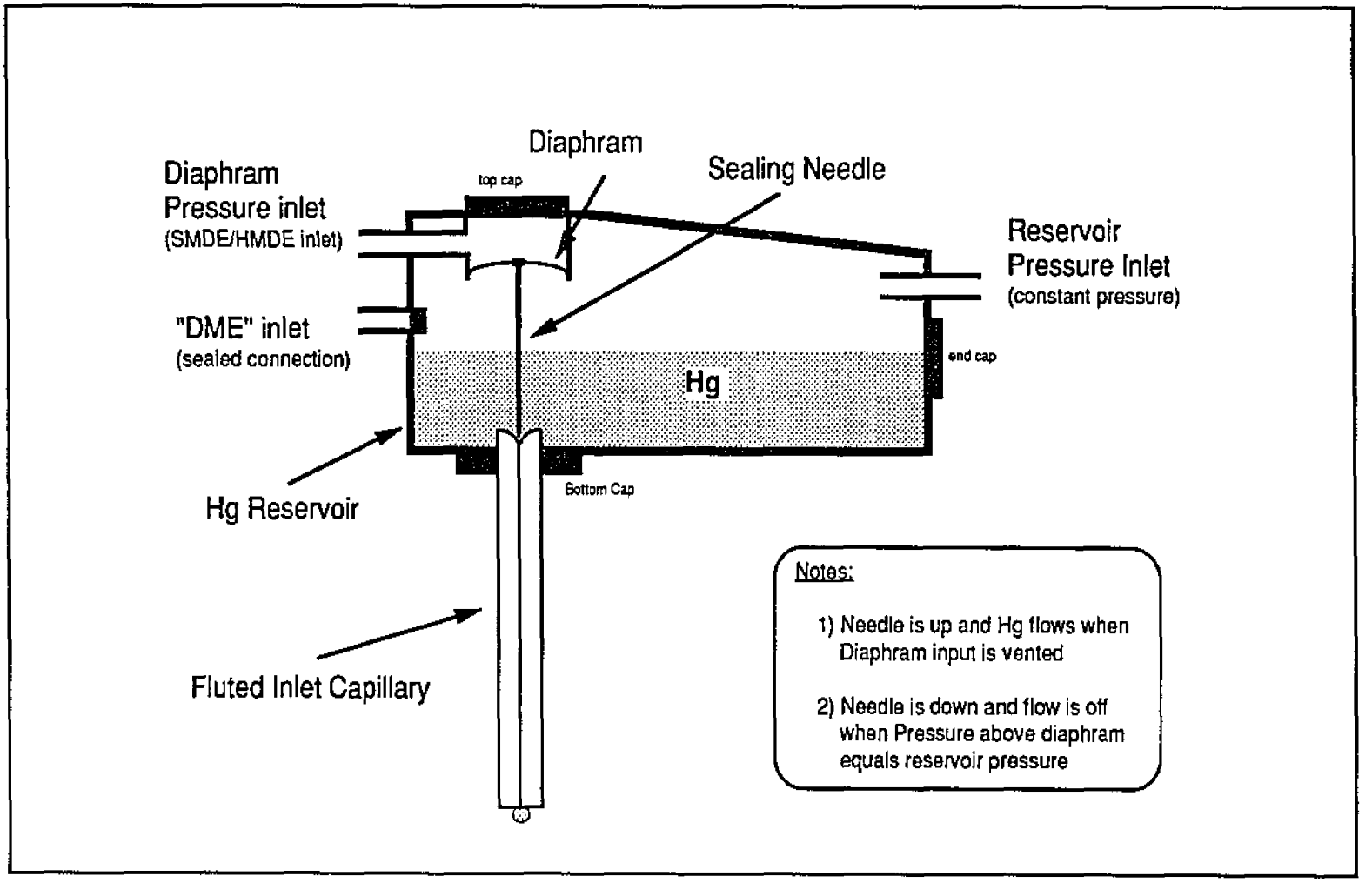

Figure 11 Simplified Diagram of the Metrohm Multi Mode Electrode

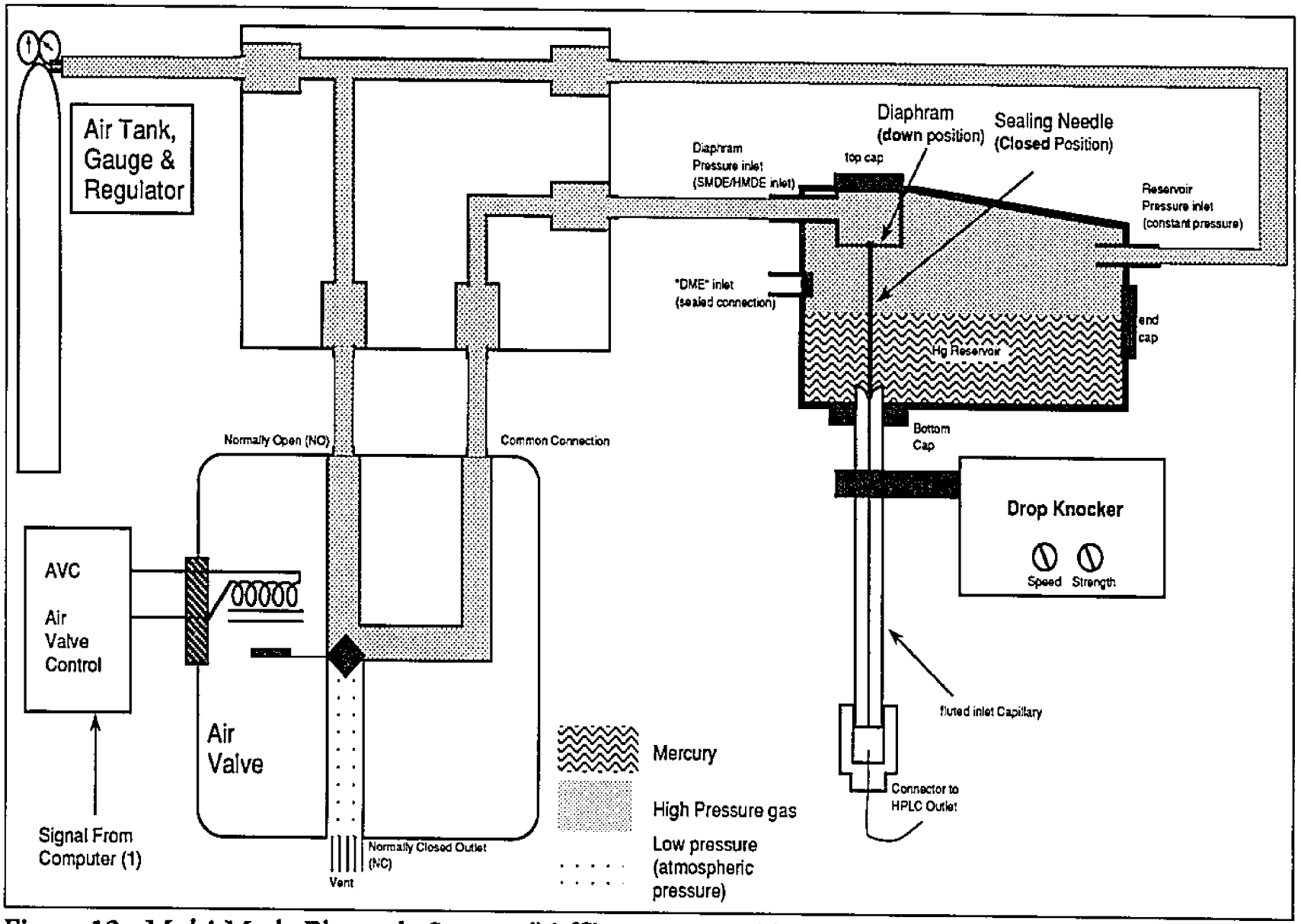

Figure 12 Multi Mode Electrode System; "Off" or Drop Hold Position

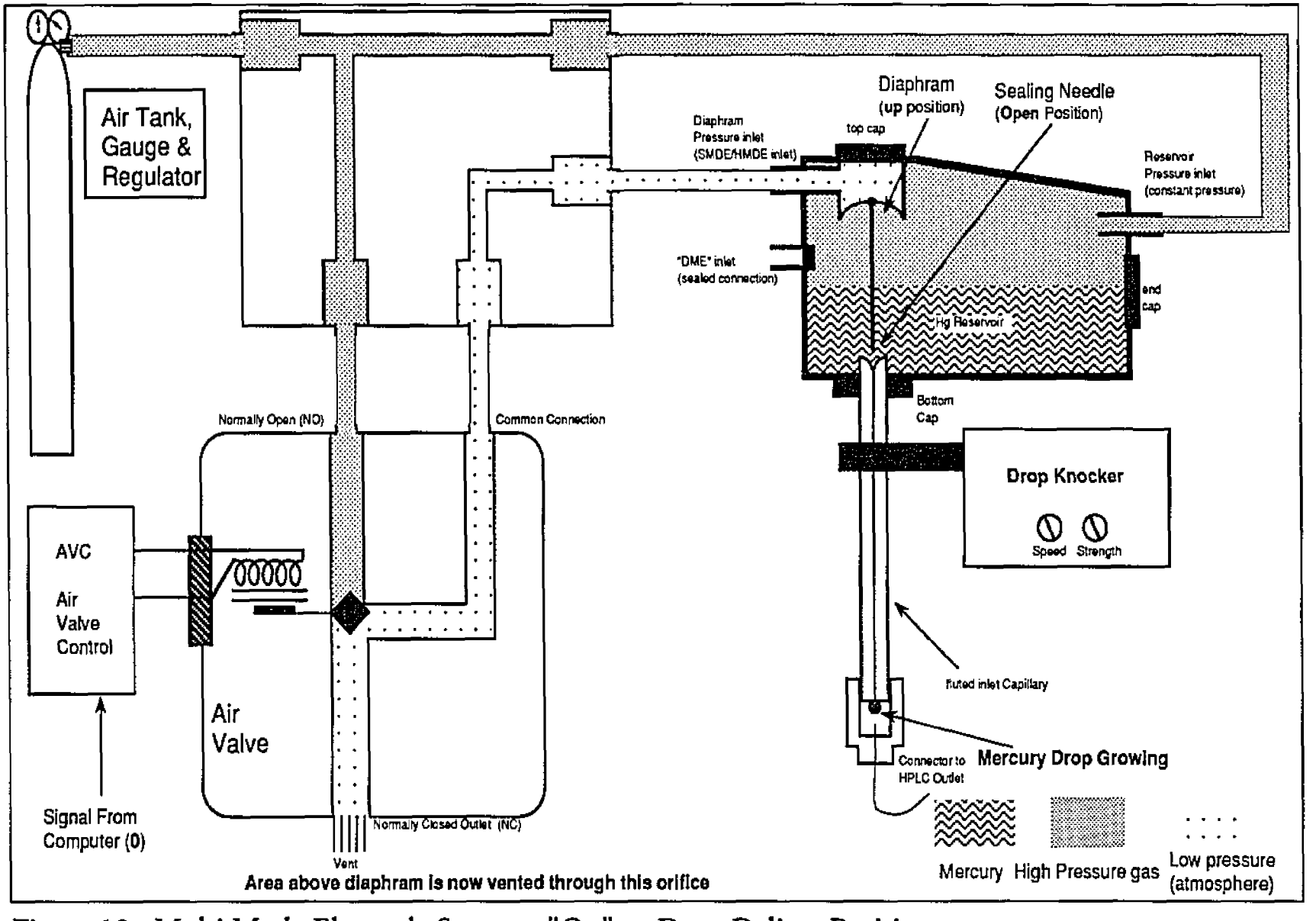

Figure 13 Multi Mode Electrode System; "On" or Drop Deliver Position

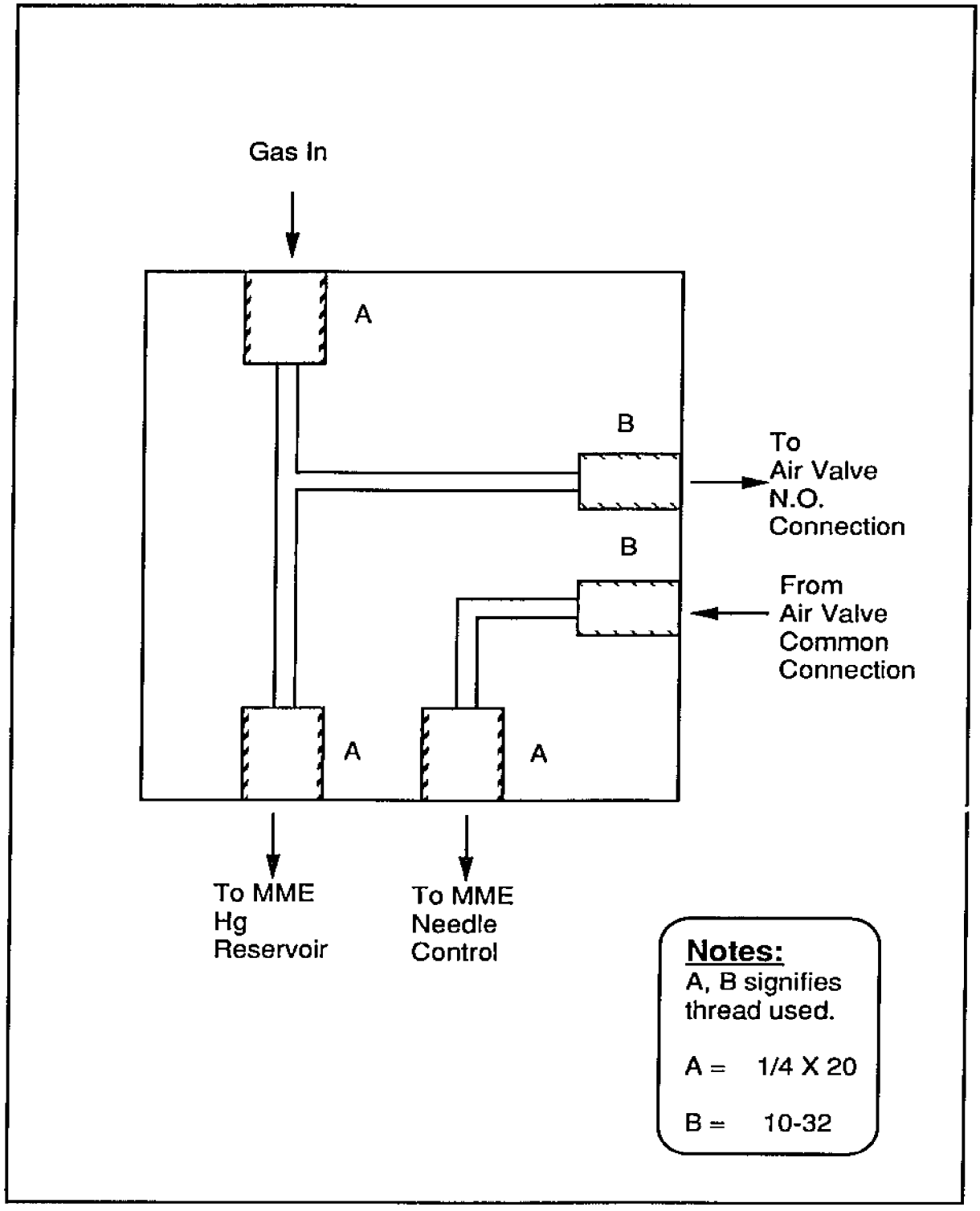

Figure 14 Pressure Router for the Air Valve

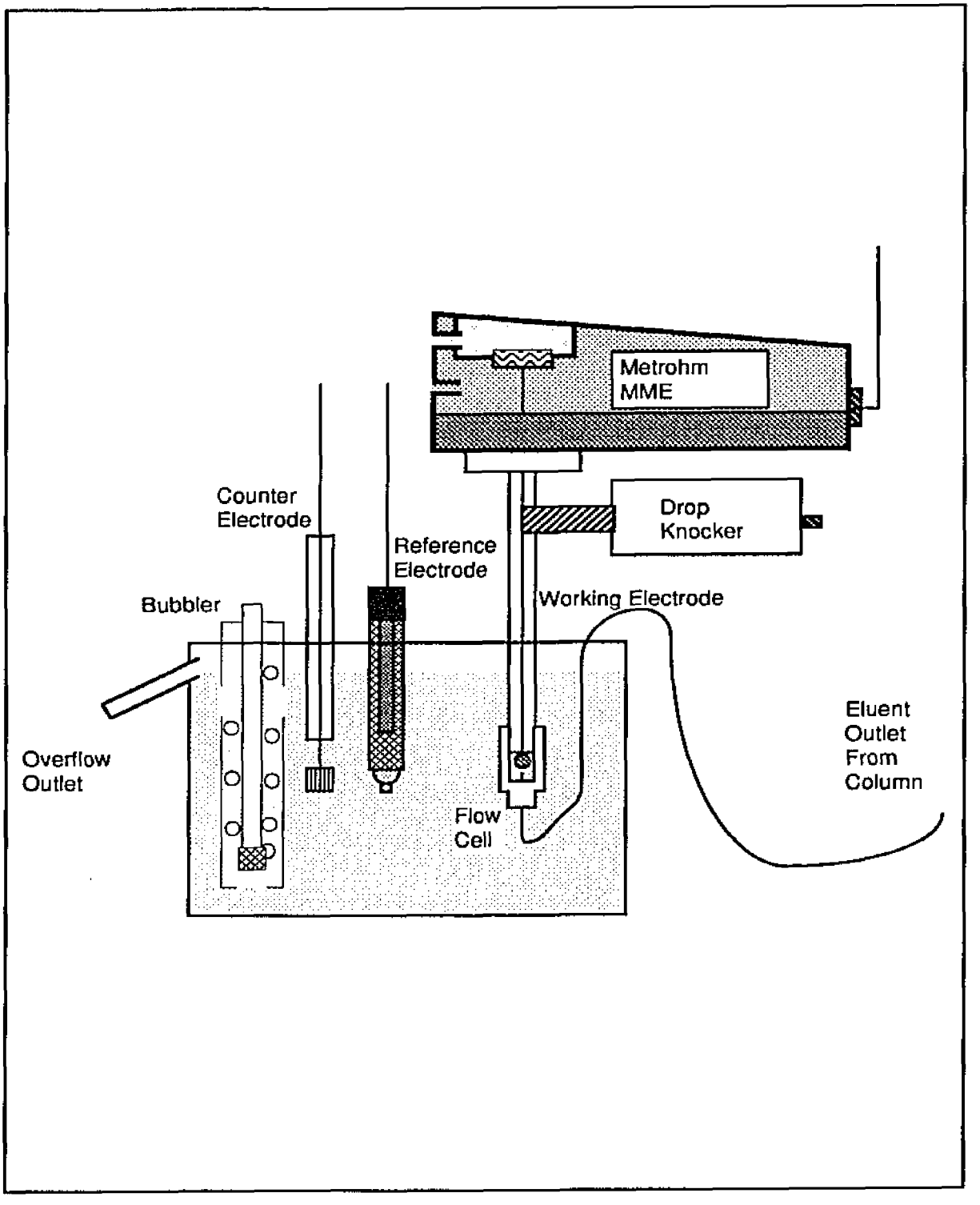

Figure 15 HPLC Mercury Electrode Flow Cell Diagram

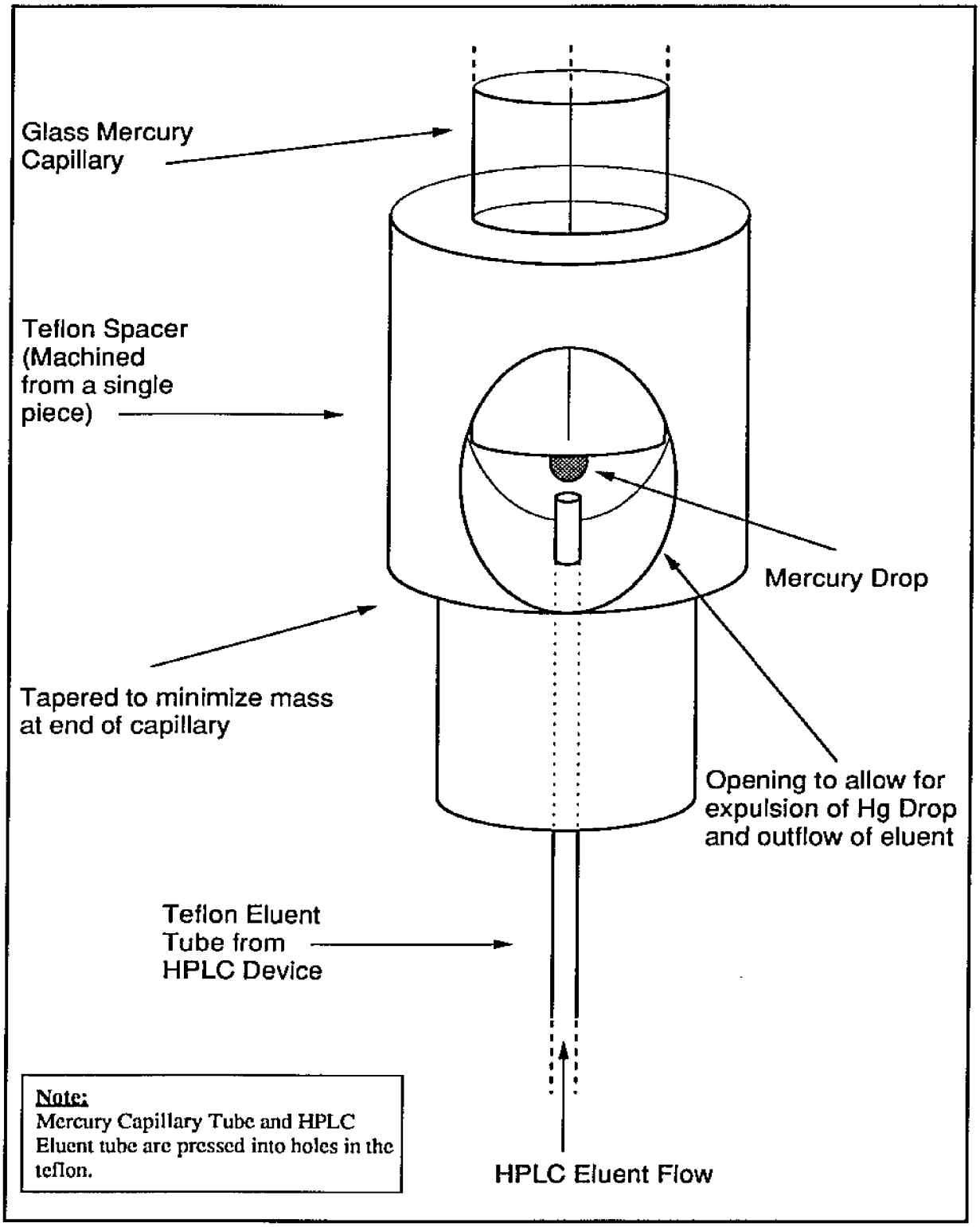

Figure 16 HPLC to Mercury Electrode Connector Diagram

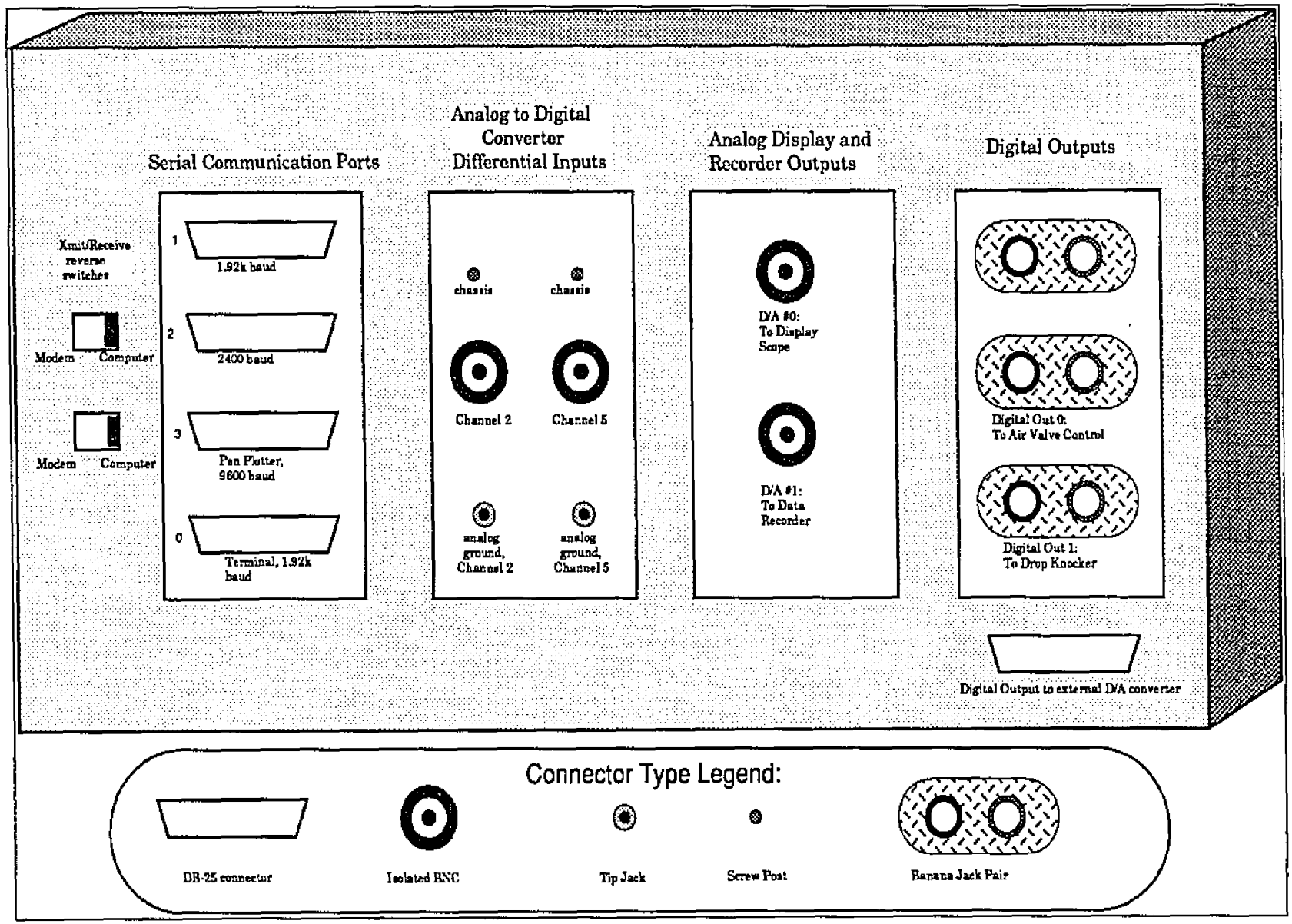

Figure 17 Placement of Connectors on Rear Panel of Computer

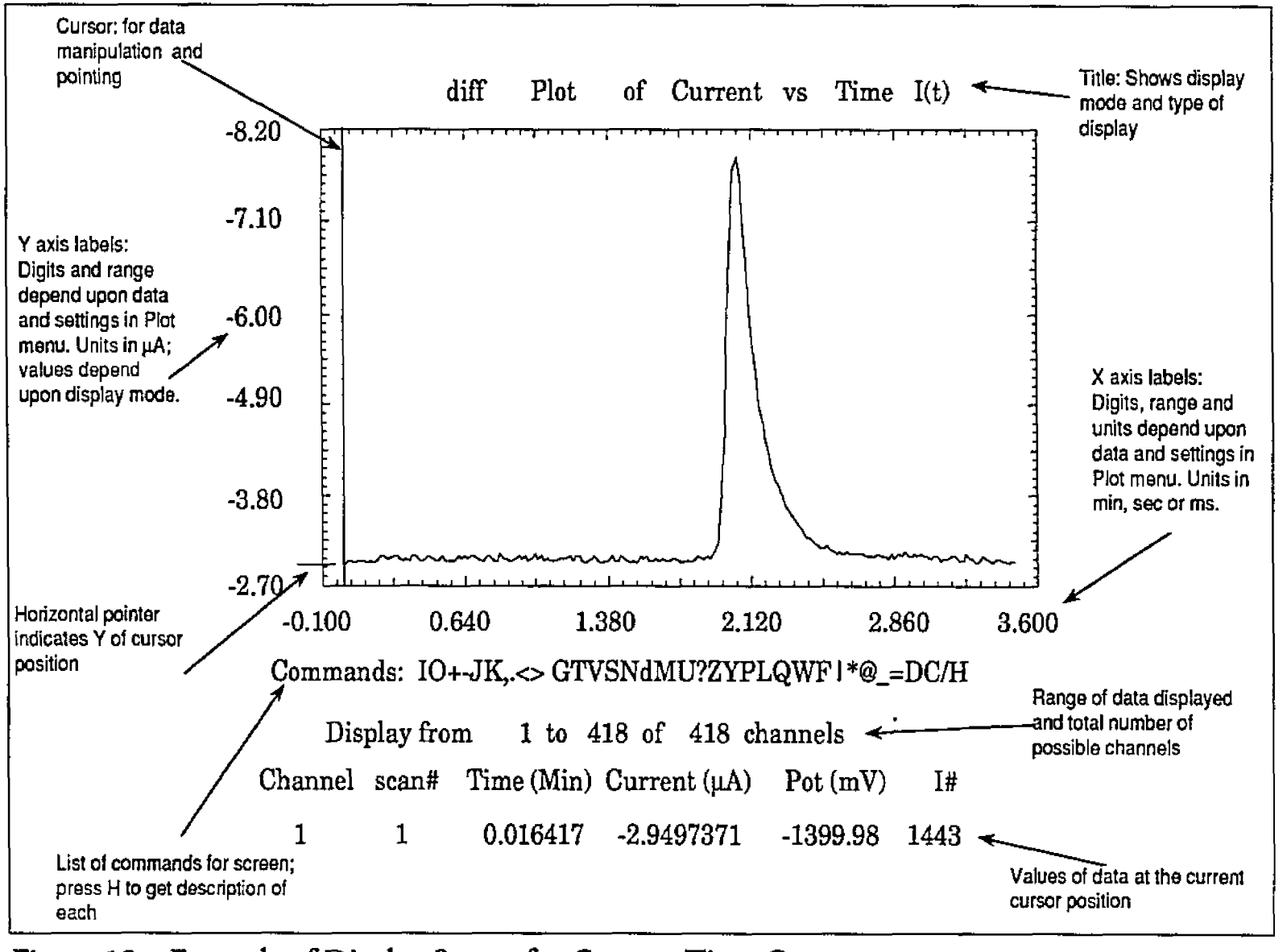

Figure 18 Example of Display Screen for Current-Time Curve

Experimental Section

Samples and Solutions

For all experiments, the buffer and mobile phase consisted of acetate buffer/acetonitrile (50:50, v/v) prepared with dissolved sodium acetate (4.1g/l) and (ca. 20ml/l) acetic acid to bring the pH to 5.0. The water was deionized and purified through high capacity ion exchange and carbon filter cartridges and then distilled. The acetonitrile was Fisher HPLC grade. The same batch of buffer was used both for the mobile phase and for dissolving and diluting samples. The cell (Figures 10, 15) was filled with the same buffer.

Experimental Procedure

The buffer in the HPLC reservoir and in the detection cell is continually degassed with argon or nitrogen. Both vessels are kept covered to minimize oxygen infiltration. The HPLC was run for at least 1 hour before each day's work. A background run was performed at the start and midway in the experiments to determine the quality of the buffer.

The mobile phase were continually degassed with argon or nitrogen. The solution in the cell was degassed between experiments, and during experiments, the flow of gas was held just above the liquid to prevent turbulence. In addition, the exhaust from the MME which consists of argon was vented into the air space in the cell.

Samples of size ca. 25 μL were injected into a 20 μl loop, with the overflow discarded. The injected samples were not degassed. The computer instrument and the HPLC was sychronized manually. This was accomplished by turning the knob on the BAS HPLC from "LOAD" to

"INJECT" while pressing the **enter** key on the computer after all parameters have been entered and the sample has been injected with a syringe into the loop. Turning the HPLC knob from "LOAD" to "INJECT" places the contents of the injection loop into the mobile phase stream.

The flow rate of the eluent from the HPLC column was determined by measuring the volume of mobile phase output for a given time, about five minutes. The eluent was collected from the detection vessel's overflow hole and the volume of the fluid was measured using a graduated cylinder. Timing was measured using the stopwatch functions and waveform timing in the **HSWAVE** program.

Instrumentation

The experiments were performed using the instrument system and **HSWAVE** program previously described. All of the experiments were performed using the Metrohm Multi-Mode Mercury Electrode (MME) and the flow cell pictured in Figure 15. Unless otherwise indicated, the experiments used the dual pulse waveform with the MME set up as a static electrode (SME). The waveforms applied were chiefly staircase, differential pulse and square wave. Polarographic experiments used the MME as a static drop electrode, with the electrode being renewed at each cycle of a waveform scan. Examples of the waveforms used may be viewed in Figures 5, 19, 7 and at the bottom of Figure 8.

Applications of the Instrument

System Deaeration Methodology:

Background Staircase Polarography of the Buffer

Helium is frequently used in HPLC as a degasser to displace other gases from the mobile phase. Other gases which may interfere with an electrochemical detector in HPLC include oxygen, which has reductive peaks at -0.2 volts and -1.2 volts, and nitrogen, which under certain conditions, will go out of solution when the eluent is decompressed at the column exit and form bubbles which may cause large current fluctuations. The instrument system used has two solutions which need degassing, the mobile phase entering the HPLC column and the solution in the instrument flow cell. A background study of the acetate/acetonitrile buffer under various conditions was performed using staircase polarography. The current voltage curves as well as the parameters of the staircase waveform are depicted in Figure 20. Five solutions were examined: 1) plain buffer used as the background solution, 2) buffer with hydrogen peroxide, degassed with argon, 3) buffer with oxygen gas bubbled through, 4) buffer with argon gas bubbled through, and 5) buffer with helium gas bubbled through.

Gases were bubbled through the liquid for a minimum of five minutes. The cell was kept loosely covered as it would be in an HPLC experiment. When the staircase voltage waveform was run, the tube used for bubbling gas was removed from the liquid and held above the surface of the liquid.

The buffer with oxygen (Figure 20) shows two waves of equal height with half-wave potentials at -0.2 and -1.25 V vs. SCE, corresponding to the waves found in the background solution. The plateaus for the oxygenated

buffer occur at values more negative than -0.45 V and at -1.47 V. The hydrogen peroxide solution shows only the second half wave at -1.24 V and a steep leveling off point at -1.34 V. The background solution before degassing, which was allowed to sit covered, showed the two half-wave potentials corresponding to oxygen. The solution degassed with argon has a flat background until around -1.64 v, where hydrogen is evolved. Helium did not fare well as a degasser, as the voltammogram shows the hydrogen peroxide and oxygen waves.

Argon is a better degasser than helium for the flow cell in the conditions of this experiment. A possible reason that argon is better might be that it is heavier than air and covers the solution, while helium is lighter than air and oxygen from the air has more of a chance to dissolve in the solution. Helium works well for degassing the mobile phase liquid, as it is easier to keep the container air tight and turbulence of the liquid in the reservoir is of little importance.

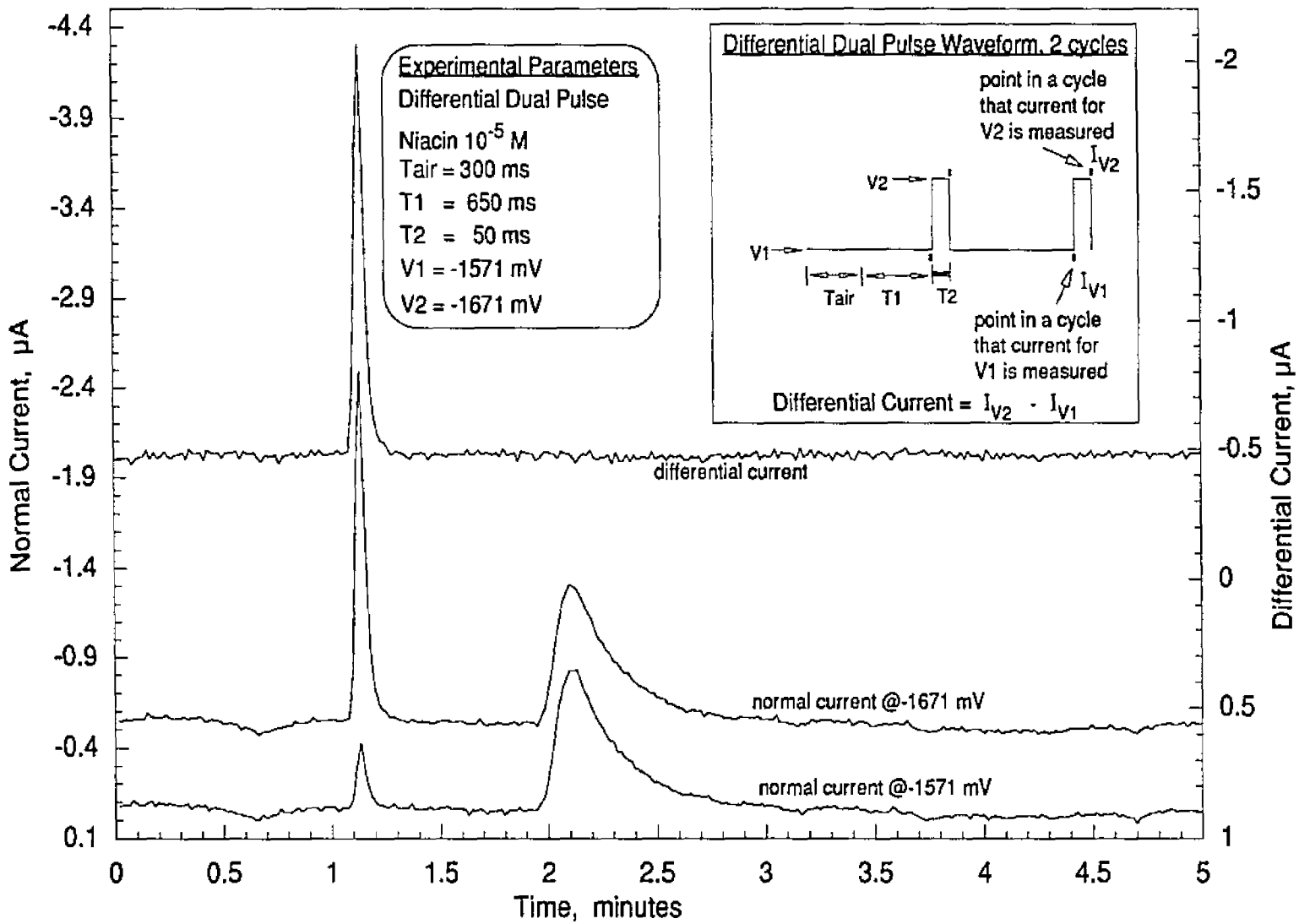

Figure 19 Differential Dual Pulse HPLC Detection of Niacin, with Potential Waveform

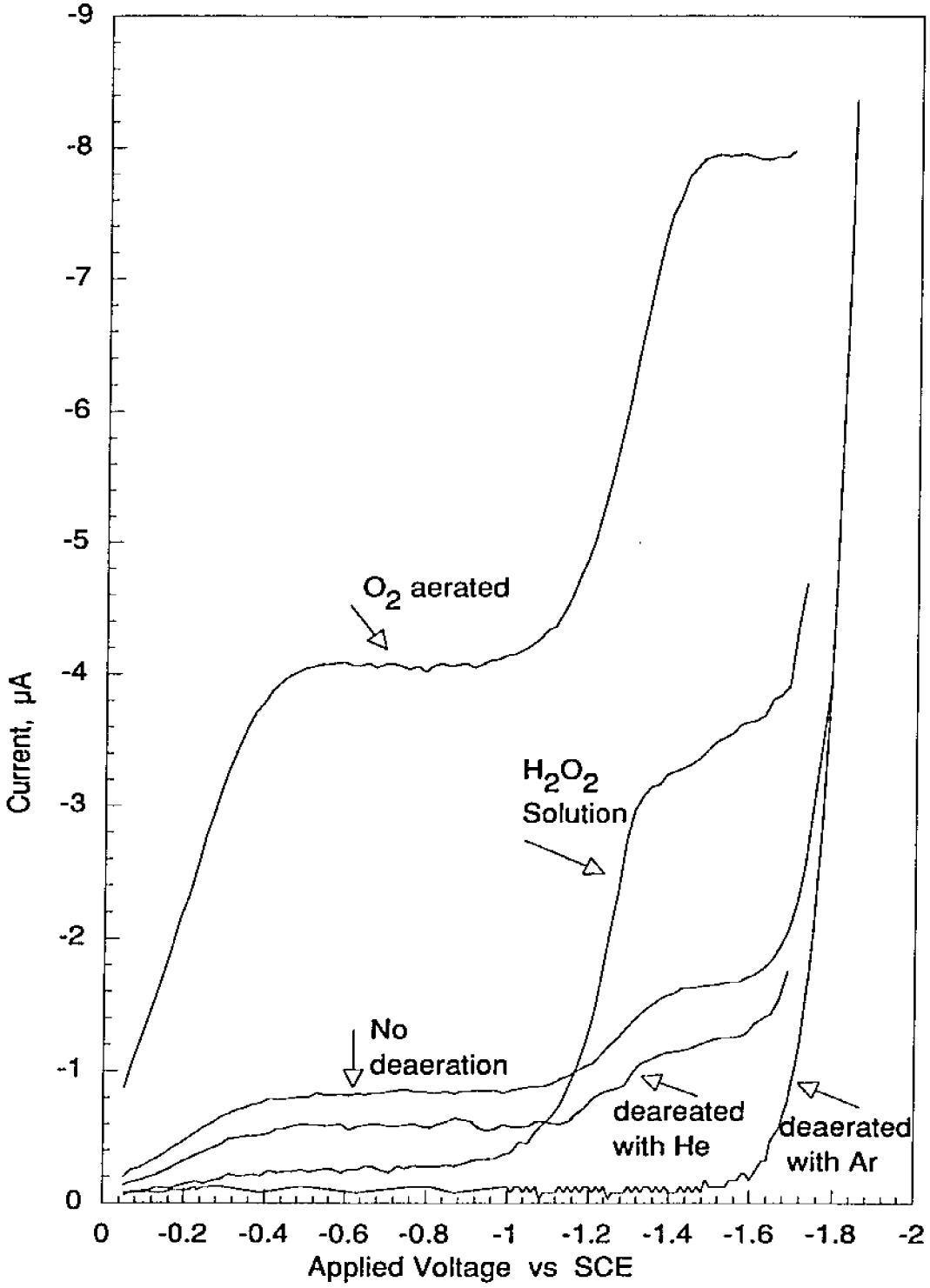

Figure 20 Staircase Polarography of the Buffer: Plain, With Oxygen, Peroxide or Argon

Differential Technique

There are several examples which illustrate the utility of the differential technique. Figure 21 shows background current before and after the pump for the HPLC is first turned on. The normal background current shifts in response to the eluent flow and to the column stabilizing, but the differential current is relatively constant. Another example is when the pump is turned on, as shown in Figure 22. In this case, acetone was injected to help clean out the column. The differential current is not sensitive to the large shifts in the normal current and discriminates against the oxygen peak, since the voltages selected for detection are beyond the oxygen plateau. The effect of injection of oxygenated buffer is seen in Figure 23. The differential current is slightly elevated when the oxygen elutes, but the response is very small. In the case of Figure 24, a sample of 10^{-5} M vitamin B_{12} was injected before the oxygen from a previous injection was allowed to eluent. The timing was such that the B_{12} eluted at the same time that the oxygen from the previously injected sample eluted. Viewing the normal data, the two peaks overlap. The differential view shows only the B_{12} peak with no contribution from oxygen. This occurs because the voltage that is being applied is on the plateau of the oxygen wave (Figure 20) which occurs at -1400 mV. When -1660 mV is applied, and the oxygen elutes, the normal current changes, but the difference current is constant. A chromatograph where niacin is the injected sample, with a retention time of 1.1 min, is shown in Figure 25. The oxygen contained in this non-deaerated sample elutes later, approximately at 2 minutes from the initial injection. If one viewed only the differential current, one would miss the oxygen peak. There is also a small oscillation that occurs before the niacin elutes which

does not show up when viewing the differential current. These examples demonstrate the selectivity of the differential method.

There is a price to be paid for examining the differential current. The level of random noise increases by a factor of two. The minimum of the noise from the A/D converter is 1 bit or the numerical value ± 0.5 bits. To take a differential, two data values are required. Combining the values results in possible errors of 0, ± 0.5 or ± 1.0 bits. Since the current is proportional to the A/D converter numerical value, the error is doubled. This is clearly evident in Figure 26 which is a chromatograph of a mixture of riboflavin and B_{12} and shows the normal background is relatively flat but the differential background is noisy. This noise can be reduced by utilizing the full range of the A/D converter or by using an A/D converter with higher resolution.

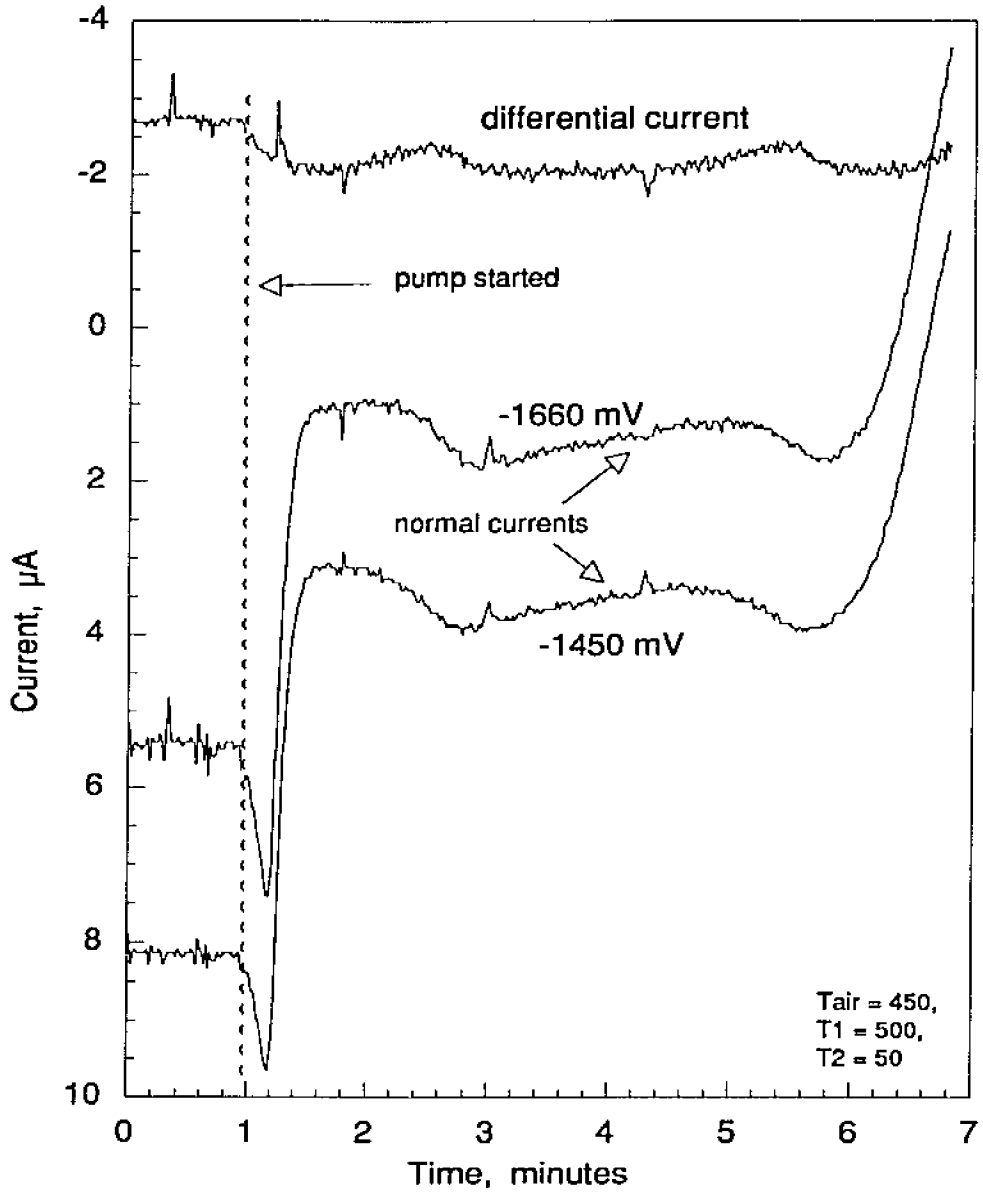

Figure 21 HPLC Dual Pulse Background, with the Pump Turned On.
Note that the differential current varies much less than the amperometric current.

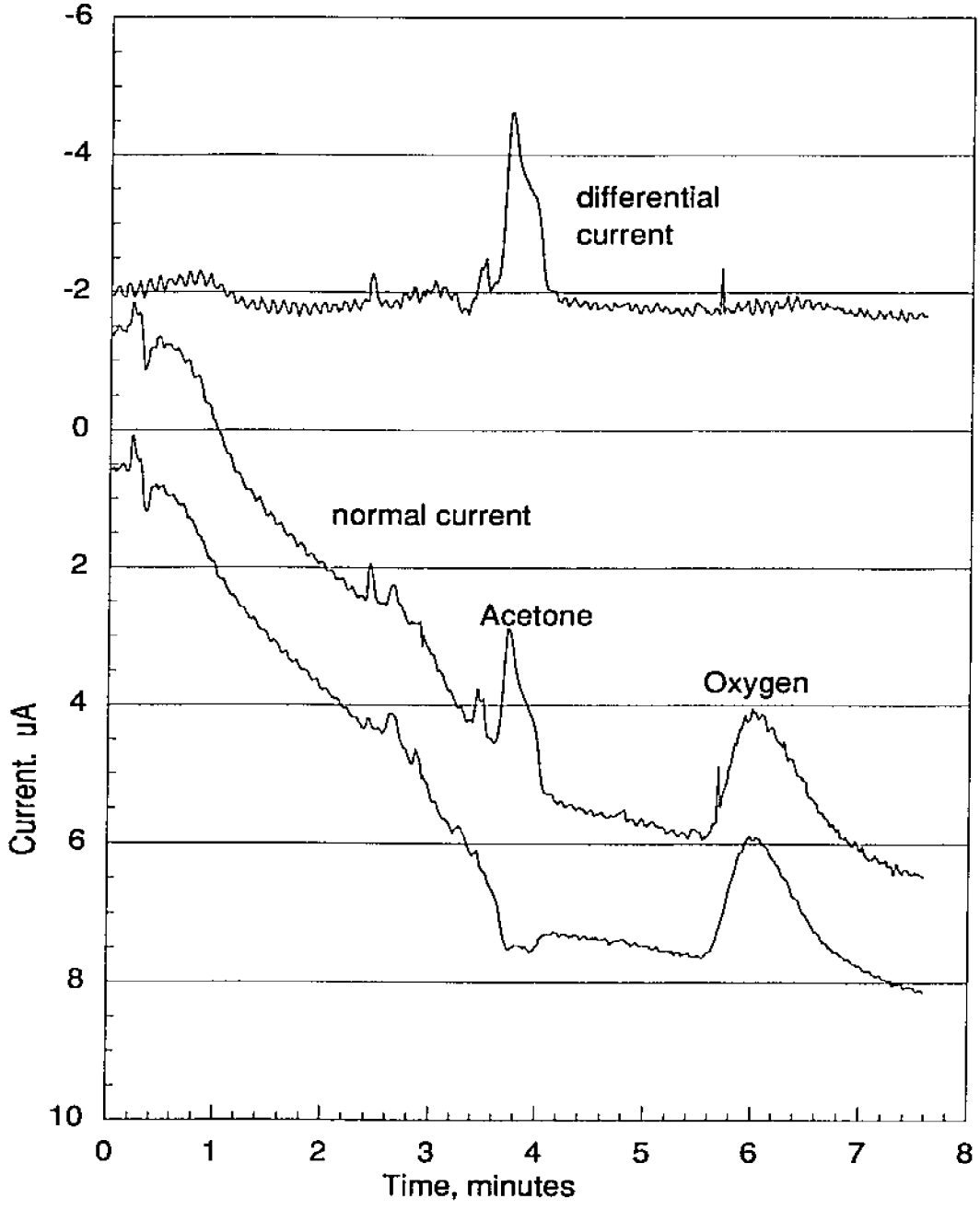

Figure 22 Column Cleaning with Acetone at Start-Up

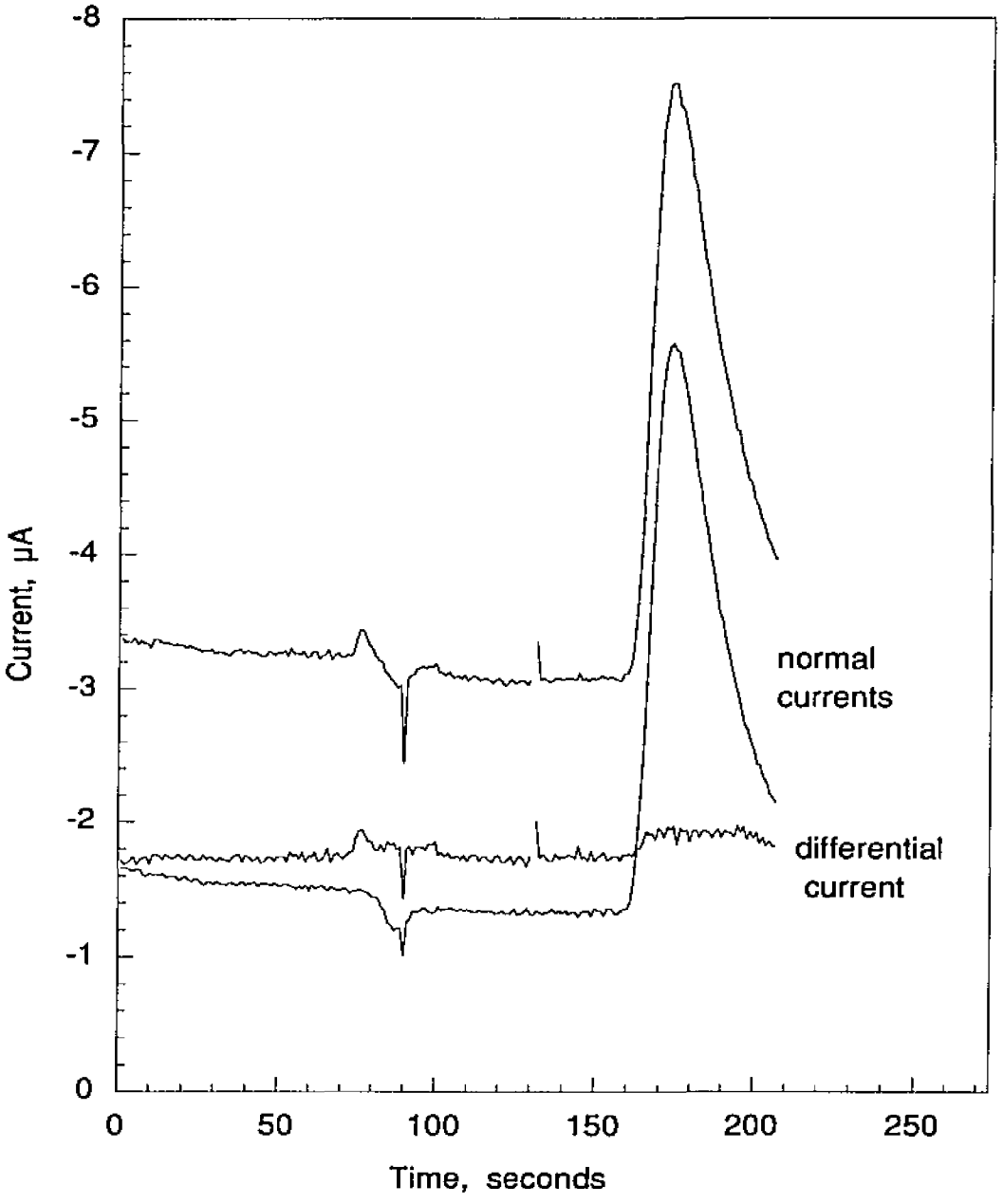

Figure 23 HPLC Dual Pulse of Oxygenated Buffer
Note that the differential current shows little response when the oxygen elutes.

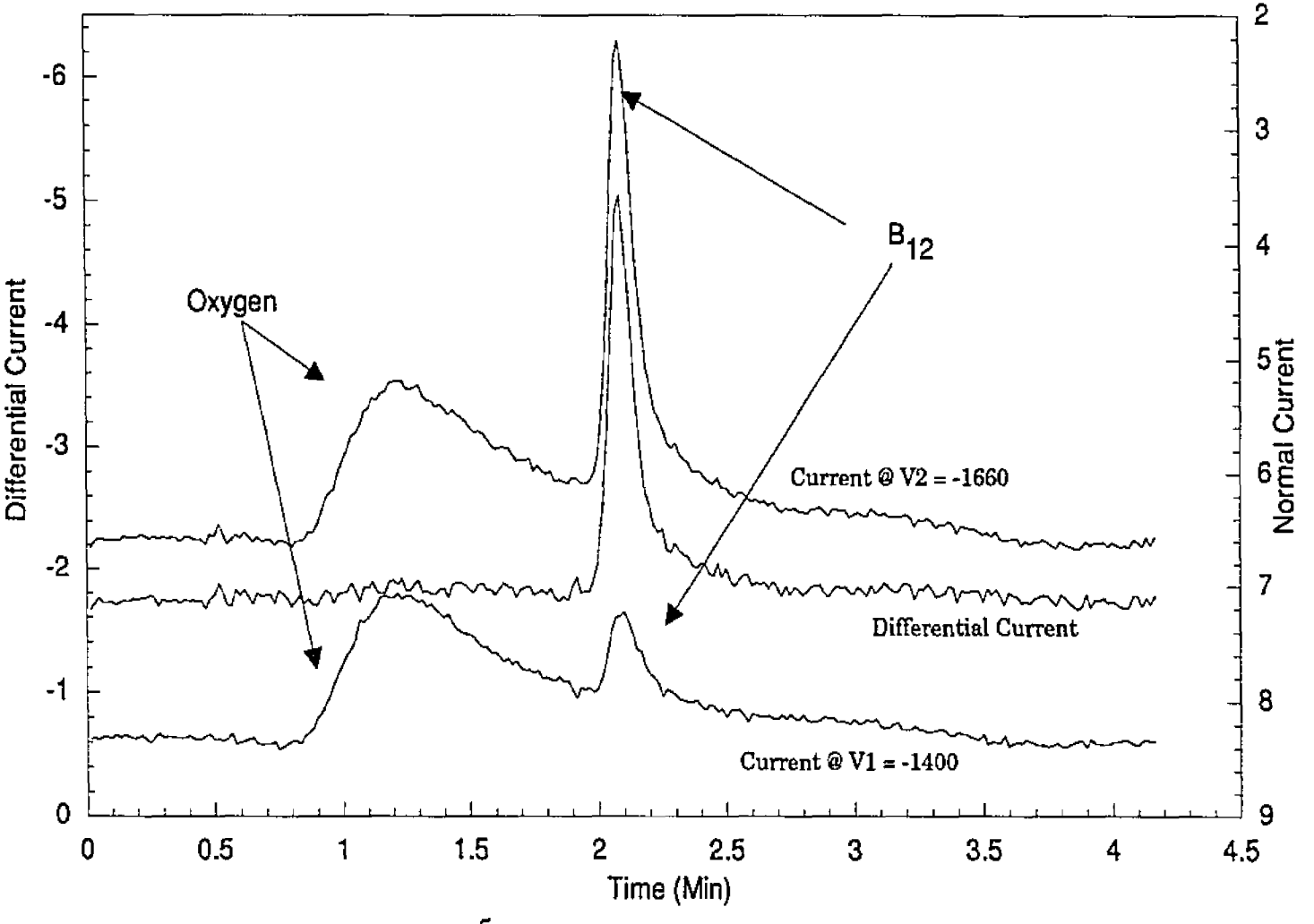

Figure 24 Vitamin B_{12} 10^{-5} M with Overlap of O_2 from the Previous Sample.

The differential current discriminates against the O_2, displaying only the B_{12} peaks

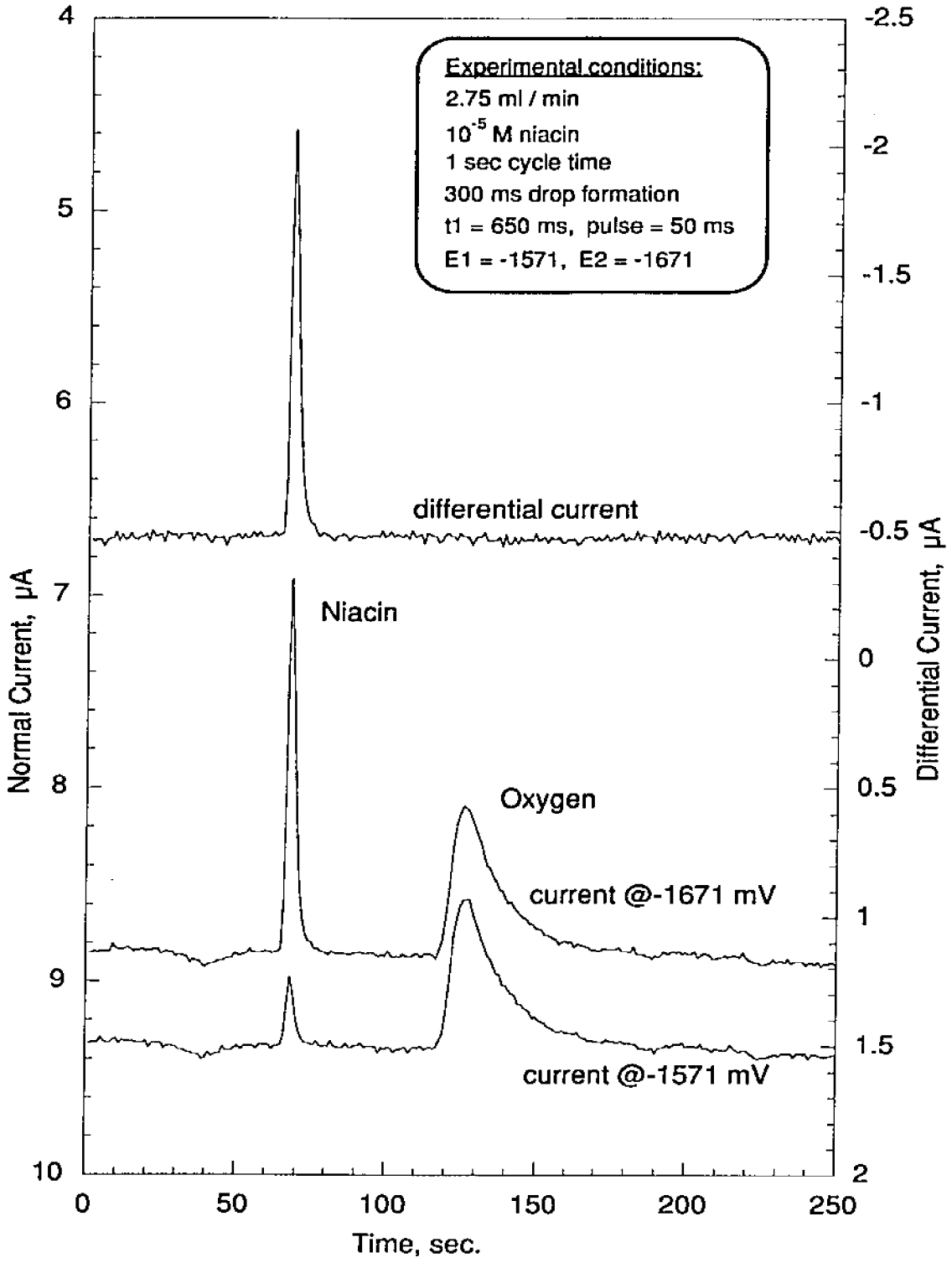

Figure 25 HPLC Dual Pulse Detection of Niacin

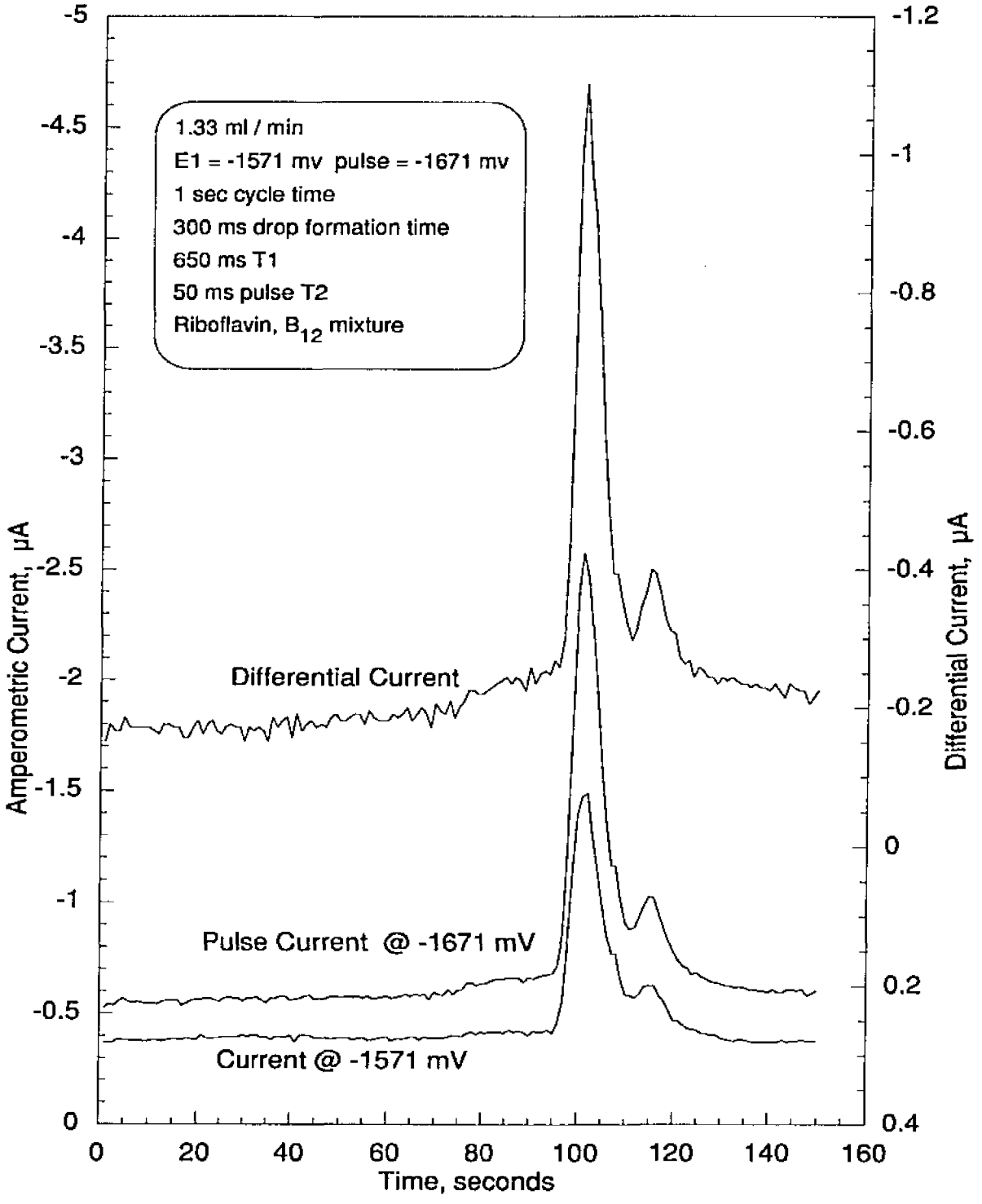

Figure 26 HPLC Dual Pulse of Riboflavin/B_{12} Mixture

Catalytic Current

The concept of the catalytic current has been described earlier and a possible mechanism is given in equation 1.1. The vitamins studied exhibit both catalytic and non-catalytic (Faradaic) electrochemical waves. Generally the Faradaic waves of the B vitamins are of low amplitude and thus do not have practical use for detection, unless the vitamin is very concentrated. For example, the top part of Figure 27 shows the Faradaic wave of riboflavin at -300mV. While there is a significant amount of current (2.2 μA), the concentration of riboflavin is 10^{-3}M which is an almost saturated solution. The catalytic current is off scale and shifted to -1200 mV. For a solution which is two orders of magnitude less concentrated, 10^{-5} M, the Faradaic current cannot be distinguished from the background noise at the same instrument settings (bottom part of Figure 27). At this concentration, the catalytic wave is -0.6 μA with a plateau that ends at -1700 mV. Another example of catalytic waves is presented in Figure 28. Vitamin B_{12} at $1 \cdot 10^{-5}$ M and riboflavin are analyzed separately using staircase polarography. The B_{12} shows a large adsorption peak at this concentration. At lower concentration, the B_{12} shows waves similar to the riboflavin in the figure. The catalytic wave provides a signal of large amplitude for detection, but the position and shape of the wave varies with concentration of the species.

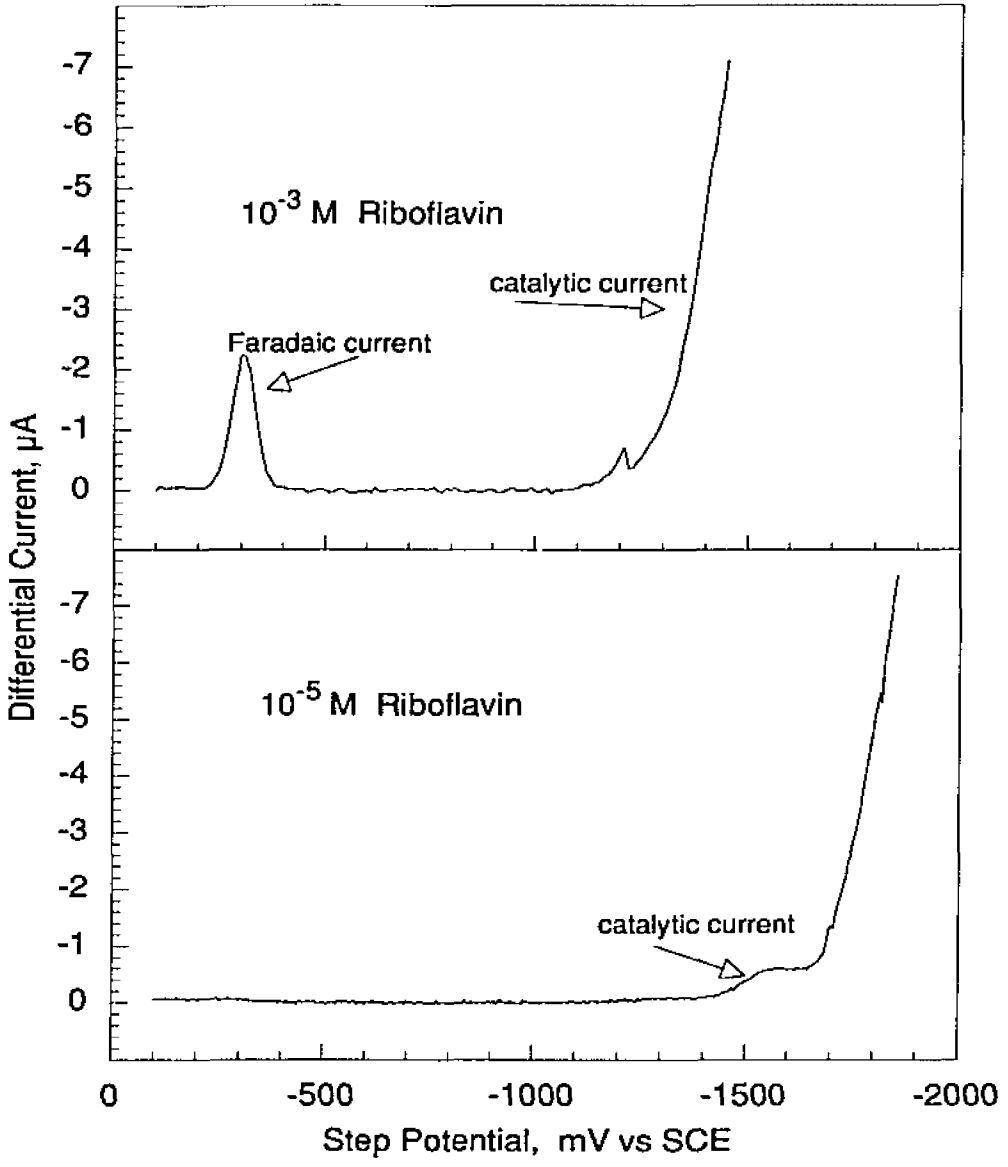

Figure 27 Differential Pulse Polarography of Riboflavin, Comparison of Two Different Concentrations

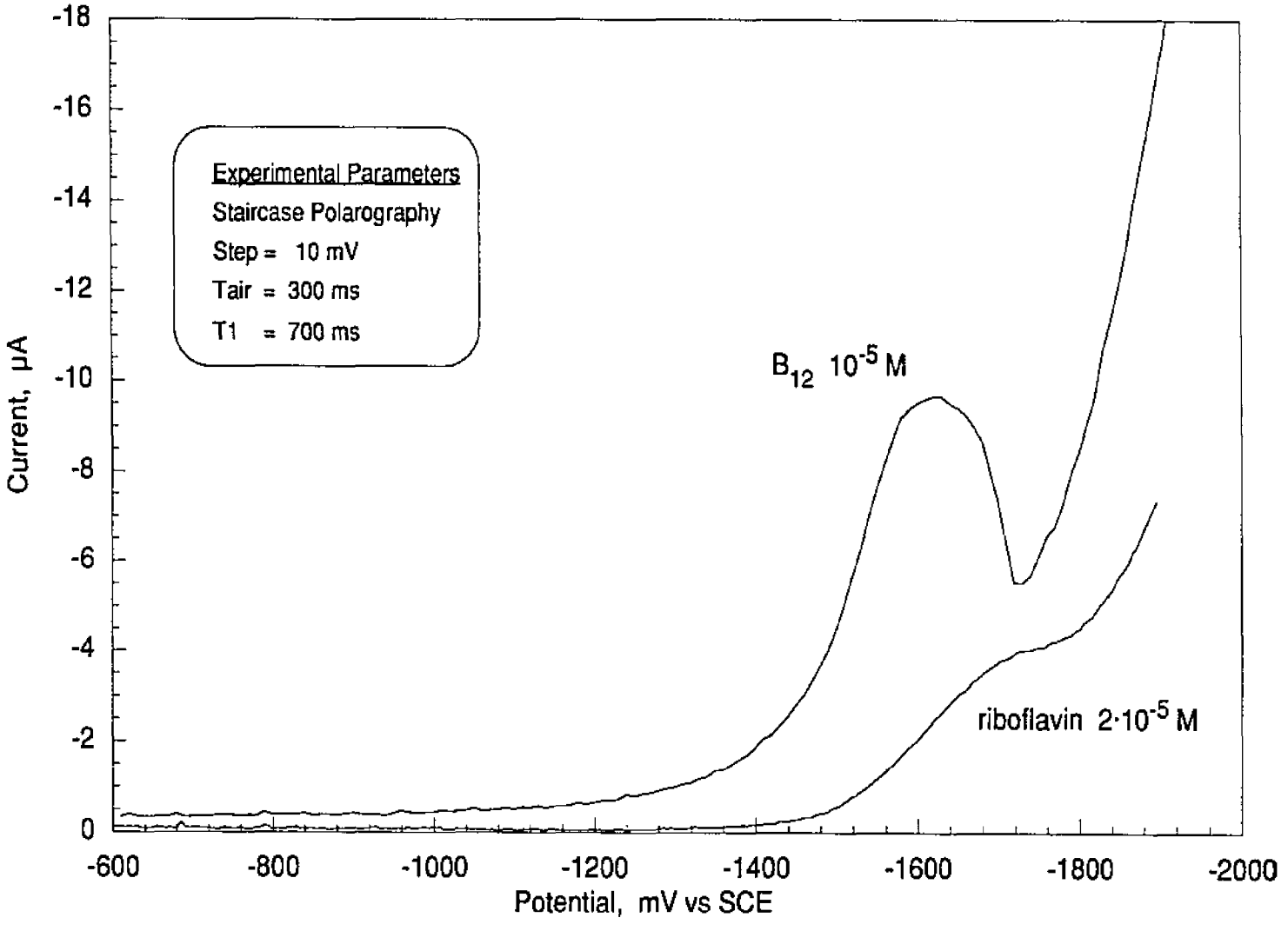

Figure 28 Catalytic Waves of Vitamins B_{12} and Riboflavin

Studies Involving Variation of Mobile Phase Flow

To demonstrate some of the HPLC properties of the system, studies were performed with the instrument at various flow rates. The flow rate was adjusted by slowly changing the pump speed which changes the column pressure. A minimum of 10 minutes was allowed after each change before performing an experiment. The column pressure was determined by noting the peak pressure on the gauge built into the HPLC device. The flow rate was determined by collecting the effluent overflow from the cell into a graduated cylinder. The time was determined by the stopwatch feature of the **HSWAVE** program. The flow rate in ml/min was then calculated. The flow rate ranged from 0.85 ml/min to 3.28 ml/min with an error in precision of $\pm$ 0.02 ml / min.

Flow Rate and Column Pressure

The flow rate was linear with (peak) column pressure for the range of pressures serviceable with a mercury electrode (Figure 29). However, flow rate values varied from this calibration whenever the instrument was shut down and restarted, by as much as 25%. Suddenly changing the pressure could also change the calibrated flow by a few percent. Therefore, experiments involving flow rate should be determined directly by flow of material rather than by column pressure.

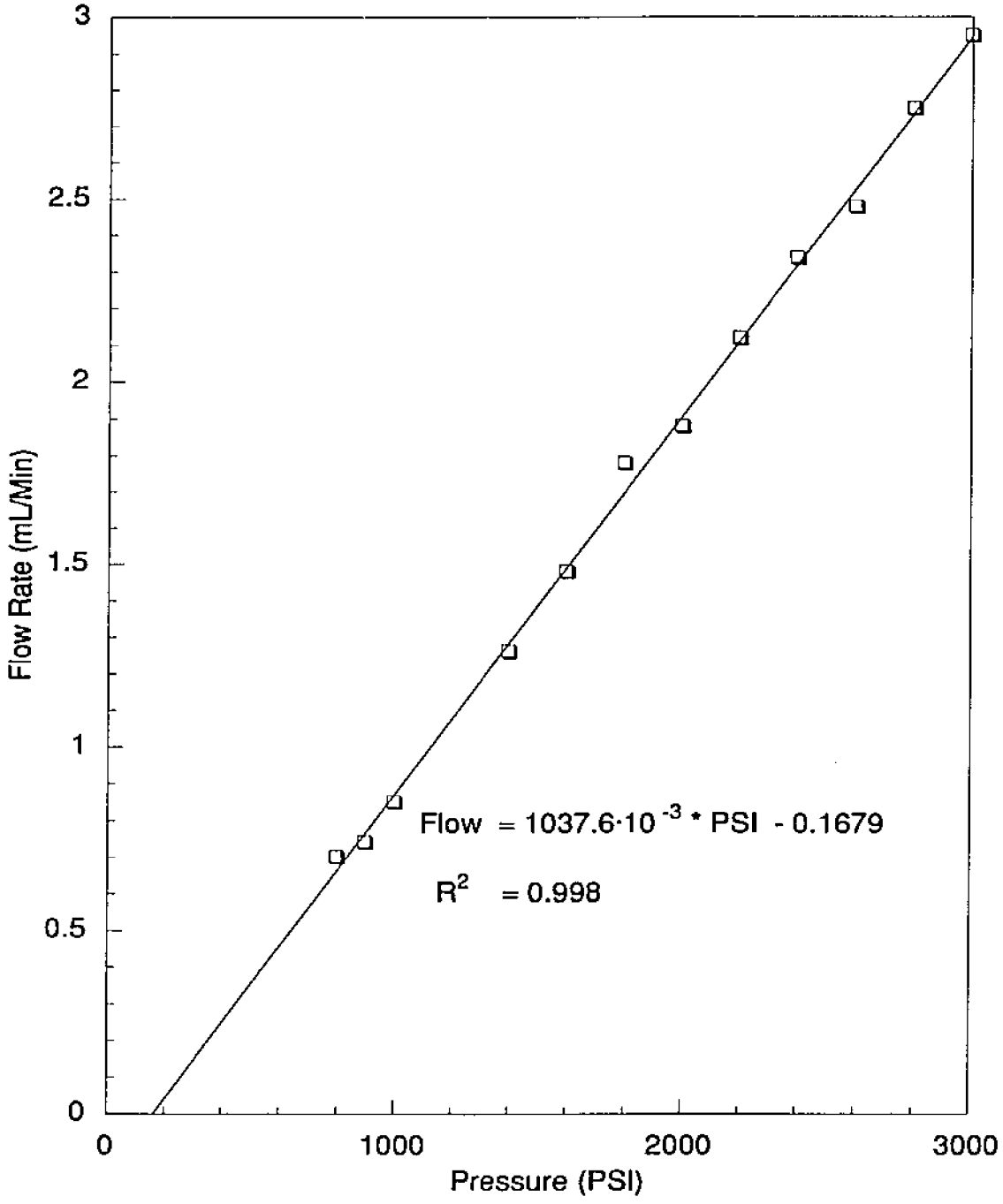

Figure 29 Dependence of Mobile Phase Flow Rate on Pressure

Flow Rate and Retention Time

Niacin at a concentration of 10^{-5} M was injected (20 µl aliquots) at various flow rates. Retention time was determined using the functions in the **HSWAVE** program to find peaks and then recording the corresponding time values. Figure 30 shows reciprocal retention time versus flow rate of the differential peak of niacin as well as the normal oxygen peak current. Both curves show a linear relationship with flow rate, with the slope of the oxygen curve half that of the niacin. A mixture of niacin and B_{12} were injected at various flow rates as detailed in Figure 31. There are three curves for niacin, B_{12} and oxygen. Comparison with the curves in Figure 30 show that the curve with the highest slope, or the peak which elutes first, is the B_{12}. The middle curve is the niacin, and the bottom curve is oxygen.

Theoretical Plates and Flow Rate

The number of theoretical plates was estimated by using the information command in the **HSWAVE** program. This command takes a selected portion of data, determines the background, and then calculates and lists the background, the range of the data, the area between the background and the curve, the retention time of a peak, the half width of a peak, and the number of theoretical plates. Equation 1 is used for the calculation of plates. This command is discussed in detail in Appendix 2.1B. The result of a study of niacin at various flow rates was a narrow peaked curve, shown in Figure 32. The peak occurred at 1.88 ml/min, with 3600 plates. At either end of the flow rate, 0.7 and 2.95 ml/min, the number of plates was calculated to be 2600.

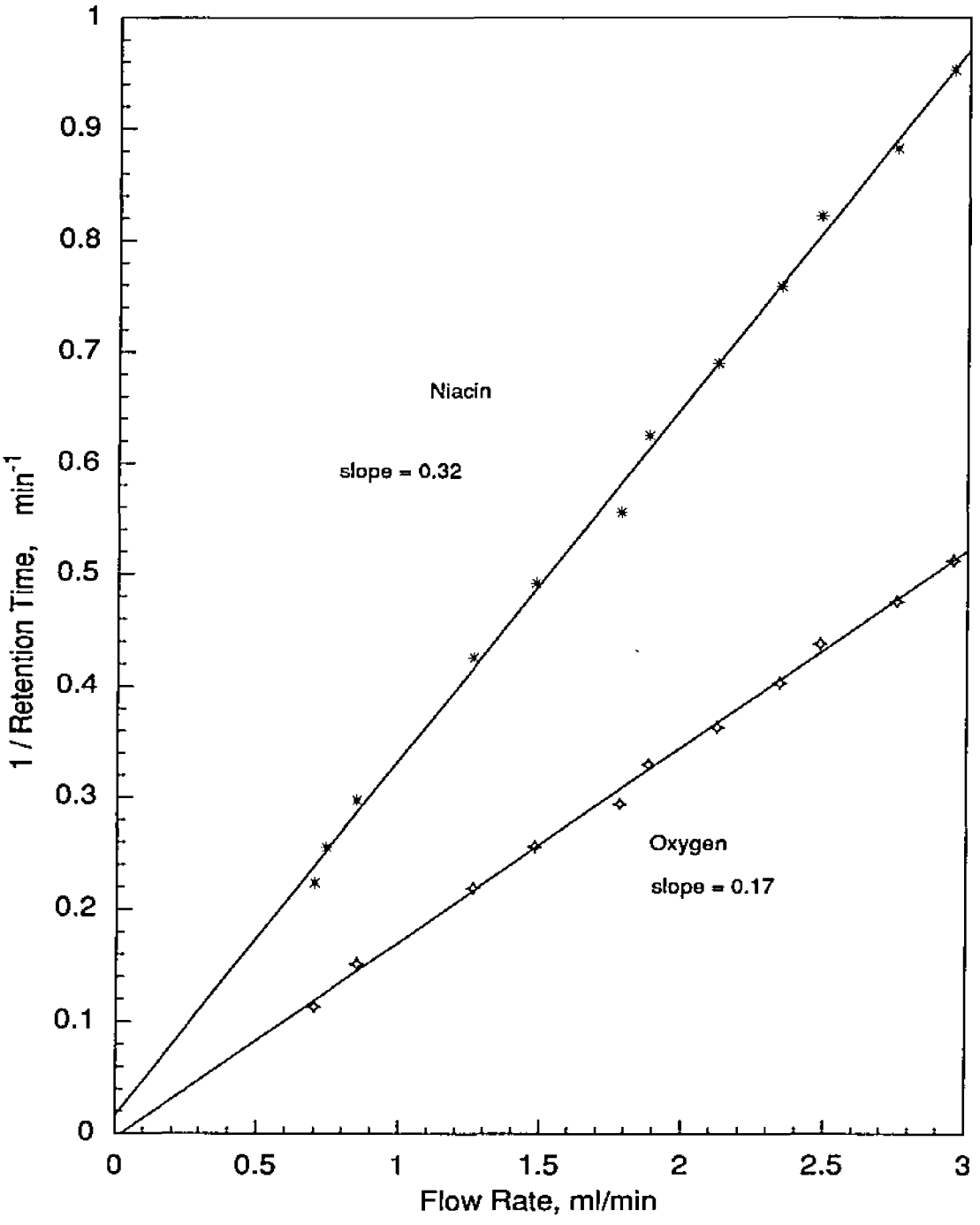

Figure 30 Dependence of Retention Time on Flow Rate for Niacin and Dissolved Oxygen

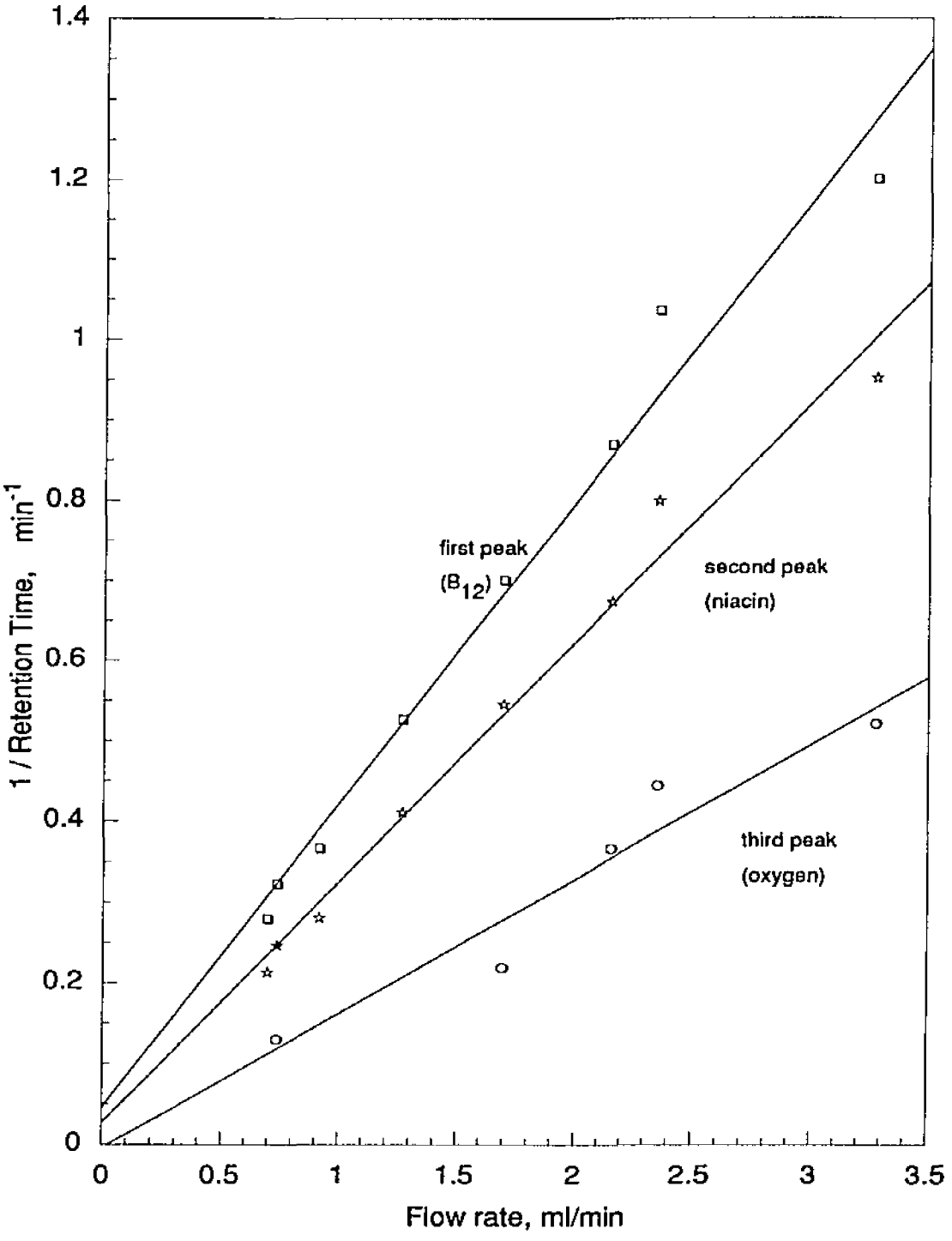

Figure 31 Effect of Flow Rate on Retention Time for a Mixture of Niacin and B_{12}

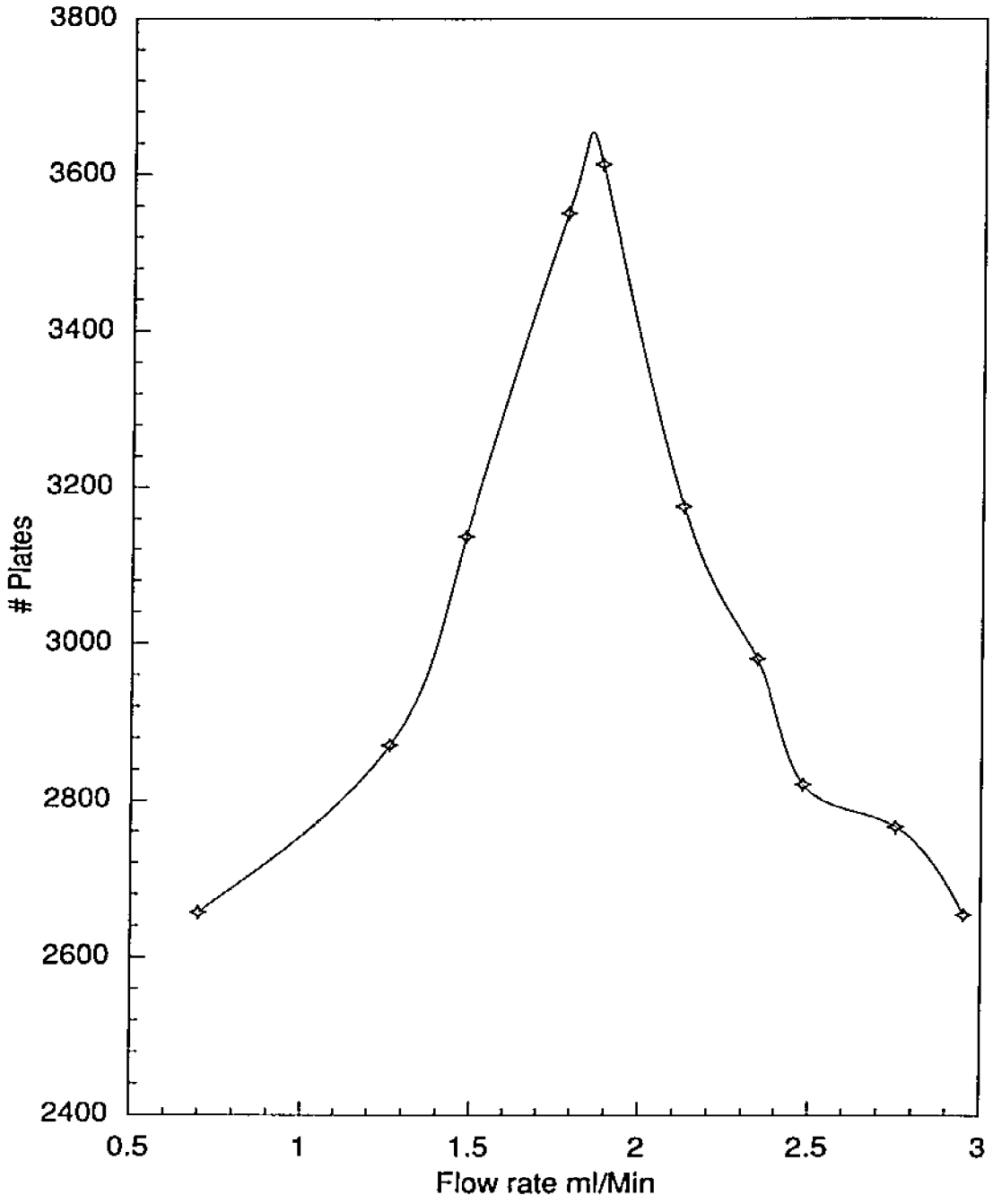

Figure 32 Dependence of Theoretical Plates on Flow Rate for Niacin

Area under the Peak Current and Flow Rate

The area under the differential current peak was examined at different flow rates. The area was calculated in the **HSWAVE** program which uses the trapezoid method for approximating the integral under the curve. Figure 33 shows that there are two regions which describe how the area changes. Below 1 ml/min, the area decreases rapidly with increasing flow rate. Above 1 ml/min, the area decreases an order of magnitude more slowly as the flow rate increases. By taking the log of the flow and the area and determining the slope of the resulting curve, it was determined that in the region of low flow rate, the area is proportional to V^{-1}, which indicates that the detector in this region is concentration sensitive. In the high flow region, the area is proportional to $V^{-0.2}$ indicating convective mass flow.[28] An empirical explanation might be that at low flow rate, most of the material is reduced at the electrode, while at high flow rate, some material does not reach the electrode and is not reduced.

Characterization of the Electrode

The electrode was examined for its properties as a wall jet or wall tube electrode. Equations for the wall tube (WT) and wall jet (WJ) electrodes have been derived in the literature for a flat disk and a growing drop electrode. Static mercury electrodes have not been examined. It is assumed that the form of the equations for the disk electrode holds for the static Hg electrode (equations 2 and 3). The nozzle size radius is 0.15 mm (manufacturer's data) and diameter of the mercury drop is 0.752 mm (determined by weight and density of several hundred drops). If only that part of the drop facing the stream contributes significantly to the current,

then the drop area is $0.5\ \pi\ D^2$ or 0.888 mm^3 Since the electrode size is larger than the nozzle, it is expected that the current will have WJ character. In the aforementioned equations, the easiest parameter to vary experimentally is the volume flow rate. From equations 2 and 3, a WT has the property that the current is proportional to the square root of the flow rate; in the WJ, the flow is proportional to the flow rate to the three fourths power. Equation 9 presents the general concept:

$$i_{peak} = c \cdot V^{x} \qquad (9)$$

and the exponent x is determined by taking the log of both sides:

$$ln\,(i_{peak}) = ln(c) + x \cdot ln(V) \qquad (10)$$

Plotting the log of the current versus the log of the flow will provide the power of the volume flow rate from the slope of the line.

The same data from the experiment examining flow rate and retention time was used (Figures 30 and 31). Peak currents were determined from the differential current peak with baseline background using the **HSWAVE** program. The baseline was determined by fitting a line between the base of the peak and interpolating to obtain the current at the time of the maximum peak. The flow rates ranged from 0.70 to 2.95 ml/min. The log of current was plotted against the log of the peak current, as shown in Figure 34. These data points were fit using linear least squares and a slope of 0.695 was determined. This value is close to the $V^{3/4}$ flow property in equation 2 which demonstrates that this electrode has wall jet character. This also demonstrates that flow is related to current, and for reproducible results, the flow rate of the system should be controlled.

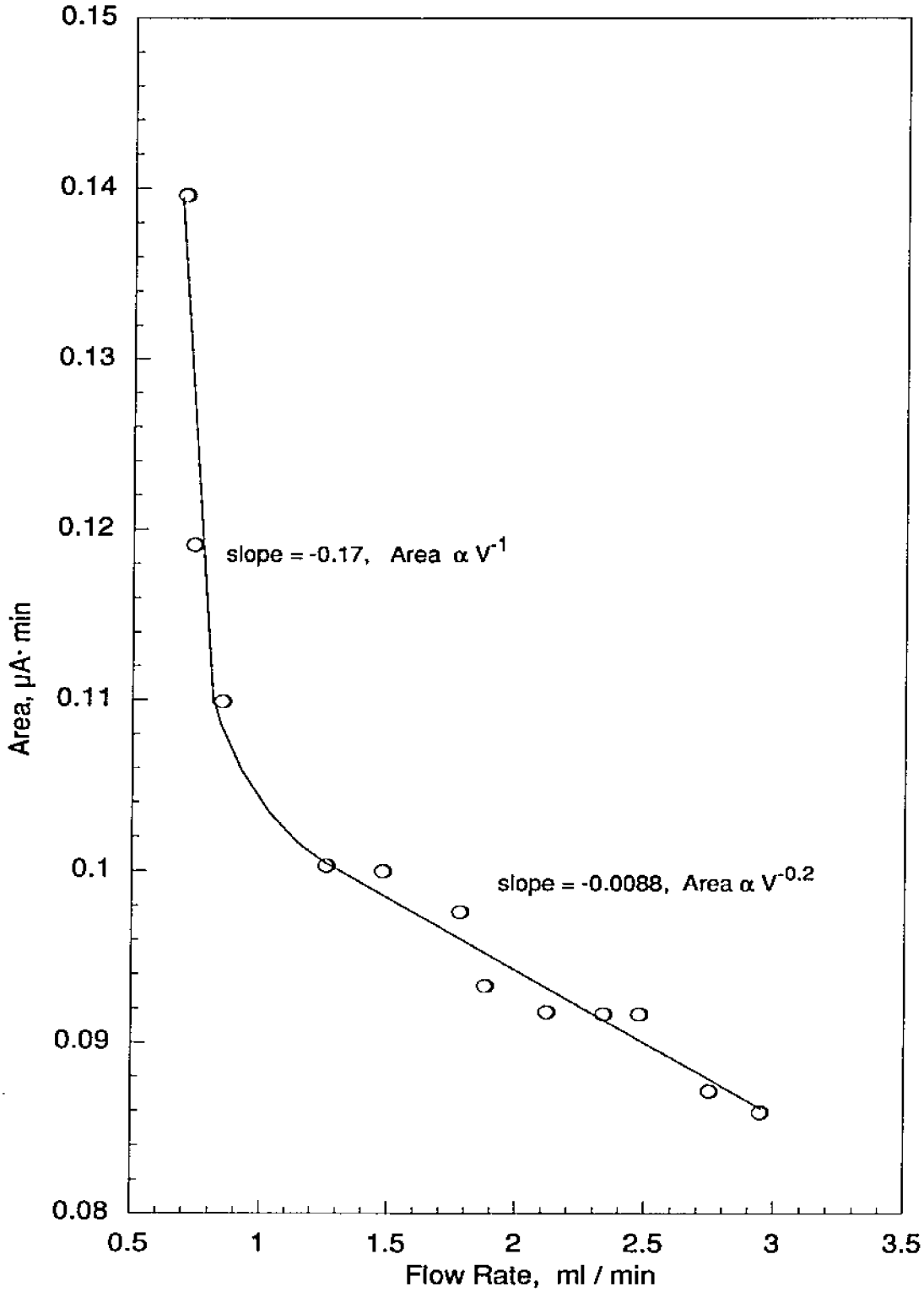

Figure 33 Differential Current Area vs. Flow Rate for $1 \cdot 10^{-5}$ M Niacin

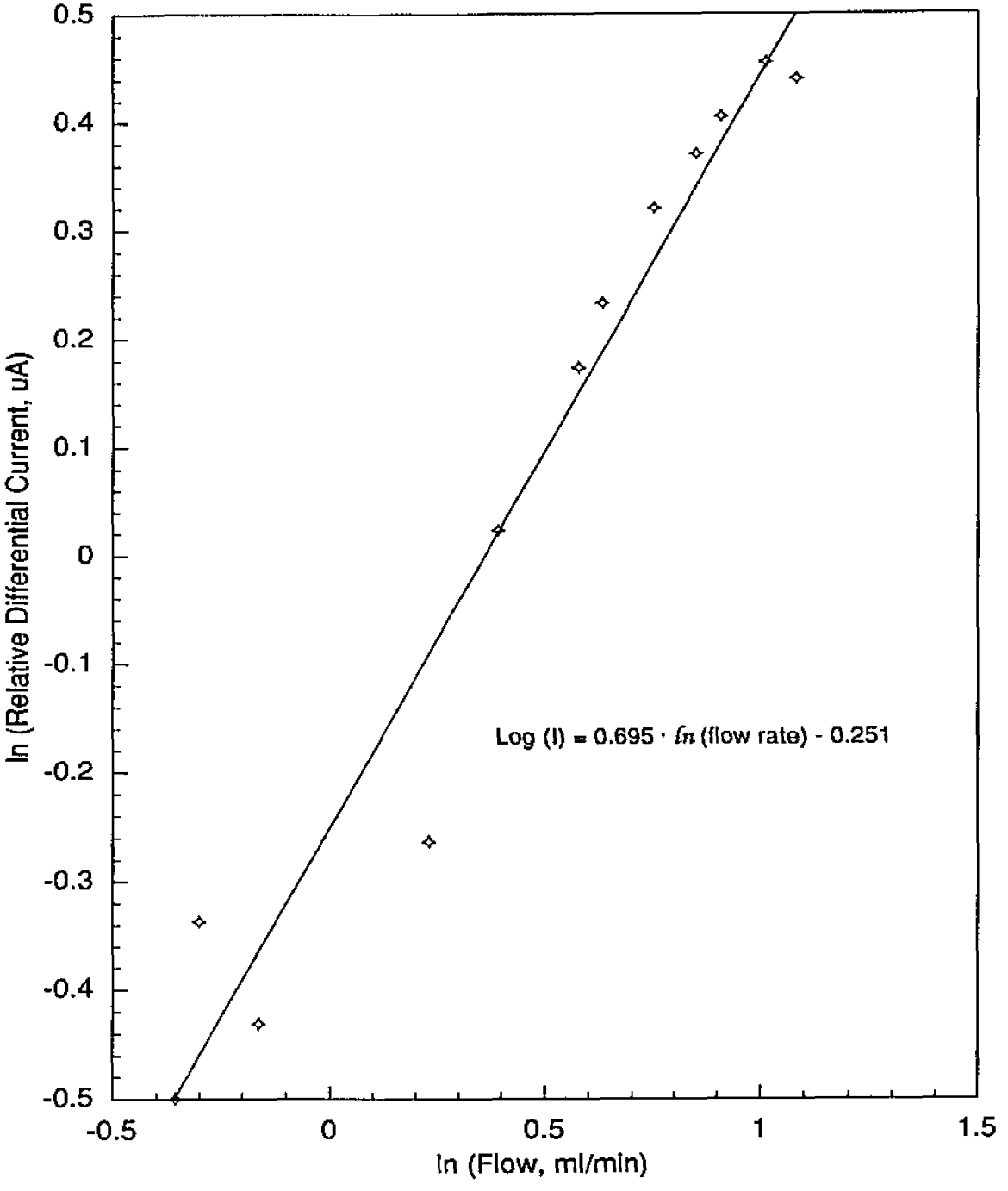

Figure 34 Niacin Flow Study: *ln* (flow) vs. *ln* (current)

Optimization of Parameters

Effect of Varying Pulse Height

The effect of varying the height of the V_2 pulse in differential dual pulse HPLC detection on a SMDE was investigated. Vitamin B_{12} at a concentration of 10^{-5} M was injected into the HPLC column. The voltage of the first potential, V_1, was set at -1400 mV. The second potential was set at a duration of 50 ms for each injection to range from -1500 to -1670 mV. The differential current was determined at the peak and the interpolated baseline was subtracted to result in the relative differential current. The result is shown in Figure 35. The current increases linearly at a rapid rate until the pulse is -1640 mV, after which the slope of the curve becomes very small. The reason for the leveling off becomes apparent if the individual background and peak currents are examined, in Figure 36. The slope of the background differential current is continually increasing as the pulse becomes larger while the slope of the pulse curve gets smaller. The theory predicts that Figure 35 should be in the shape of a sigmoid.[29] Other studies in our laboratory with more points both at smaller and larger pulse heights correlates with theory.

Effect of Varying Pulse Starting Point

This experiment studied the effect of varying the starting point for the pulse with the dual pulse waveform. The first potential was varied between -1500 mV and -1600 mV, while the second applied potential was always kept at a value of the first potential plus -100 mV. A mixture of vitamins B_{12} and riboflavin was injected into the column for this study. Both vitamins show a peak when the value of the first potential is -1550 mV and the pulse is -1650 mV, with a gain of current in this example of 0.2 μA (see Figure 37).

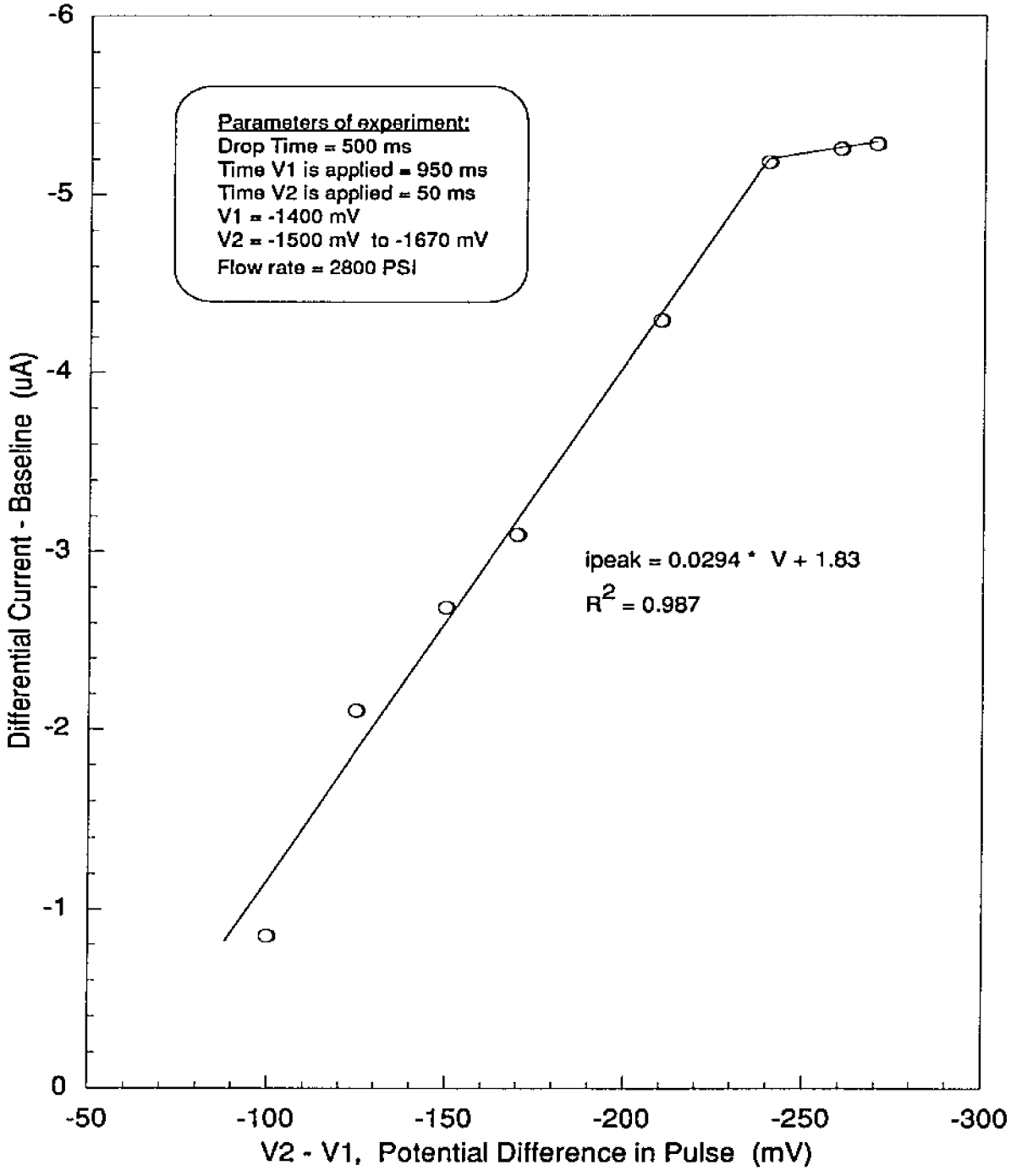

Figure 35 Dependence of Relative Differential HPLC Current on Pulse Height for Vitamin B_{12}

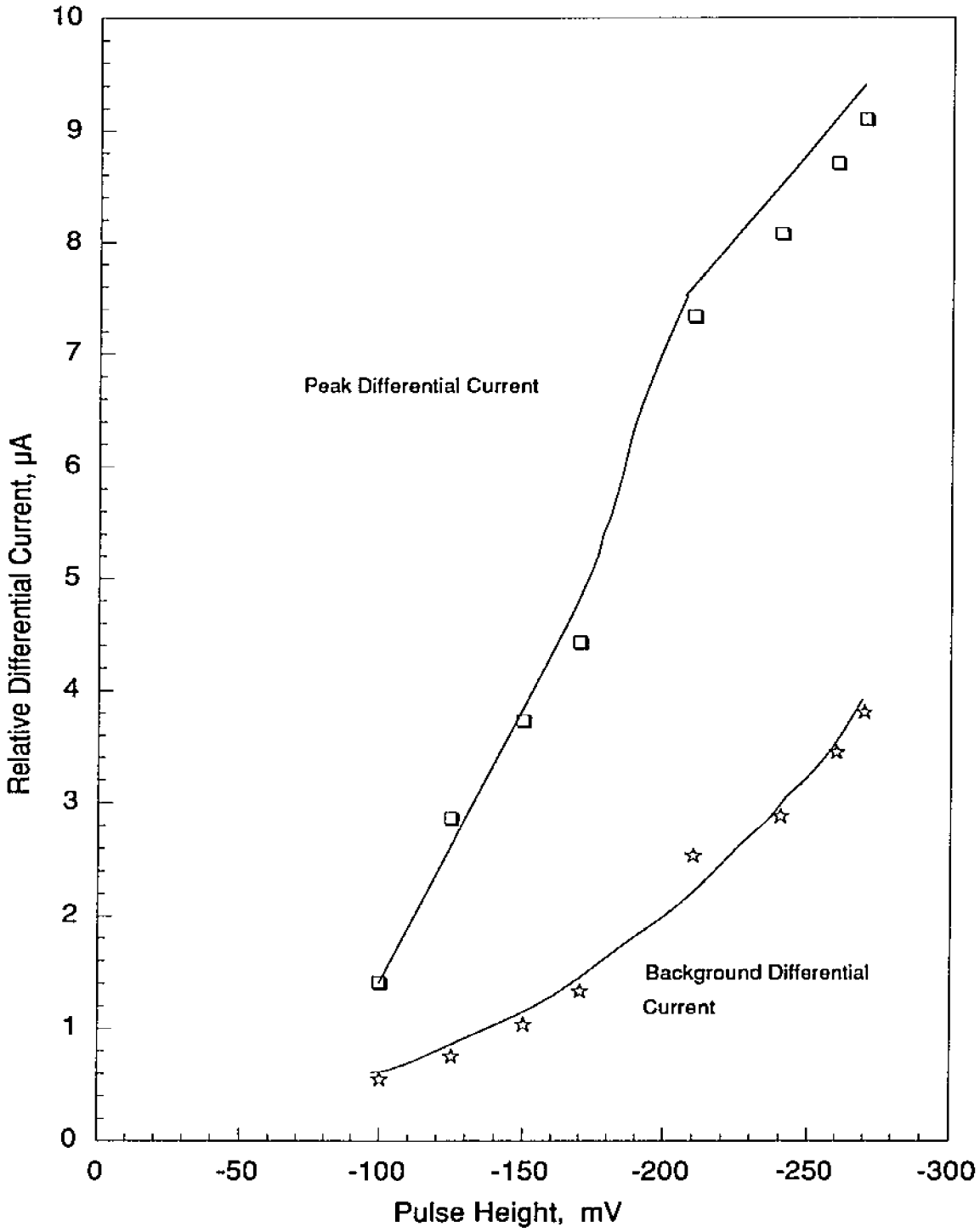

Figure 36 Peak and Background Differential Current Dependence upon Pulse Height

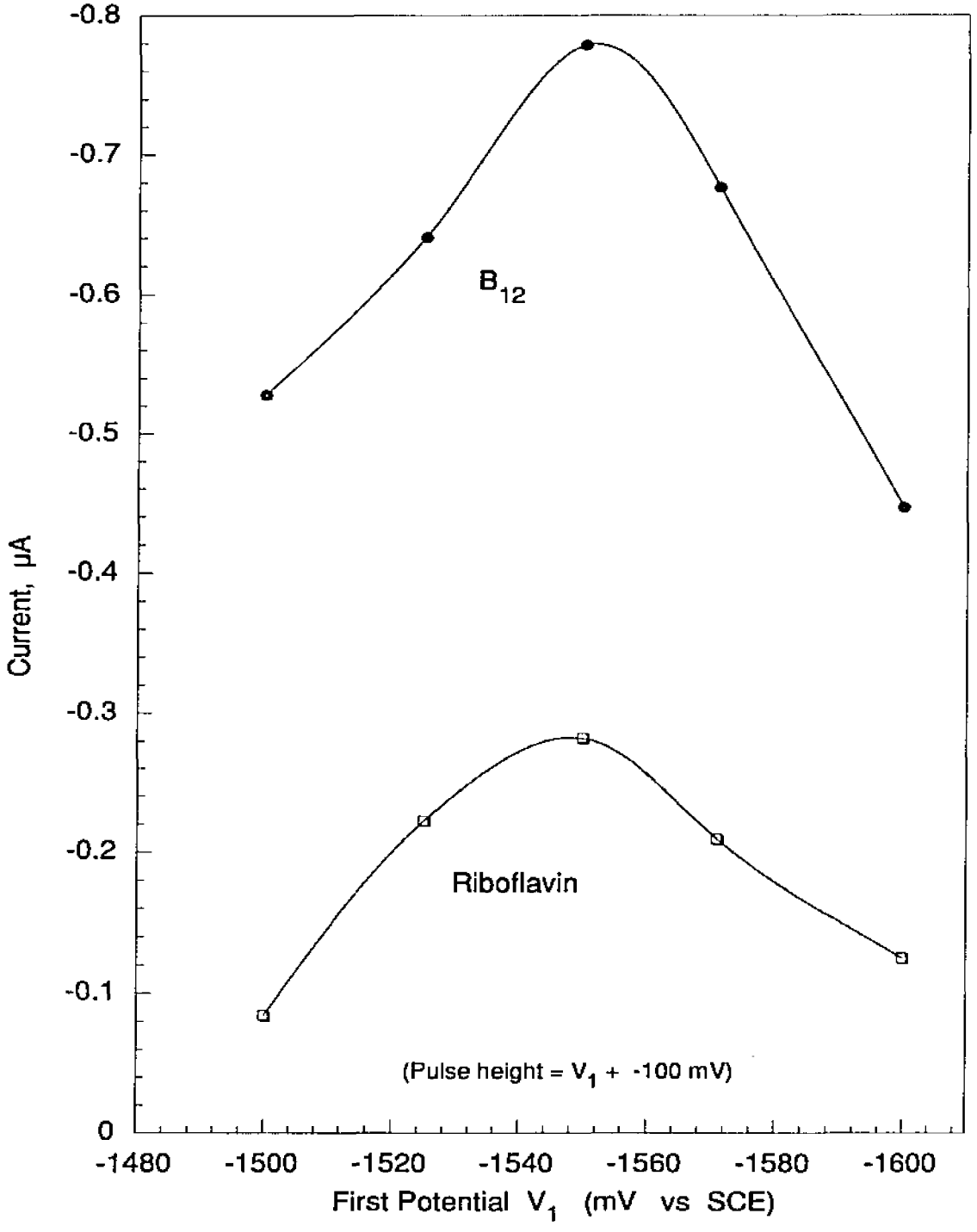

Figure 37 Effect of Dual Pulse Starting Potential on Peak Current at Constant Pulse Height

Effect of Varying Pulse Time

The effect of changing the pulse time on the HPLC dual pulse detection of vitamin B_{12} was examined. The first potential value, V_1, was set to -1400 mV and the pulse, the second potential value V_2, was set to -1660 mV, with the V_2 pulse time ranging from 15 ms to 700 ms. The drop formation time was set to 300 ms, with a constant total cycle time (drop formation time plus V_1 time plus V_2 time) equal to a constant 1 second.

The current at short values of V_2 time, from 15 ms to 75 ms, shows a reciprocal square root dependence on pulse time, as shown in Figure 38. In this region, current is Faradaic diffusion controlled and is very sensitive to pulse timing. This is to be expected from the response of a first order catalytic process to a two step potential waveform.[29] Changing the pulse time yields no effect on the current at 75 ms and longer, up to 700 ms, because at that point the current is controlled by the solution hydrodynamic control. This may be seen in the inset diagram in Figure 38.

Mixtures

Four B vitamins (B_{12}, riboflavin, niacin, and thiamine) were injected individually and as a mixture into the HPLC column operating at a flow rate of 1.78 ml / min. The detection waveform was differential dual pulse, set at -1571 mV for 950 ms (including drop growth time) and -1671 mV for 50 ms. These potentials are at a point where the differential techniques discriminates against oxygen and is on the catalytic wave of the vitamins. The concentrations were each $1 \cdot 10^{-5}$ M, except riboflavin, which was $4 \cdot 10^{-5}$ M. The chromatographs of the individual injections are depicted in Figure 39. B_{12} eluted first, at 1.65 minutes. Riboflavin followed at 1.88 min;

then niacin appeared at 2.07 min and the thiamine came last at 2.13 min. A mixture of these vitamins, at a quarter of the concentration of the individual injections, was injected and the chromatograph is depicted in Figure 40. The B_{12} and riboflavin are well separated with minimal overlap, but the niacin and thiamine overlapped to form one peak.

A mixture of two of the vitamins were then analyzed, B_{12} and riboflavin. As depicted in Figure 41, the voltammographic waves overlap each other, with riboflavin being very broad compared to B_{12}. The mixture was injected into the column and detection was set for square wave. There are several representations of the results. Figure 42 gives a three-dimensional overview of how the current voltage curves evolve over time. Viewing all of the data at the same time gives an overall view of the experimental result, but adds complexity to the image. Using the **HSWAVE** program, slices may be taken from the curve and chromatograms displayed in Figure 43, which maximize either the B_{12} or the riboflavin peak. Figure 44 shows the distinct individual current voltage curves of B_{12} and riboflavin extracted from the data in Figure 42. The first few current voltage curves in that figure show the background. Then, the curves grow and decay due to the catalytic current of B_{12} as it passes the electrode. After the B_{12} peak subsides, the remaining curves show the riboflavin catalytic waves as it elutes.

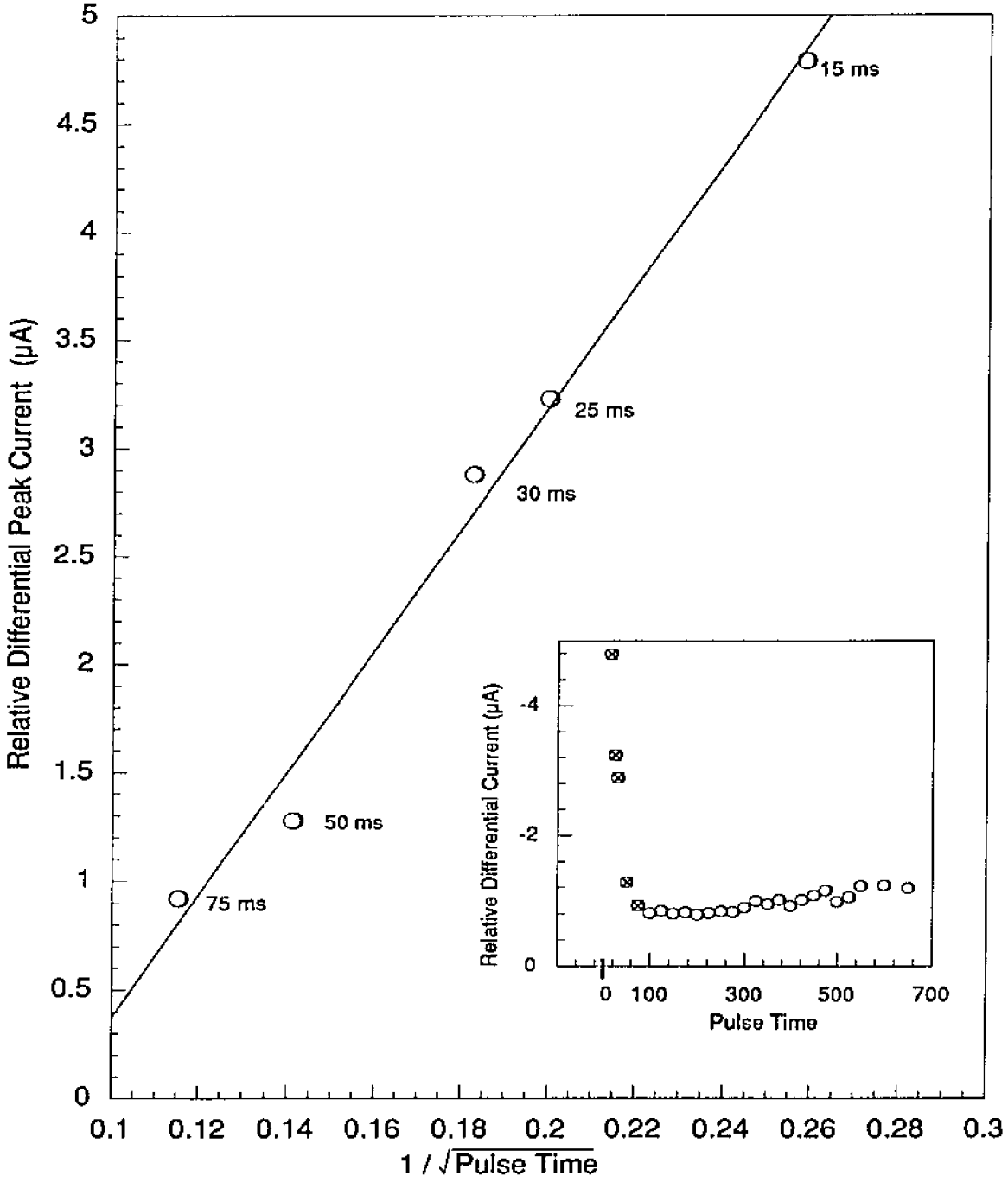

Figure 38 Effect of Pulse Time on Vitamin B_{12} Current

HPLC with dual pulse detection of vitamin B_{12} current plotted vs reciprocal square root of pulse time to demonstrate diffusion controlled region at short values of pulse time. Inset plot shows that longer values of pulse time has constant current dependence.

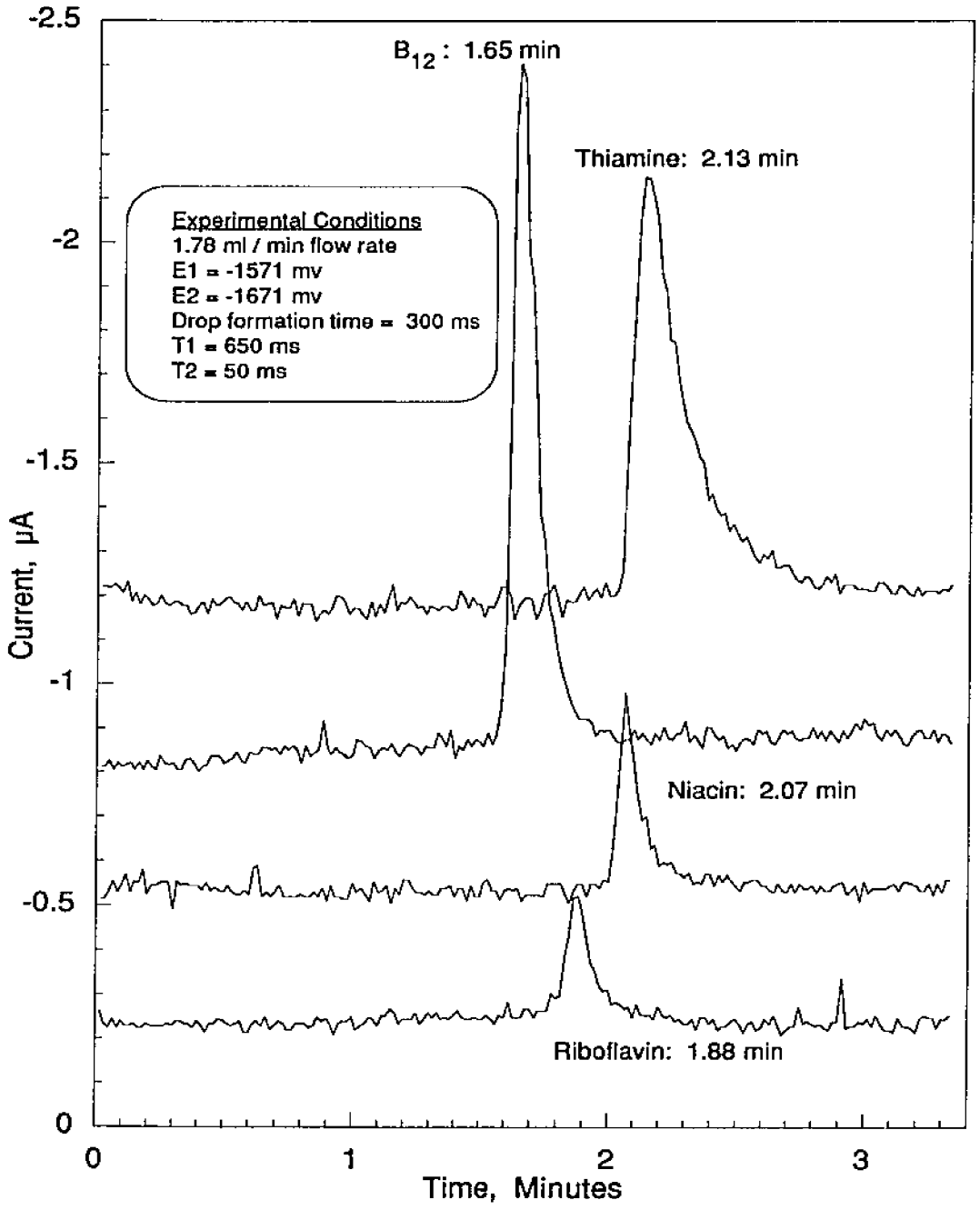

Figure 39 Dual Pulse HPLC of Seperate Samples of B_{12}, Riboflavin, Niacin and Thiamine.

All concentrations are $1 \cdot 10^{-5}$ M , except riboflavin which is $4 \cdot 10^{-5}$M. Backgrounds are offset in this graph for better visibility.

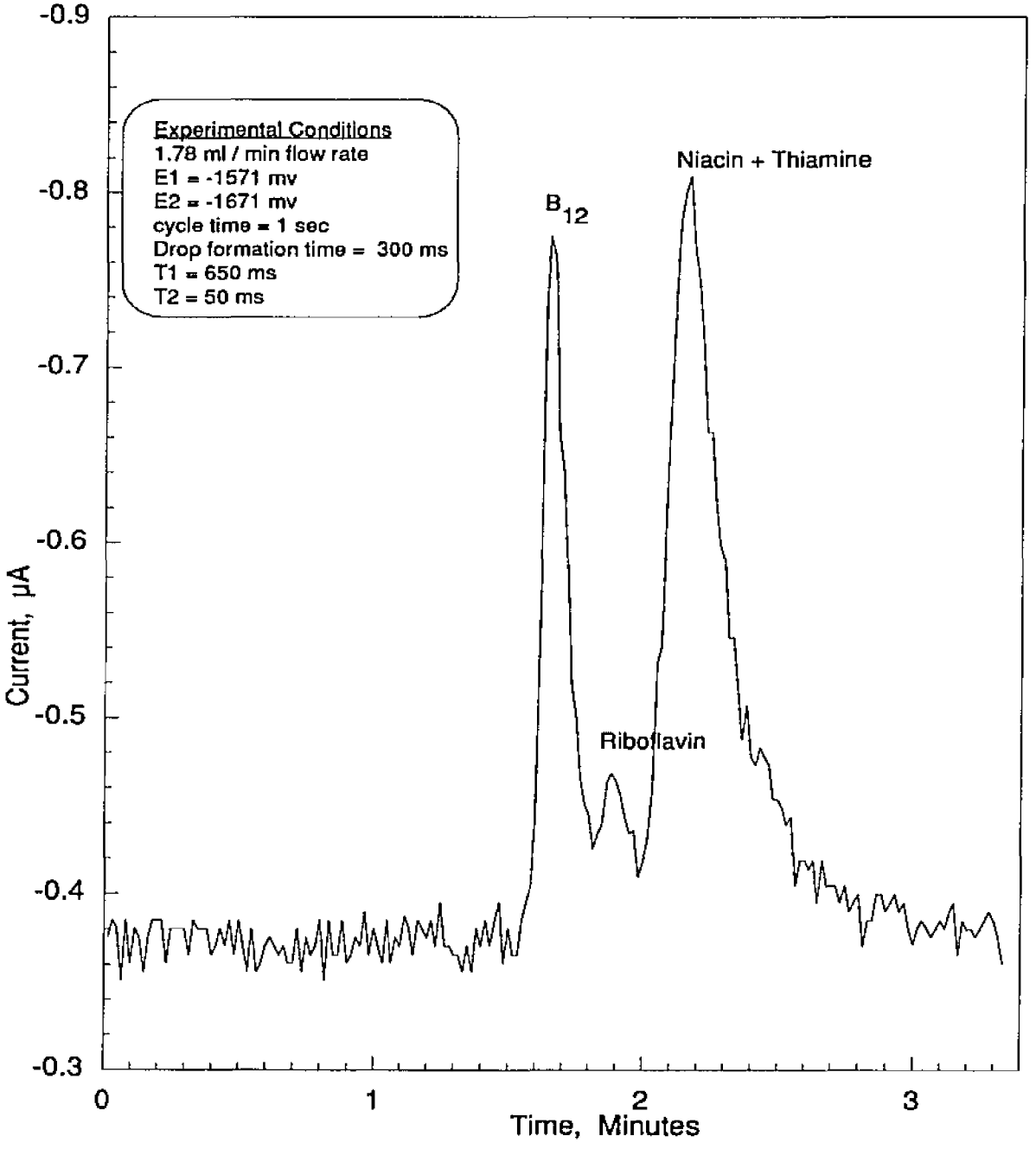

Figure 40 Dual Pulse HPLC of a Mixture of B_{12}, Riboflavin, Niacin and Thiamine.

All are $2.5 \cdot 10^{-6}$ M except riboflavin which is $1 \cdot 10^{-5}$ M.

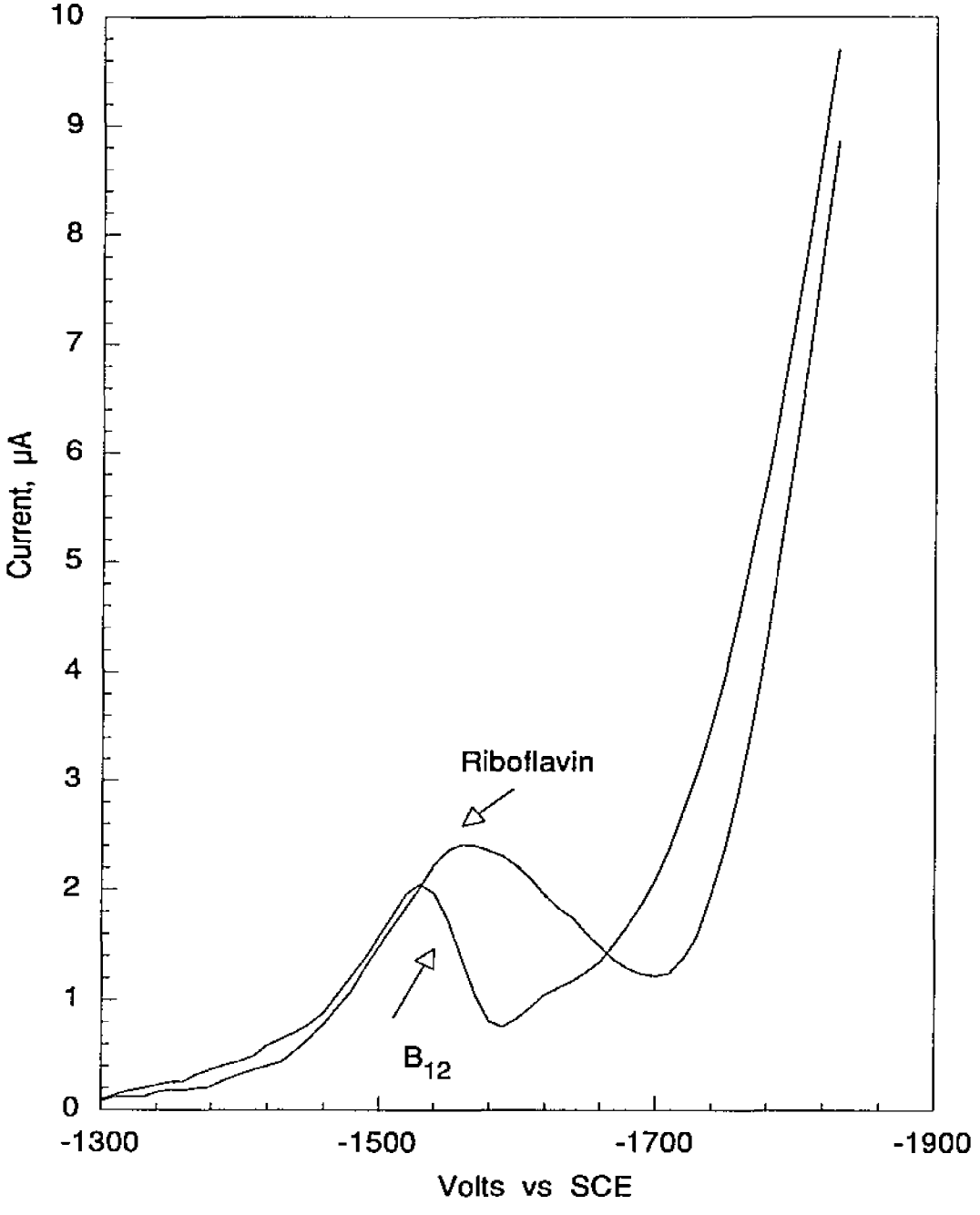

Figure 41 Square Wave Current Voltage Curves for the Catalytic Process of B_{12} and Riboflavin

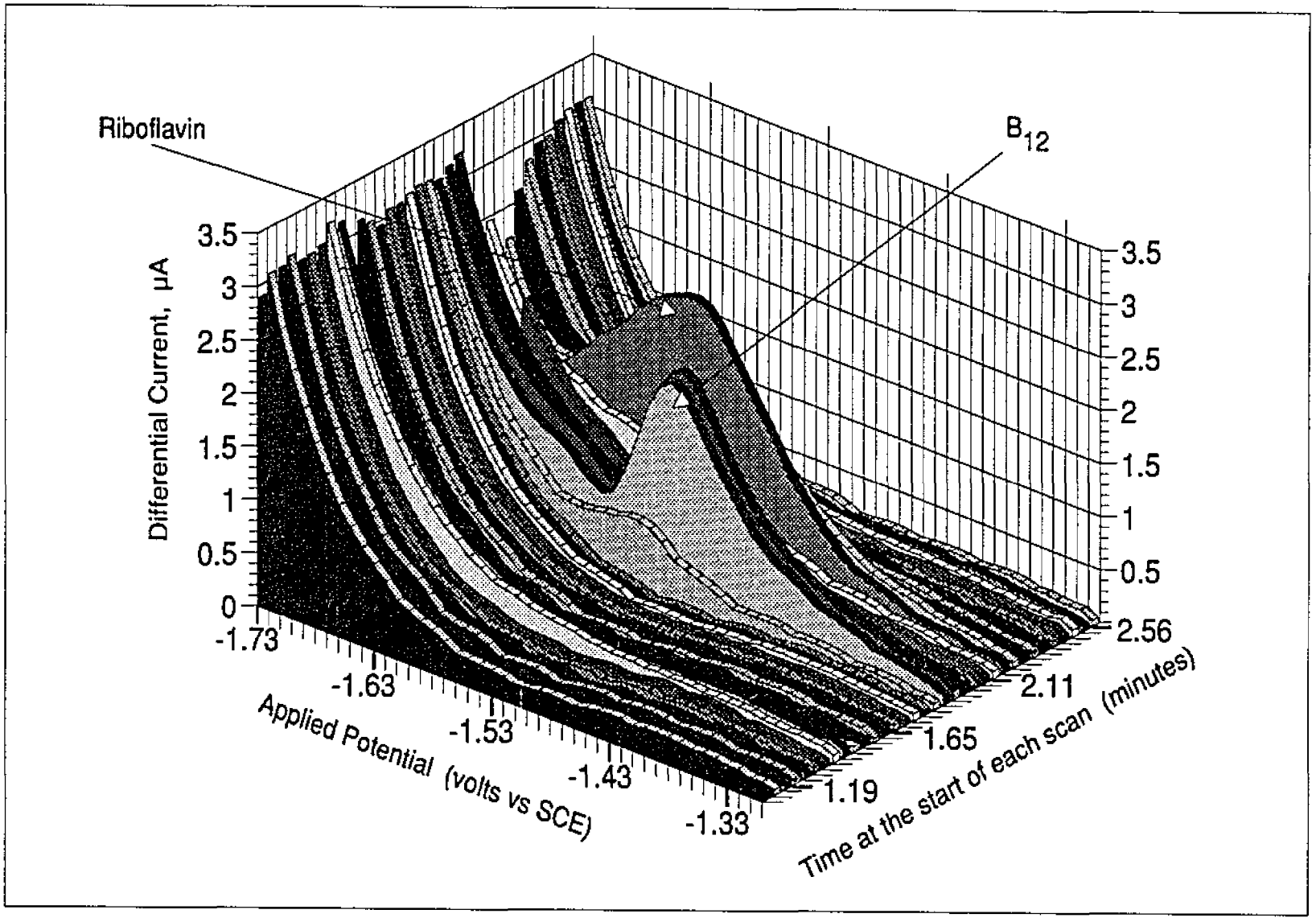

Figure 42 Current-Potential-Time Plot of Square Wave HPLC of a Mixture of B_{12} and Riboflavin

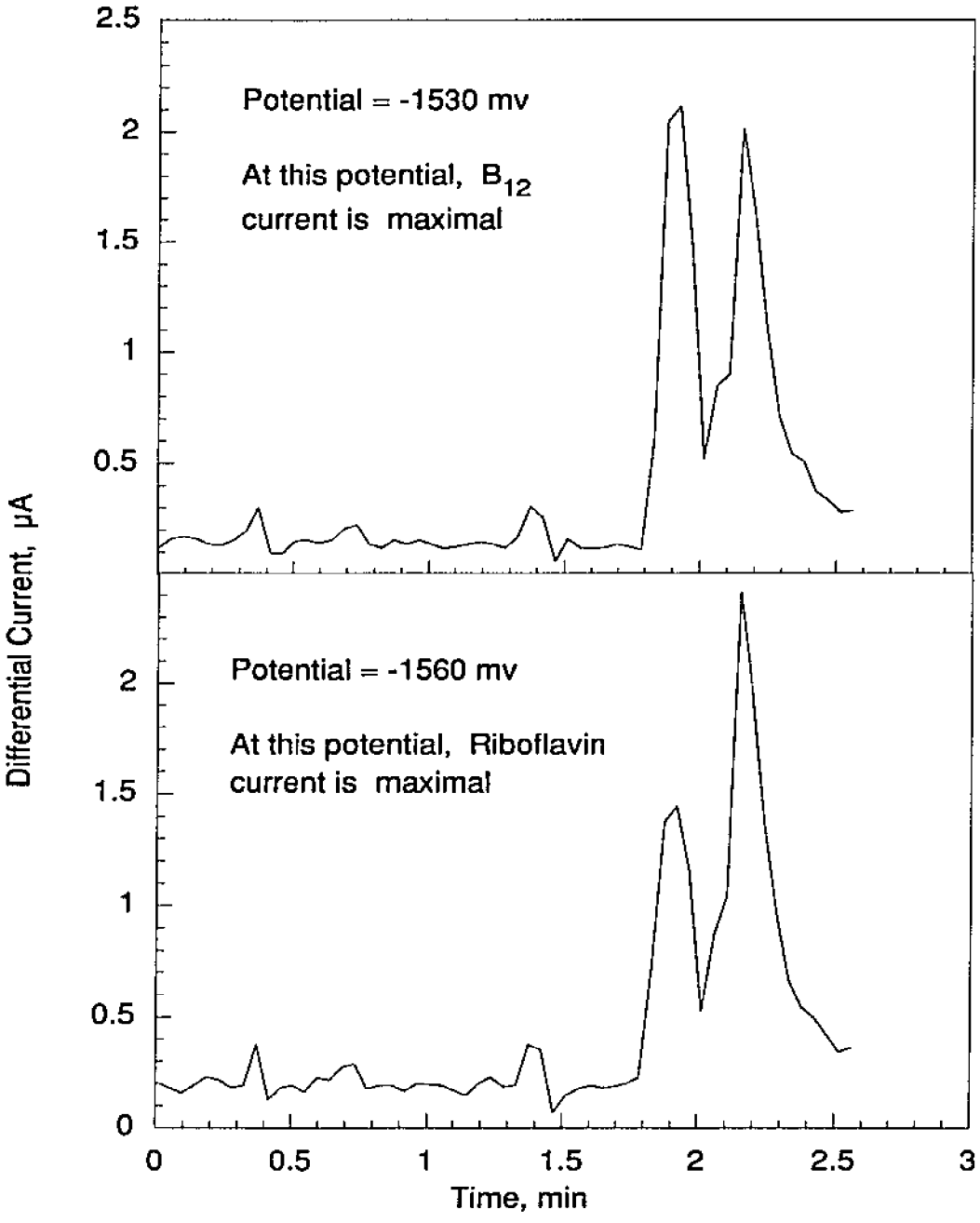

Figure 43 Current-Time Plot Slices at Constant Potential of Square Wave HPLC of Riboflavin and B_{12}

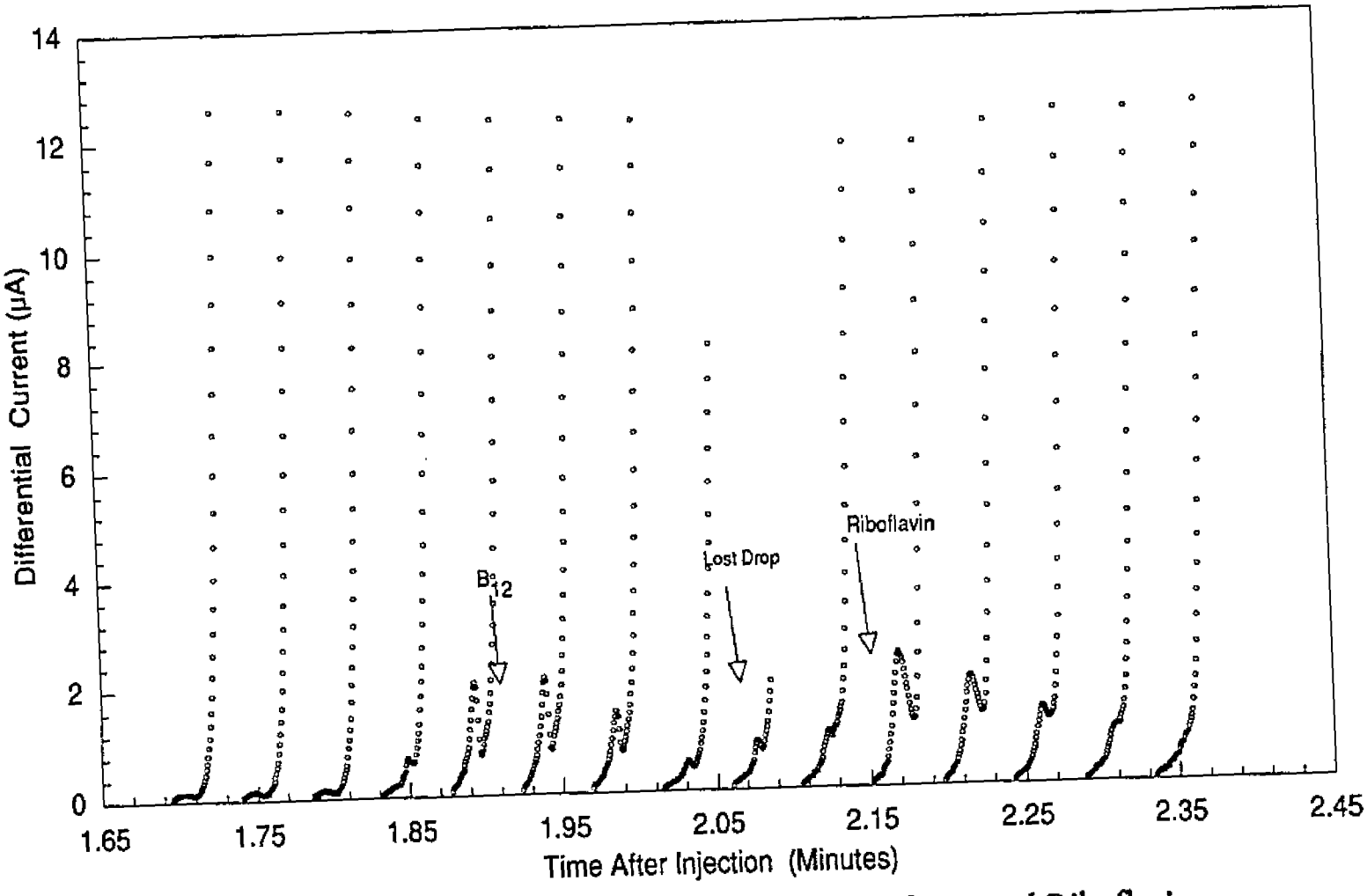

Figure 44 HPLC Square Wave Current-Voltage Curves of B_{12} and Riboflavin.

Each scan is on different mercury drops. Truncated curves are due to lose of the mercury drop before the scan completed.

Concentration Calibration Curves

The detection limit for vitamins B_{12} and niacin was determined by injecting varying concentrations at a constant flow rate. The calibration curves obtained are plotted in Figures 45 and 46. Both curves show linearity for lower concentration, and higher concentration show current drop off from linearity, but still dependent upon concentration. The B_{12} has the larger lower linear dynamic range of three orders of magnitude, while the niacin was experimentally detectable to two orders of magnitude. Niacin is linear to higher concentrations, with $3 \cdot 10^{-5}$ M being linear and $5 \cdot 10^{-5}$ M not far off the calibration curve. B_{12} is linear up to $1.25 \cdot 10^{-5}$ M, with large deviations from linearity at higher concentrations. At very high concentrations ($>10^{-4}$ M), the differential pulse current becomes independent of concentration as would be expected for an electrochemical process which involves adsorbed electroactive species.[17]

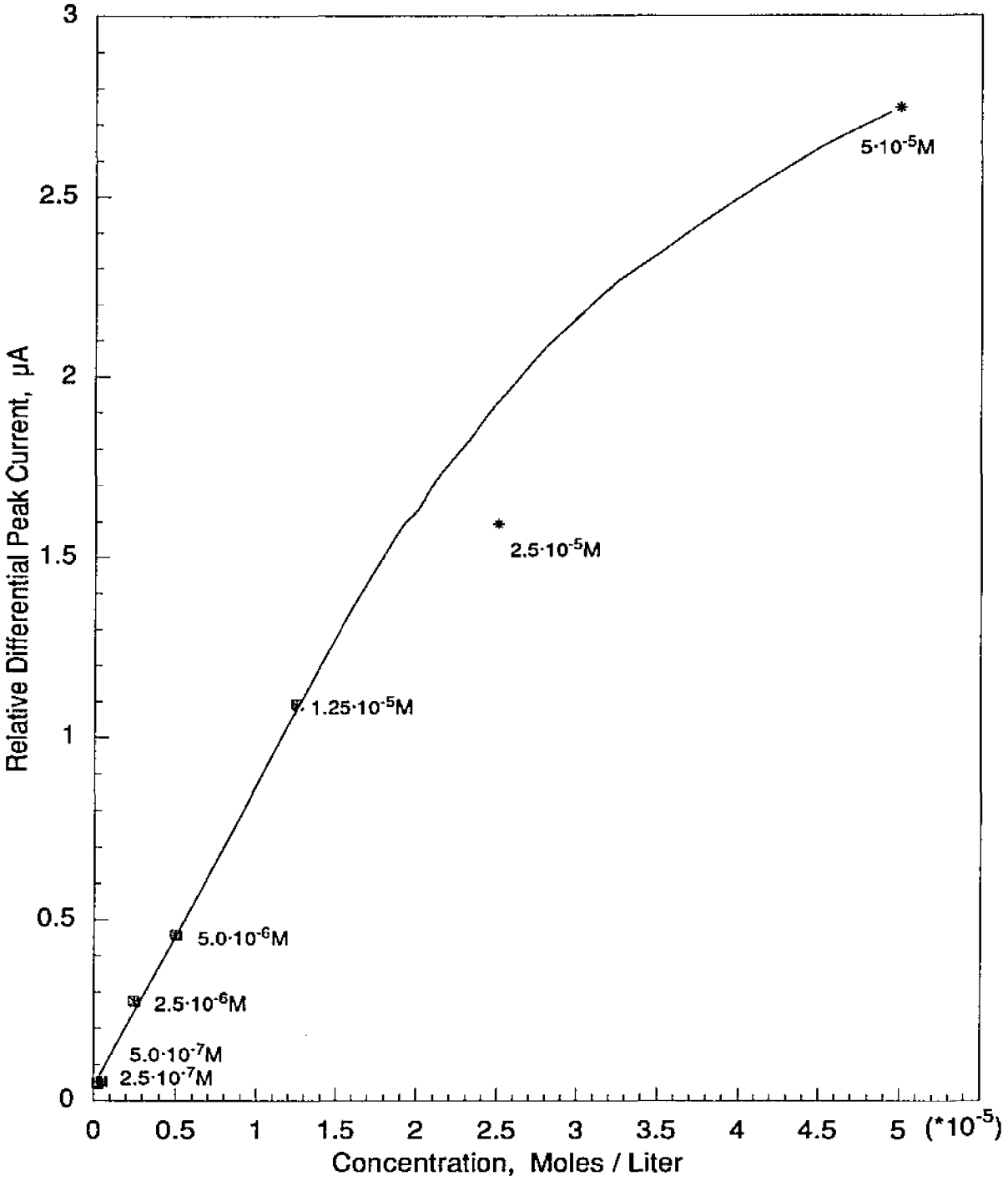

Figure 45 Concentration Calibration Curve for Vitamin B_{12} Using Dual Pulse HPLC with Static Mercury Electrode Detectionn

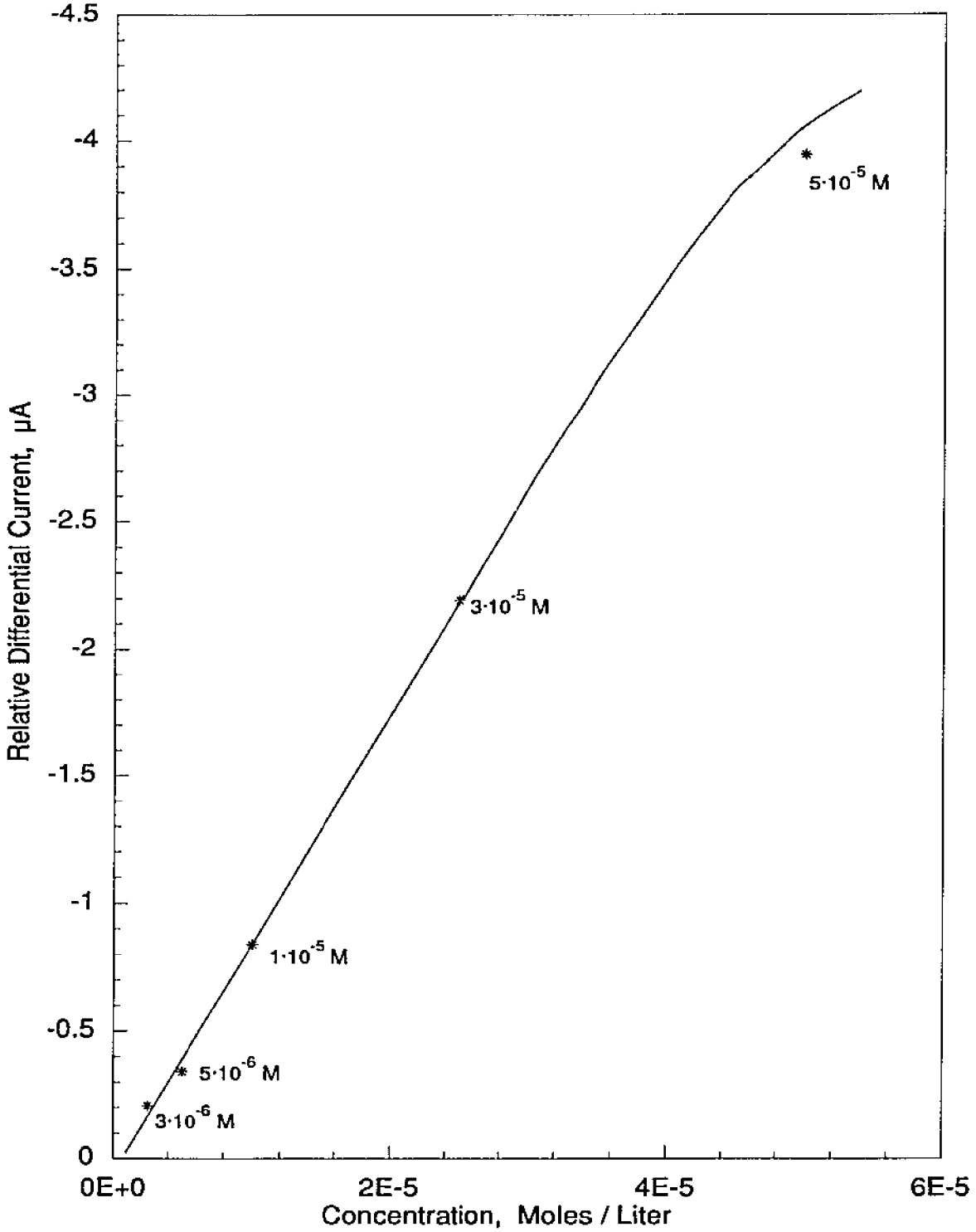

Figure 46 Concentration Calibration Curve for Niacin Using Dual Pulse HPLC with Static Mercury Electrode Detection

Conclusion

The computer based voltammetry instrument developed in this thesis has been demonstrated for just a small set of its capabilities, mainly in its function as a detector for HPLC using the catalytic waves of B vitamins. In this set of experiments, oxygen in the sample did not require deaeration, as the oxygen eluted later. However, it was shown that by using the differential technique, oxygen could easily be discriminated against, at the potentials utilized. The flow electrode has wall jet characteristics as described by the dependence of current on flow rate to the 0.7 power. Pulse time shorter than 100 ms give large increases in current. Finally it is possible to examine the overlapping catalytic current-voltage curves of B vitamins using a HPLC as a separation device.

Appendix A Automatic Titrator
(for section 1)

1.0A The Automatic Titration Instrument Hardware

1.1A Circuits & Details of Buret & pH Meter Modifications

The instrument was built with a computer as the main control. In order to interface the computer to control and acquire data from the titration, custom designed circuits were added to the motorized buret and the pH meter. This hardware is described in detail in the following sections. Information concerning the integrated circuits may be found in references (26) and (27).

1.1.1A Control of Reagent Delivery Relay Circuit Details

There is a "remote" selection on the front panel function switch on the Thomas Scientific Model 258 motorized buret which allows the forward buret movement (reagent delivery) to be controlled by an external switch. Since the voltage to control the motor is not compatible with the output control signal from the computer, a relay circuit was mounted internally in the buret chassis and attached to the external switch contact. This allows the TTL level signal from the computer to turn the buret motor on and off.

The circuit diagram for the relay circuit is in Figure 1A and parts locations are in Figure 2A. The circuit has been designed to prevent superfluous operation of the buret when it is generally not desired; when the computer is off, when the computer is first turned on, and when the connector is not attached, either purposefully or accidentally. The relay and buret is activated when the input to the circuit is at TTL low or near zero volts. The circuit and relay may be simply tested by connecting the input banana jack to ground, which will activate the relay.

The power to control the relay is from a wall transformer, described in Appendix 1.1.3A. Looking at Figure 1A, the voltage is dropped to an acceptable level by three diodes in series, D1,D2, D3. It is filtered by capacitor C1. The signal is inverted and amplified by transistor Q1 and then amplified and inverted again by Q2. Since Q2 carries the most current, it is heat sinked to prevent possible overheating. Resistor R1 keeps the input high and thus the relay off when computer power is off or the leads to the computer are disconnected so that the buret will not move in these cases. The output of the computer is normally high when it is turned on as well, so this design takes superfluous movements of the buret into account. Diode D4 protects the circuitry from inductive spikes when the relay is turned on and off. Capacitor C2 and diode D5 prevents spikes form the motor. The buret motor is kept at a separate ground from the circuit to minimize noise and current loops.

1.1.2A Measurement of Buret Volume Tachometer circuit details

The method used to measure the quantity of aliquot delivered was to add a chopper to the DC motor and a homemade tachometer. This method is the least expensive of several possible methods. There is no contact of the flowmeter with the solution and no possibility of contamination. The accuracy of this method depends on the precision of the bore of the glass tube of the buret and the number of vanes on the chopper. The tachometer was installed inside the case of the motorized buret. Since the power supply for the motorized buret was found to be noisy, an external power supply was used (see Figure 3A).

The tachometer is relatively simple, consisting of a Light Emitting Diode (LED), a photo transistor, an integrated circuit and a few resistors, capacitors and diodes. The circuit diagram is in Figure 4A and the parts location is in Figure 5A. The actual counting and timing of the signal is

performed by the computer. The LED is a high intensity red type with a small angle of light dispersion. The photo transistor is NPN. They are mounted inside the instrument in sockets on a PC board, leads bend such that the active sites of each device face each other and they are separated by about 5 mm. An opaque tab is mounted on the motor's shaft (with epoxy) and the PC board holding the tachometer electronics is aligned in such a manner that the tab will interrupt the light beam four times each rotation, but would not contact the tabs. The photo transistor has electrical leads for both its emitter and collector. The base is the light sensitive surface. When illuminated, a positive potential is developed (referenced to the emitter) which turns the transistor on. The photo transistor is configured as a switch: when the light is blocked, the transistor is off and the voltage out is the voltage through the collector resistor, 4.9V. When the beam is not interrupted, the light from the LED causes saturation and the transistor conducts, effectively bringing the collector near ground : ca. 0.17 volts. These two voltages are within the levels required for a TTL signal (0.8V maximum for low; 2 volts minimum for high). The alignment of the two devices are important; if the alignment is off, the low signal (0.17V) may rise to unacceptable levels. There is an easily accessible test point should alignment be required. In future designs, an optical switch or optical interrupter module should be incorporated to eliminate problems with alignment.

The phototransistor collector is connected to a 74LS14 hex Schmitt trigger inverter. A Schmitt trigger has hysteresis so that a slowly changing or noisy signal will not cause superfluous outputs. The rise and fall time of the signal from the phototransistor was found to be ~120 μS. This is relatively slow for a TTL signal, therefore this circuit was used to eliminate false triggering. The integrated circuit has six Schmitt triggers which were used to condition the signal. The first inverter (input pin 11) cleans the signal (for sharp rise time and elimination of most noise). The output of the

first trigger is capacitively coupled to the next inverter (pin 9). This second inverter is configured as a one shot to provide a pulse of constant width. The one shot's duration is controlled by the coupling capacitor and the discharge resistor. A fast response diode (1N914 type) is used to clamp the input for negative going transitions, which might otherwise eventually damage the integrated circuit. The output is then buffered by the third inverter (pin 5). The output of the 3rd inverter is connected to the inputs of the two last inverters. One inverter (pin 3 input) is used as the output driver. When there is a signal, the output goes from normally high to low. The other inverter (pin 1) goes to an LED mounted in the front of the buret to serve as an indicator and diagnostic for the operation of the tachometer. When the motor is in operation, the LED will blink whenever the tachometer light beam is interrupted by the chopper.

1.1.3A Circuit for Powering Buret Modifications

The power supply for the buret modifications could not be derived from the low voltage DC power in the buret, since the motor generated spikes large enough to disrupt the circuits previously described, even when suppressing diodes and bypass capacitors are installed. The power supply (Figure 3A) comes from a simple wall transformer which has an internal diode bridge and capacitor to convert the 120 VAC to (9 ~ 12 volts) DC. The output goes to a phono jack connection on the buret. Inside the buret, there is a circuit board which was added with the voltage regulator circuit. The regulator circuit consists of a protective diode (to prevent damage if a wall transformer of the wrong polarity is attached), then to 1000 μF Capacitor for additional filtering, and then to a LM7805 5 volt regulator integrated circuit, which maintains the voltage at 5 volts ± 0.1%. The output is then filtered by various bypass and storage capacitors in the relay and tachometer circuitry for noise suppression.

1.1.4A pH Meter Amplifier

The pH electrode is connected to a Corning model 125 pH meter to provide buffering of the high impedance signal and provide a continuous display of the pH. The output of the meter is connected to the computer via an analog to digital converter (A/D) in the computer. The output of the pH meter was meant for an older strip chart recorder which operates at full scale ±5 mV. The A/D input range is ±5.115 Volts. Therefore, an amplifier was added inside the pH meter to multiply the output by 1000 to match the range of the A/D. The ±5 mV signal output at external banana jacks were disconnected and the amplified signal was connected to these terminals. The circuit is extremely simple, with an LF13741 operational JFET amplifier and a 100Ω input resistor, a 100kΩ feedback resistor to provide amplification of 1000 times, and a capacitor across the power supply which comes form the pH meter. This circuit is mounted inside the chassis of the pH meter.

1.2A Computer Hardware Requirements

The computer peripheral hardware required for this experiment consists of the items in Table 1.1A. The table has a column, labeled "minimal functional requirements" for each item. This should be used as a reference if another titration instrument will be built using a computer other than a MINC-11 or the LSI-11 series and if a minimum of reprogramming is desired. Note that the type of board does not always directly pertain to the function that is used in this instrument. For example, the clock board is not used as a clock, but as a counter, and any counter with the properties listed in the table may be substituted in a new design of this instrument. The serial lines are used to drive terminal, printer and plotter, but other computers such as the Macintosh use a monitor instead of a terminal, and the IBM PC usually uses a parallel port instead of a serial line to drive a printer. The software aspect of this subject may be found in the Appendix 2.4A. It is not necessary to use the same graphics terminal, as most modern computers and Basic languages have their own graphics specifications and drivers.

DEC #	Board Type	Minimum Functional Requirements
ADV11	12 bit A/D	12 bits, ± 5 volt input range, high input impedance, < 40μS conversion time, only one channel is needed
KWV11	Real Time Clock	16 bit counter with Schmitt trigger input. Computer can read counter at any time without altering count. Computer can initialize the counter. Minimum 1kHz count rate input.
DRV11	16 bit output port	Only one bit is used. Output should be TTL levels, with no handshaking required.
DLV11J	Serial Ports	This is used for the terminal, plotter and printer.
VT105	Graphics Output	Graphics Display, VT105 compatible desirable

Table 1.1A Computer hardware requirements for the TIGHTC automatic titrator.

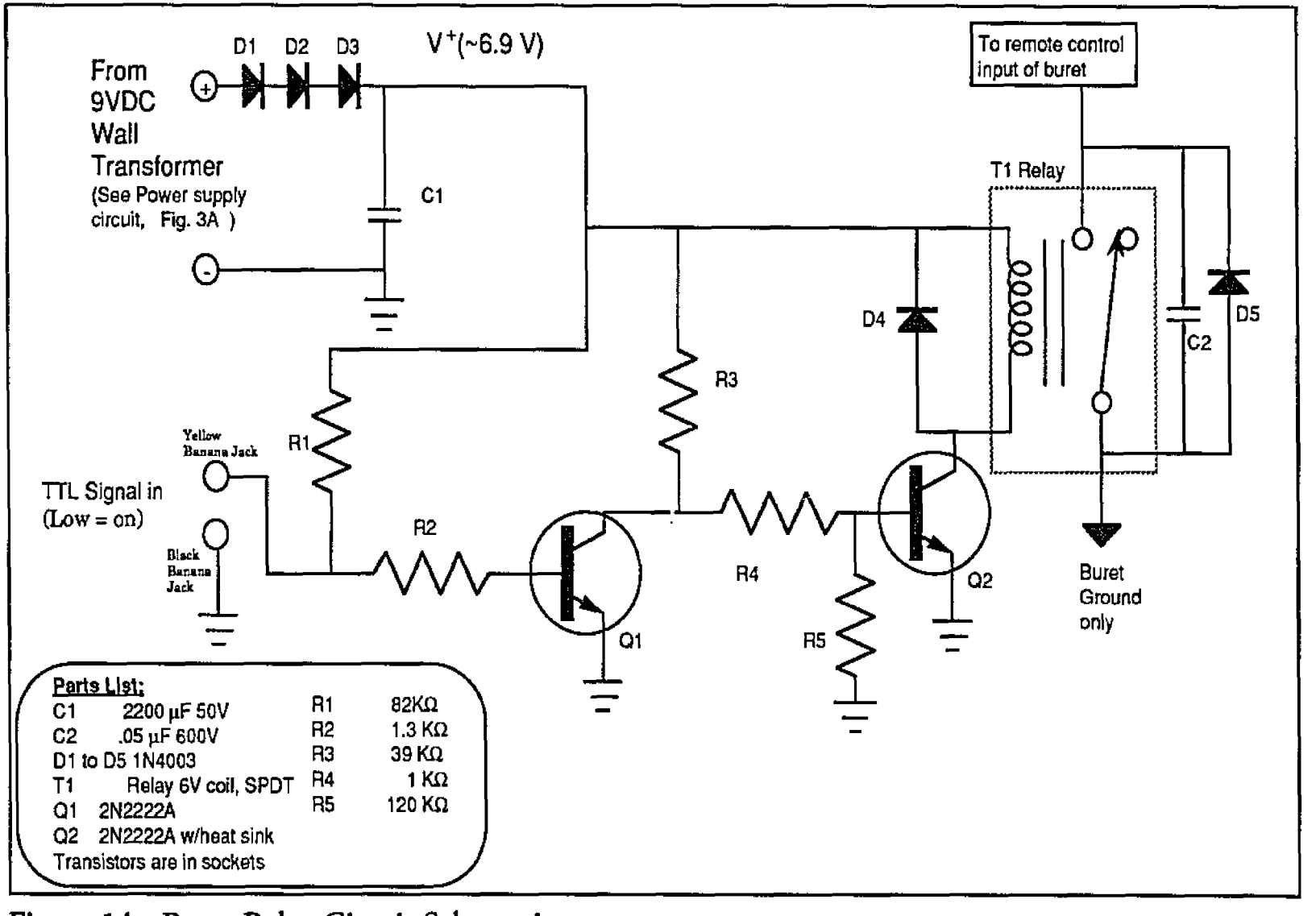

Figure 1A Buret Relay Circuit Schematics

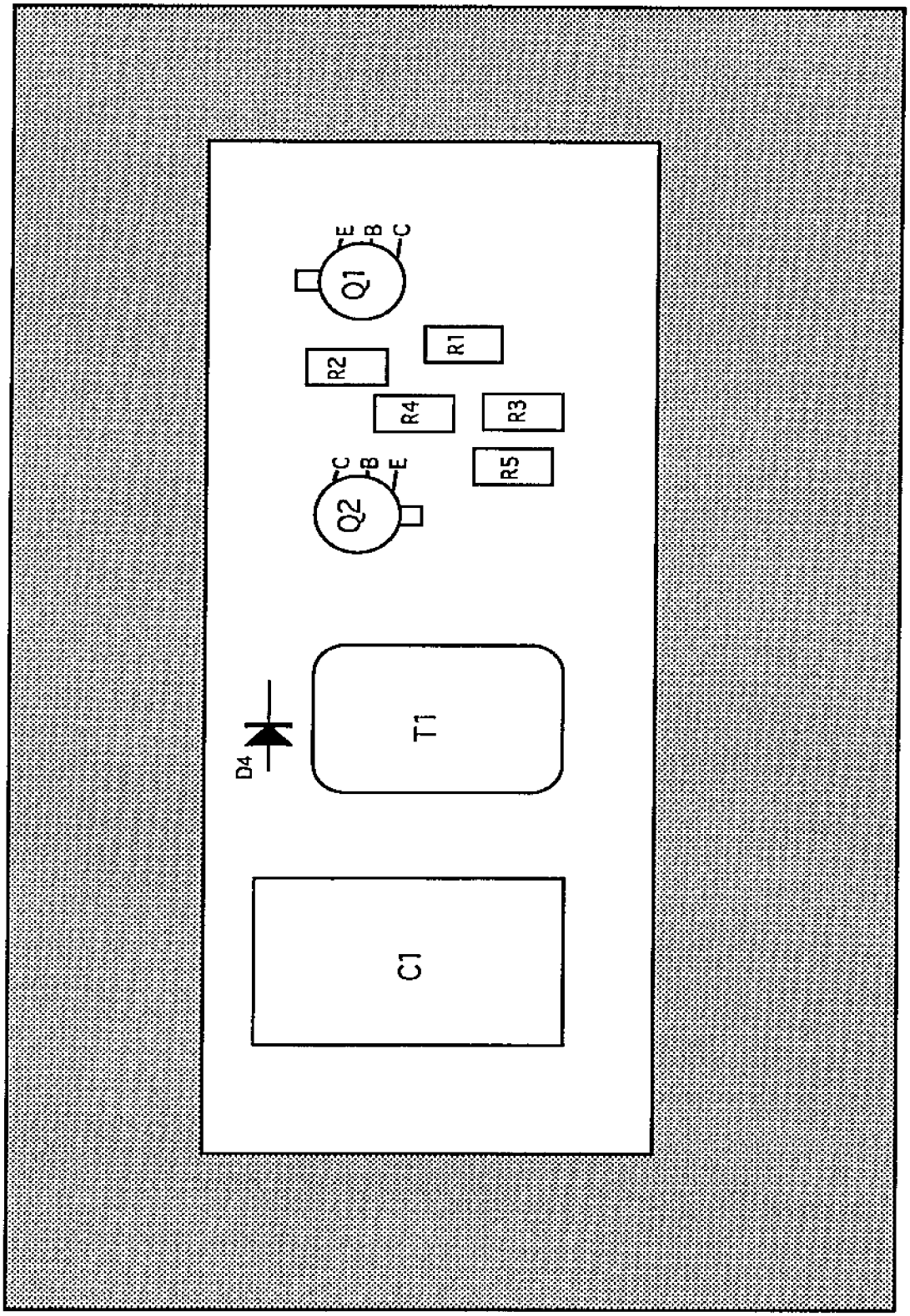

Figure 2A Approximate Location of Parts for the Relay Circuit

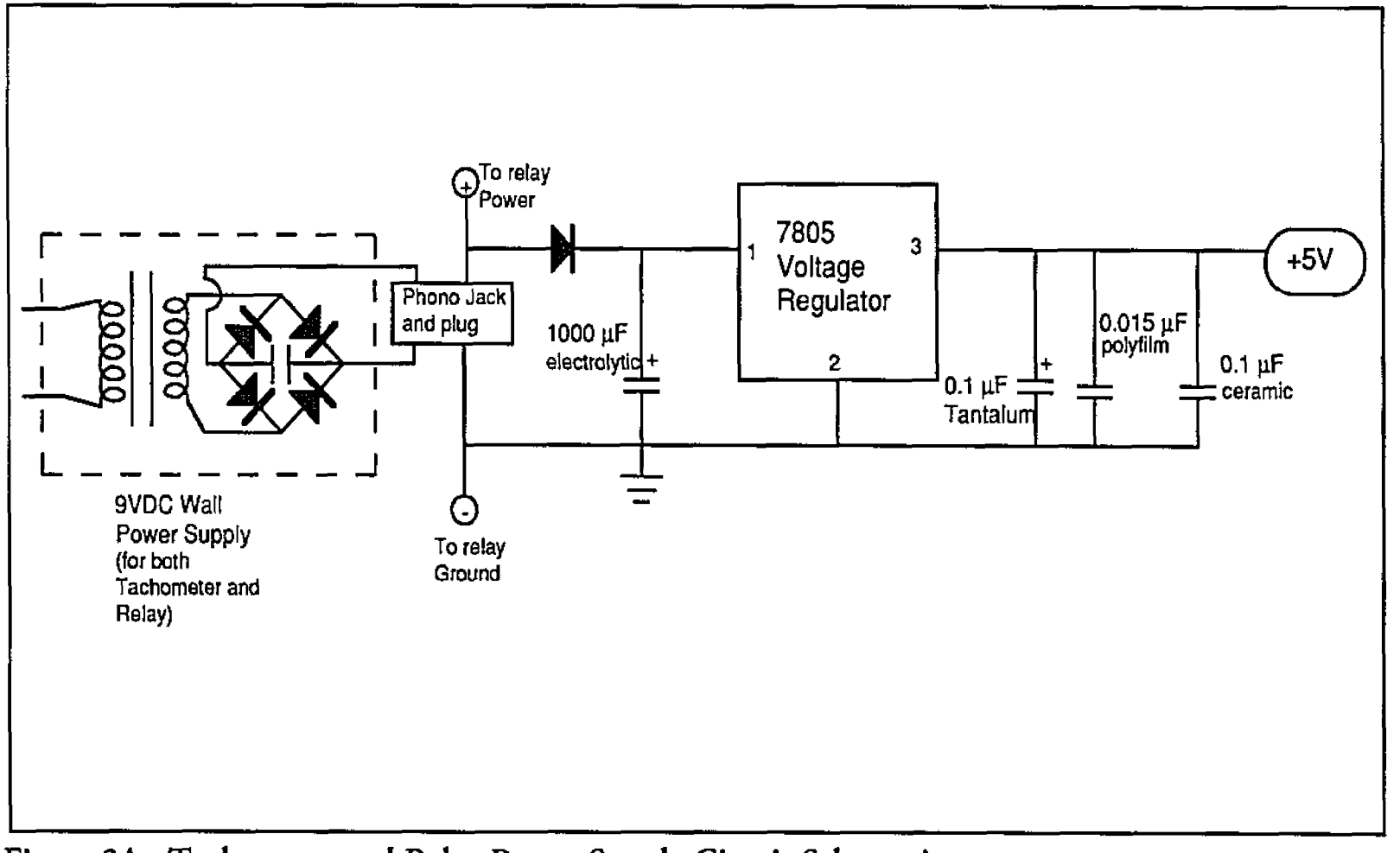

Figure 3A Tachometer and Relay Power Supply Circuit Schematics

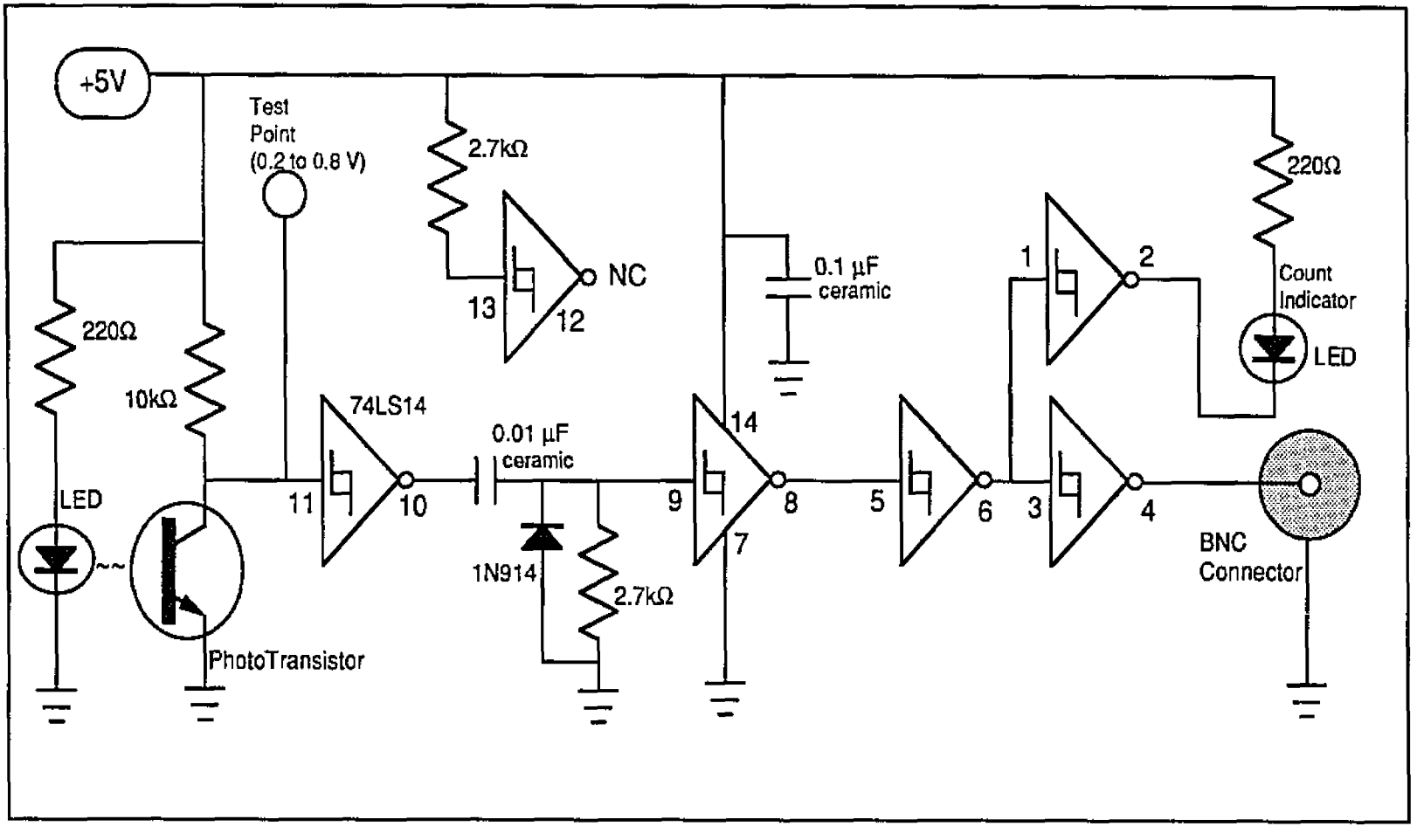

Figure 4A Tachometer Circuit Schematics

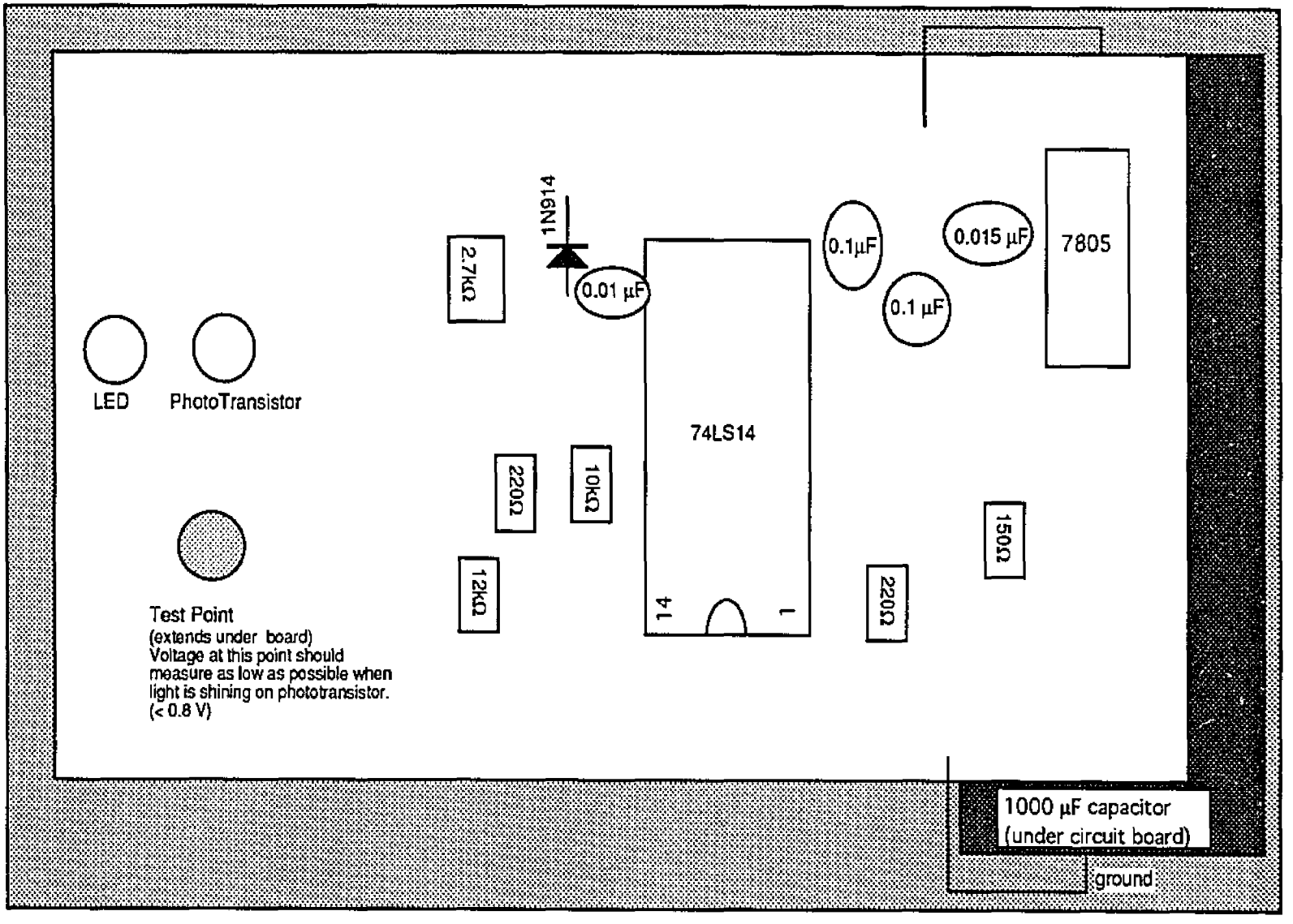

Figure 5A Approximate Location of Parts for the Tachometer Circuit

2.0A The Auto Titrator Instrument Software

2.1A Description of Titration Control Methods

This section discusses in detail some of the original algorithms and equations used in the titration program **TIGHTC**. Summaries and overviews of these algorithms, which may provide additional understanding, are discussed in the main body of the text. Parameters names are given in quotes. Parameters such as "pH maximum deviation slope" and "pH noise" are described in Table 1.1 in Section 1.

2.1.1A Method Used to Obtain a Value of pH

a) First, the average change of a minimum of forty samples must be less than the criteria "pH maximum deviation slope". In the experiments performed for this thesis, the value used was $4.0 \cdot 10^{-3}$ volts. If the average is greater than the parameter "pH maximum deviation slope", another set of readings is repeated until the criteria is met.

Explanation: When the readings are larger than "pH maximum deviation slope", the pH is assumed to be changing quickly and not yet near the settling point.

b) Then, sets of 8 values are read in and averaged. At least 20 sets of these 8 values are weighed and averaged, each set weighed by the factor: 0.925(n-i) Where n is the number of sets and i is the number of the ith set. At the 20th set, the standard deviation is calculated. If the standard deviation is greater than the pH noise criteria, "pH noise", another 20 sets are averaged. If more than 400 sets are measured and the standard deviation is still larger than "pH noise", then the standard deviation is displayed along with the message:

Noise in pH measurement: deviation = ...

The average value is erased and step a) is repeated.

Explanation: This procedure requires that the noise on the measurement be below the criteria "pH noise". This helps compensate for any noise in the

electrode or electronics or from the chemistry and serves as a secondary check that the measurement has settled to its final value.

c) When the standard deviation has gone below the threshold, "pH noise", the program waits 0.1 sec., the average and standard deviation are reset and then steps a) and b) are once again repeated. If the second iteration meets the criteria, then the weighed mean of the voltage defined above is returned to the program as the pH measurement. Otherwise the entire process is repeated.

Explanation: The procedures are repeated to confirm that the readings have settled.

In the above procedures, data is acquired in a "burst" mode: the time delay between data values depend upon A/D timing and instruction times. Insertion of a waiting period between acquisition of data points and sets was tested and results were no better than when no waiting period was used. Under different circumstances, this may not be the case, so this caution should be considered when using the above algorithm.

2.1.2A Method to Determine and Predict Proper Aliquot Size

The proper aliquot size in this instrument is the amount of reagent that should be added so that the change in pH will be the same as the parameter "pH change". The value of "pH change" is usually set to 0.1 pH unit. The decision process used by the instrument to determine aliquot size is based mainly on the numerical derivative calculated from the previous two data points, the change of the pH of these points, and the parameters listed in Table 1.1: "pH change", "minimum aliquot", "maximum aliquot" and "pH change correction". The following describes exactly how the software determines and predicts the proper aliquot size.

The goal of the titration decision process is to maintain pH changes to be as close as possible to the value in "pH change" . First the previous value of the actual delivered reagent volume is stored in a variable,

old_volume. Then a value is calculated, "modify", in units of pH. This value reflects the amount that the previous delivery overshot the goal in "pH change". If the previous change in pH was less than the target, then change is set to 0. If the pH change was larger, "modify" is set to the difference between the goal and the actual change, up to the value of the goal. The value of "modify" is in the following range:

$$\text{"pH change"} \leq \text{modify} \leq 0$$

Then an offset, " counts", is determined from the difference between "pH change" and "modify" divided by the derivative of the data:

$$\text{volume} = \left| \frac{\text{"pH change"} - \text{modify}}{\frac{\Delta pH}{\Delta volume}} \right|$$

At this point, " volume" contains the volume which would have been correct to meet the goal for the previous data point. This value will be decreased such that large derivatives will decrease the number of counts more than smaller derivatives.

$$\text{volume} = \frac{\text{volume}}{1 + \text{"pH Change correction"} * \left| \frac{\Delta pH}{\Delta volume} \right|}$$

volume at this point is approximately proportional to the numerical derivative, so the equation is of the form:

$$\text{volume} = \frac{W * \frac{\Delta pH}{\Delta volume}}{1 + U * \frac{\Delta pH}{\Delta volume}}$$

As opposed to when there is no correction, the equation would be of the form:

$$\text{volume} = G * \frac{\Delta pH}{\Delta volume} + F$$

The latter case means that the volume is proportional to the derivative. This correction is applied to correct for the fact that at large derivatives the pH will change faster and in a non linear fashion than at small values. For small values of the derivative, " volume" is almost unchanged. When the derivative is large, " volume" levels off and becomes almost constant.
If the first derivative is zero, maximum delivery from "maximum aliquot" is used:

$$\text{IF } \frac{\Delta pH}{\Delta volume} = 0 \quad \text{THEN} \qquad volume = \text{"maximum aliquot"}$$

Then the amount to delivered is checked to determine if it is beyond reasonable values. If so, "minimum aliquot" or "maximum aliquot" is substituted:

```
IF volume >"maximum aliquot" THEN
                    counts = "maximum aliquot"

IF volume < "minimum aliquot" THEN
                    volume = "minimum aliquot"
```

The signal that specifies maximum delivery is a tilde (~), and when pressed by the chemist, regardless of the volume calculated, the delivery will be the amount in "maximum aliquot":

```
IF M9<>0 THEN       volume = "maximum aliquot"
```

Where M9 is a flag that indicates, when non zero, that the tilde was pressed earlier. Then the aliquot is prevented from exceeding four times the previous quantity:

```
IF volume > 4 * old_volume THEN
                    volume = old_volume * 4
```

The final result is used to calculate the volume that the buret should output for the next delivery. The value in volume is the point at which the motor will be turned off by the program. The motor will still be spinning, so a percent compensation, "Spin Down percent", is used to reduce the volume.

volume to be delivered = volume - volume * "Spin Down percent"

The actual volume, which may be slightly more or less than "volume", is measured by the tachometer count and stored with the data.

2.1.3A Method Used to Determine the Actual Aliquot Delivered

The amount of reagent to be delivered is determined by the criteria in Appendix 2.1.2A. A target is determined by:

Target pH Change = Amount Desired * (1 - "Spin Down")

The aliquot is determined by starting the buret and continually examining the number of counts on the counter which come in from the tachometer. If the buret doesn't move in 0.2 seconds, the amount that it previously moved (volume of reagent) is recorded and an error is flagged (This may occur if there is a bad connection or if the buret is empty). Otherwise, when the target has been met, the buret remote is set to off and the counts are examined until there are no more counts for 0.5 seconds (the motor in the buret has inertia and takes time, less than a second, to stop spinning. Then the program pauses for "Mix time" (the default is 2 seconds), and then reads and stores the counter reading as the final amount of reagent delivered.

2.1.4A pH Calibrations

The pH electrode outputs a voltage which is directly proportional to voltage in the range in which it is considered useful, pH 1 to pH 12. In a pH meter, this voltage is converted to the number that we recognize as pH. Since different pH electrodes have different characteristics, and these characteristics can vary over time[28] and with different temperatures, there are controls on the meter which change the conversion factors from voltage to pH number. Usually, this consists of two dials: "zero" (also called "offset") and "temperature" (also called "slope"). The zero dial is commonly used with pH 7.0 buffer to offset the conversion line, and the "temperature" dial is used with pH 4.0 or 10.0 buffer to rotate the conversion line with the pH 7.0 point fixed. The output of the pH meter (which is attached to the computer) is a voltage which is proportional to pH. The computer needs the conversion factors by which it can translate the voltage that it reads into a pH value. The equation in the computer software is possibly the same equation that the meter implements in hardware,

$$E = pH * C + K \qquad (2.1A)$$

or,

$$pH = \frac{E - K}{C} \qquad (2.2A)$$

Where:

E	is the voltage from the electrode or the meter
K	is an offset ($\frac{K}{C}$ is analogous to the zero control)
C	is the slope ($\frac{1}{C}$ is analogous to the temperature control)

The values for K and C are calibrated before a titration begins. Since K and C are unknown, a minimum of two buffers are required to determine these values. **TIGHTC** gives the choice of doing a two point titration, entering K and C values, using previously determined values or doing a multiple point calibration. The multiple point calibration is performed by recording values of pH and voltage and then performing a linear least squares to fit the best line to those values. Previously determined values may be included in this calibration.

2.2A The Computer Environment of TIGHTC

The programs to control and acquire data from the titration experiment were written and run on a Digital Equipment Corporation MINC-11 computer with an LSI-11/23 microprocessor using MINC Basic. The language is an extended Basic, with most of the extensions designed to control the input / output devices available for this computer. The disk storage which is used to start Basic and the titration programs requires some interaction from the chemist (i.e., the program does not start immediately; some information is needed to be entered into the computer).

2.3A The Program TIGHTC and Support Programs

The program was written in several parts, and there are several support programs. These are listed in Table 1.2A. The programs which are required to run the experiment are **TIGHTC**, **TIGHTZ** and **TIGHTY**. The main program is **TIGHTC**, which performs all of the calibrations, control and data acquisition. **TIGHTZ** does all of the plotting, storing and data output. Another part of the program, **TIGHTY**, is only for editing the data and is an optional component of the program. The reason that the program was written in several parts is that all parts would not fit into the 56k memory of the computer all at one time, so an overlay scheme was used. When one part of the program was no longer needed, the next part would be called in and written over the old program in memory. This is all almost transparent to the operator. The only thing that the operator will notice is a wait time (~60s) when the different sections are called into memory.

Program	Version	Description
TIGHTC	5.75	This is the main program for the titration. It performs all the experimental control: it controls the buret and acquires data.
TIGHTZ	5.81	This is the overlay for TIGHTC. TIGHTZ is called in when the experiment has been completed and display, storage, analysis and output of data are required. Do not run TIGHTZ directly.
TIGHTY		This is another overlay for TIGHTC. It allows for editing and smoothing of data. Do not run TIGHTY directly.
TIGHTD	5.70	This program is used when it is not desired to run an experiment, but it is desired to examine titration data stored from previous experiments . This program initializes variables so that the TIGHTZ and TIGHTY overlays can be called in.
TIGHTM	5.61	This program is an older version of TIGHTC. It is a manual version of the program. It requires the operator to manually decide the quantity of reagent delivered.
NOISE		This program looks at the noise coming in over the A/D converter, does some simple statistics and plots the data and lists the results.
TITADC		This program demonstrates how to write a program to read data in from the A/D converter.
TITONC		This is a simple program which demonstrates how to write a program to control the relay which turns the buret on and off. It is also used to help calibrate the conversion factor for motor turns to reagent volume, as well as trouble shooting.
TACCAL		This is a more powerful program than TITONC for calibrating flow rate.

Table 1.2A List of Programs and Files for Titration Experiment.

2.4A Portability of the Programs, Overview

Portability of the programs is a subject which concerns the problems of implementing the titration programs on another computer. This is of some concern for this instrument, as the MINC-11 has not been produced by DEC in many years. There are two things to consider when porting the programs: the hardware and the software of the target computer.

For minimal algorithm rewriting, the target computer must have hardware which performs the same functions as in the MINC-11. The minimum requirements are a counter, a digital output line and a 12 bit A/D converter. This topic is discussed in Appendix 1.2A and listed in Table 1.1A.

The software will need some changes. Elements of the Basic language varies with computers, and with the author of the language. MINC-11 Basic consists of elements which can be found in other versions of Basic, along with its own specialized built in extensions. These extensions, which make up the bulk of the variability of the language, are mostly functions which control the input-output devices and manipulation of the graphics display. The other difference is the environment when first starting up the computer, running programs, and editing and manipulating the file directory.

The data acquisition and control instructions may have their equivalents in other systems. If not, PEEK and POKE instructions might take their place. The other sections involve printing and plotting and may need a more extensive rewrite for the configuration of the computer for other idiosyncrasies of the computer such as opening files and printers and printer device names. The overlay structure might be abandoned, as new computers have much more memory than this MINC-11. Graphics and terminal functions on the MINC Basic are meant for a DEC VT105 terminal, whose graphics standard is not common. However most of the graphics functions should have a direct equivalent.

Much of the code has comments for function and algorithm. There are manuals for the Minc, references 30 to 32, which describe all of the statements in MINC Basic. Reference 29 describes much of the peripheral devices, and may give some additional insight into programming.

3.0A Operation of the Instrument

3.1A The Basic Monitor

The Basic monitor allows you to manipulate files and run programs. When you are communicating with the Basic monitor, you will get a prompt which is displayed: **READY** .

3.2A Starting and Operating the Instrument

The configuration of this instrument uses two removable floppy disks (names that you input to the computer when referencing the disks are DY0: on the left, DY1: on the right). The instrument should in most cases be started by putting the titration startup disk in DY0:, and your data disk in DY1:. When the instrument is first started, the RT-11 system and then MINC Basic is loaded into memory and run. You will get prompted for the date, and then you will get the Basic monitor. Since the **TIGHTC** program is large, memory space allocated for I/O operations must be allocated to program space by invoking the **EXTRA_SPACE** command. Then, any of the programs listed in Table 1.2A may be run; to start the titration, **RUN TIGHTC** is entered.

An experiment is performed by using controls on the buret to load reagent into the buret. The material to be titrated (substrate) is weighed out, dissolved and continuously mechanically stirred in an appropriate vessel. The program **TIGHTC** is started, the electrode is calibrated according to directions in the program, a few simple parameters are given to the computer and then the computer performs the titration. The end of the titration is determined by a preset pH value. It is up to the operator to decide when the titration has been completed. This may be done from pH values or by visually inspecting the curve that the computer presents in real time. The computer will pause the titration automatically when the buret is empty. Then data may be plotted, printed, stored or manipulated.

3.3A Examples of Experimental Runs and Output

3.3.1A Titration Experiment, A Sample Run

The following pages show a example of what may appear on the screen when performing an experiment. This is intended to demonstrate operation of the program and aid understanding of each step. Italicized text does not appear on the screen and is presented here as a description of what is happening. Underlined values are values which might be entered. Everything else is exactly what will appear on the terminal screen.

First screen (Introduction)
Automatic Titrator : TIGHTC.BAS

*** Version # 5.75 auto tach gds
by George D Sukenick
volume determined by buret tachometer

(pause, then the screen is cleared...)

Second screen (instructions)
TIGHTC INSTRUCTIONS:
1) Manually fill Motorized Buret with titrant.
2) Remove all bubbles, remove backlash from buret.
3) Set buret direction to REMOTE.
4) Turn stirring motor on.

Values in braces are default:
If you don't enter a value and press return, you will select the default value.

This program determines the pH by looking at the voltage output of the pH electrode (or meter) and by applying this equation : E = K + C * pH
where:
E is voltage output and K, C are constants which must be determined before this experiment can be run.

Determination of the constants may be done either by using values of the constants previously found or, preferably, by program calibration

Do you want to:
1) 2 point Calibration of K & C under program guidance"
2) Multiple point"
3) Enter K & C"
(a 4th choice is given if values were previously calculated):
4) Use same K & C as the last run ?
enter a number {1} ";
return is pressed for choice #1, calibrate with two buffers

We will now calibrate.
pH Meter instructions:
(Always place the pH meter on standby and rinse electrode when moving electrode between solutions. Take off standby when electrode is in the solution. Do not change CAL SLOPE or TEMP when you start the computer calibration.)
This calibration requires several standard buffers

1) First calibrate the METER by inserting the electrode in pH 7.0
   Adjust the CAL knob until the meter reads 7.00
2) Then, insert into another standard buffer (e.g., 4.0) and adjust the SLOPE knob so that the correct reading is obtained.
3) Now, we will determine K & C.

Place the electrode into a buffer solution"
wait for equilibrium and enter the exact pH "; 10.0
The pH of the solution is entered when the reading it appears stable.
In this case, the pH buffer was 10.0.

* *

4) Now place the electrode into a different buffer (e.g., pH 7.0)
   wait for equilibrium and enter buffer's pH "; 7.0
The pH of the second buffer solution is entered.
In this case, the pH of the buffer was 7.0.

Now, the screen is cleared. The computer will use the calibration values and report a pH value. If the pH value is not close to the correct value, you should do the calibration again. The values for C and K will vary depending upon the electrode and the meter calibration.

The value for the pH coefficient C is 0.744342

and the value for the pH constant K is -5.10212

This program will now test and measure the pH of the present solution.

The EQUATION is : VOLTS = -5.10212 + 0.744342 * present solution pH.

Test with above constants gives a pH of 7.0

What would you like to do now:
1 Recalibrate
2 Test again
3 Continue to next step
enter a number {3} ";
Press return to get the third choice, continue

Enter molarity of titrant if known, or an estimate if not known {.1};
Enter Molarity; this is used in calculating # moles used; if you do not have a value, just press return.

Enter Flow conversion: ml/#counts {.001875}

Enter a value only if you have recalibrated the conversion from pulses to ml.

Calibration complete. Ready to titrate.
Rinse and place pH electrode into solution
Enter pH Stop value {-1000}

Enter the value of pH that you want the titration to stop at. If you are not sure, enter a value beyond the final pH (e.g., if the titrant is acid, enter a number such as 0; if the titrant is base, use a value such as 14), and then you can later stop the titration manually. The pH stop value is to provide a convenient method to stop the titration, but it is not the only method which can end the titration. Note that once the value is reached, you cannot continue the titration.

To change internal parameters before starting, enter CHANGE.
If parameters are ok, press return to start titration {START}

Press return to start titration or enter the word " START". If you wish to change internal parameters (see Table 1.1), you may enter CHANGE.

When the titration has started, the screen is cleared, a graphics area is set up, the titration is started and data is plotted on the screen as it comes in. Appearing on the top of the screen:

press * to pause titration, # to change parameter
- to deliver max @ dpH deriv criteria

This instruction tells you that at any point in the titration, you may enter . to stop the titration, # when you want to change a titration parameter (see Table 1.1), .: to deliver a maximum aliquot (for each - pressed) and .@ to change the delta pH derivative criteria.*

The bottom of the screen has room for viewing the last 3 values of data:

#	Volume	pH	Voltage	Deriv 1	Deriv 2
1	0.0169	11.452	3.4223		
2	0.0469	11.4411	3.4139	0.3633	
3	0.3019	11.366	3.3580	0.2945	-0.48280

In this example, the first 3 deliveries were made. All listings of data consist of data channel #, volume delivered, pH at that volume, Voltage (proportional to pH) at that volume, first numerical derivative calculated that volume and pH and the previous, and the second numerical derivative calculated from that volume, pH and the previous two volumes and pH's. Only the first two values d0 not have entries for derivatives, as there is not enough information to calculate them.
When the buret is empty or you have pressed . to end the titration, you will get this message:*

Titration pause
Enter STOP to call in storage/display or press RETURN to continue

If you press return, the titration continues. Entering .STOP will stop the titration and then:

TITRATION ENDED .... TIME NOW IS :: 4:00:00
Would you like to re-do this titration (Yes or {No}) ;

Press return to continue...

Calling the display program into memory

Now, TIGHTZ is being called into memory. This takes about one minute. Then a menu will appear:

TIGHTZ VERSION 5.81 gdsukenick OPTIONS:
(if the word READY appears on the screen, enter GOTO 10 or RETURN)
(if you get an error concerning OPEN, enter CLOSE#1 \ GOTO 10)

0 To start a new titration"
1 pH vs. volume Display and Plot"
2 First Derivative Display and Plot"
3 Second Derivative Display and Plot"
4 List all data on your terminal"
5 List endpoints on the terminal"
6 Output the data to the printer"
7 Store data on a diskette"
8 Replace data with data from disk"
9 Leave this program"
10 Recall data, Print, Plot (8,6,1,2,3)"
11 Store, Print, Plot, then new titration (7,6,1,2,3,0)"
12 Advanced option menu"

Enter a number (0-12)

To select any of the options from the menu, enter the number on the left of the option. In most cases, option 11 would be the one to choose.

The advanced menu has the following screen:

Advanced options:

20 Edit data
21 Fit data
22 Find percent; normality
23 Select display: dual/single
24 Return to previous menu
25 Start New Titration directly
26 Stop

Enter a number (20-25)

3.3.2A Sample Data Report

The following page shows a sample of a data report with information concerning the titration produced by **TIGHTC**. The first line has the name of the file where the data is stored (if any). The second line contains comments entered when the data was stored. The next five lines contain parameters of the titration, in the same order as that listed in Table 1.1 . Following that is the date and time that the data was stored. The tenth line lists the number of data points. Then the flow rate conversion factor, in ml / pulse and the reagent molarity, and coefficients of the pH-to-voltage equation are listed.

After this information, there is a list of data in five columns. The columns consist of the channel number, the volume at the channel, the measured pH, and the calculated first and second derivatives. At the end of the data list are columns of points at which the second derivative changed sign (suspected endpoints). In the list is the nearest channel number and the volume, pH, and moles interpolated to the point where the second derivative is zero.

Data points are stored on disk in a form similar to the data report. The first and second derivatives are not stored (as they can be easily calculated form the data) and the volume is stored in units of tachometer counts. The program which reads the data would convert counts back to volume by multiplying the data by the flow rate conversion factor, and calculate first and second derivatives by using the Appendix 4.1A equations.

<u>Method to interpret data:</u>

Look at list of possible endpoints, find the maximum value for first derivative. In the example, -1.05301 is the maximum derivative for the first endpoint. This is near data channel # 33. Volume(i) lists the volume determined by the interpolation of the second derivative to zero. This case it is 11.5947. This is the volume that should be used, but it should be first checked for reasonableness. Look up the values around channel 33, 32 to 34 we see that it is the maximum derivative and the volume in this case is on 33, and the volume at #33 is not much different, 11.6025.

DY1:NA2CO3.G2H
Na2CO3: 0.15204 g
$Parameters:
4.00000E-03 , 4.00000E-03 , .1 , 7.50000E-03 ,
2 , .02 , 2 , .4 ,
0 , 0 , 4 , 7.10525 ,
.281906 , 2.31939 , 4 , 10 ,

; 23-JUL-88 16:19:54
OF DATA POINTS, 100
FLOW RATE USED =, 1.87500E-03
TITRANT MOLARITY as entered from terminal, 0.12246
equation used to CONVERT VOLTAGE TO pH: E = K + C * pH
WHERE K=, -5.00641 C=, .744283
(volume delivered & pH at each point):

#	Volume	pH	1st Deriv	2nd Derivative
1	0.0000	11.400	0.000	0.0000
2	0.0188	11.396	-0.224	0.0000
3	0.0619	11.383	-0.292	-2.2035
4	0.3806	11.305	-0.244	0.2658
5	0.7688	11.213	-0.237	0.0177
6	1.1719	11.123	-0.224	0.0330
7	1.5919	11.030	-0.220	0.0097
8	2.0194	10.944	-0.199	0.0494
9	2.5031	10.854	-0.187	0.0274
10	3.0262	10.763	-0.173	0.0268
11	3.5850	10.671	-0.165	0.0158
12	4.1644	10.581	-0.154	0.0185
13	4.7981	10.484	-0.152	0.0031
14	5.4300	10.390	-0.148	0.0067
15	6.0863	10.293	-0.148	0.0005
16	6.7406	10.192	-0.154	-0.0097
17	7.3650	10.094	-0.157	-0.0043
18	7.9744	9.989	-0.171	-0.0238
19	8.5106	9.888	-0.189	-0.0300
20	9.0131	9.782	-0.210	-0.0414
21	9.4481	9.678	-0.239	-0.0604
22	9.8381	9.570	-0.275	-0.0879
23	10.1700	9.460	-0.331	-0.1546
24	10.4306	9.360	-0.384	-0.1786

25	10.6800	9.245	-0.463	-0.3124
26	10.8656	9.138	-0.574	-0.5086
27	11.0194	9.035	-0.668	-0.5569
28	11.1544	8.927	-0.801	-0.9190
29	11.2650	8.830	-0.879	-0.6338
30	11.3850	8.688	-1.177	-2.5876
31	11.4506	8.603	-1.298	-1.2952
32	11.5256	8.503	-1.338	-0.5722
33	11.6025	8.385	-1.529	-2.5200
34	11.6606	8.297	-1.523	0.0977
35	11.7300	8.194	-1.473	0.7753
36	11.7975	8.098	-1.426	0.6818
37	11.8706	8.011	-1.196	3.2790
38	11.9569	7.920	-1.051	1.8149
39	12.0506	7.827	-0.987	0.7119
40	12.1425	7.748	-0.862	1.3544
41	12.2550	7.665	-0.740	1.1871
42	12.3806	7.584	-0.643	0.8140
43	12.5363	7.495	-0.572	0.5063
44	12.6994	7.416	-0.481	0.5718
45	12.8963	7.333	-0.422	0.3270
46	13.1194	7.251	-0.366	0.2651
47	13.3819	7.165	-0.327	0.1612
48	13.6800	7.081	-0.280	0.1676
49	14.0269	6.994	-0.250	0.0931
50	14.4113	6.908	-0.225	0.0676
51	14.8388	6.821	-0.203	0.0563
52	15.3019	6.733	-0.189	0.0305
53	15.8063	6.644	-0.175	0.0284
54	16.3575	6.552	-0.166	0.0166
55	16.9425	6.468	-0.144	0.0399
56	17.6156	6.363	-0.155	-0.0824
57	18.2025	6.272	-0.155	0.0002
58	18.8156	6.174	-0.158	-0.0051
59	19.4175	6.073	-0.168	-0.0165
60	19.9744	5.973	-0.179	-0.0177
61	20.5200	5.865	-0.197	-0.0333
62	20.9737	5.769	-0.212	-0.0307
63	21.4238	5.659	-0.243	-0.0674
64	21.7913	5.553	-0.290	-0.1166

65	22.1044	5.446	-0.338	-0.1411	
66	22.3744	5.338	-0.402	-0.2173	
67	22.5938	5.230	-0.489	-0.3568	
68	22.7831	5.114	-0.615	-0.6146	
69	22.9200	5.012	-0.747	-0.8127	
70	23.0475	4.893	-0.926	-1.3531	
71	23.1375	4.786	-1.192	-2.4450	
72	23.2181	4.663	-1.521	-3.8561	
73	23.2706	4.579	-1.608	-1.3000	
74	23.3325	4.434	-2.350	-12.9783	
75	23.3662	4.350	-2.486	-2.8462	
76	23.4112	4.206	-3.191	-17.9084	
77	23.4394	4.108	-3.484	-8.0031	
78	23.4769	3.974	-3.563	-2.4094	
79	23.5106	3.862	-3.330	6.5230	
80	23.5444	3.759	-3.057	8.1144	
81	23.5744	3.677	-2.706	10.9975	
82	23.6119	3.587	-2.403	8.9723	
83	23.6512	3.505	-2.084	8.3184	
84	23.7037	3.409	-1.832	5.4905	
85	23.7563	3.331	-1.483	6.6403	
86	23.8181	3.250	-1.310	3.0220	
87	23.9025	3.155	-1.130	2.4556	
88	23.9963	3.071	-0.896	2.6260	
89	24.1087	2.987	-0.740	1.5126	
90	24.2325	2.908	-0.640	0.8511	

POSSIBLE ENDPOINTS:

(i) means value is interpolated to point 2nd derivative = 0

Approx #	Volume(i)	pH(i)	Moles(i)	1st Deriv	2nd Deriv
15	5.4648	10.389	6.69216E-04	-0.1481	0.0005
33	11.5947	8.397	1.41989E-03	-1.05301	-2.5257
55	16.7767	6.494	2.05447E-03	-0.1443	0.0399
56	17.5864	6.368	2.15362E-03	-0.1558	-0.0182
57	17.6197	6.364	2.15771E-03	-0.1556	0.0002
78	23.4500	4.064	2.87169E-03	-3.5633	-2.1386

3.4A Performing an Experiment

3.4.1A Calibration of the Flow Rate

The relation between motor turns of the buret and the amount delivered may be calibrated. One method would be to use the program **TITONC** which reports the number of signals received by the computer. **TACCAL** is another program which is an aid in this calibration. **TACCAL** uses several delivery runs to calibrate, and values are stored on disk and printed. It is best to deliver a large amount of reagent, to reduce errors due to errors in weighing and counts. Start the program by entering

RUN TACCAL

Prepare a dry beaker, enter the initial mass, weigh, enter final mass, and repeat. If the delivery time needs to be changed, enter the time value when prompted. When finished, the data is stored on the disk.

Using the data, determine mass / turn. From the temperature, find the density of the liquid from a reference such as the CRC Handbook[20] and divide to determine ml / turn:

$$\text{flow rate calibration} = \frac{\frac{\text{mass (g)}}{\text{clicks (buret turns)}}}{\text{density of liquid (g/ml)}}$$

The value obtained should be around 0.001875 ml/turn.

3.4.2A Performing a Titration

If the program isn't already running, start it by entering:

RUN TIGHTC

Dissolve the material, place a stirring bar into a (ca. 200ml) beaker on the stirring motor and adjust the stirrer to a reasonable mixing speed.

It is a good idea to calibrate pH before each run. When performing the calibration, first use the buffer with pH most different from the material to be titrated. Follow instructions from the program, washing the electrode between each standard solution. When calibration has been completed, place the electrode into the solution to be titrated and lower the delivery tube

so that it is just barely is above or touching the solution. Start the titration. When the titration has been completed, if the stop value for pH hasn't been reached, you may press shift 8 (*) and enter **STOP** to manually end the titration. Store data on the disk, plot and list it. If the buret runs empty before the titration has finished, you may refill it and continue the titration. The program will detect when the buret has stopped delivering and will wait for you. Remove the electrode and the delivery tube slowly, and rinse the outside into the main solution. Point the delivery tube to a waste beaker and switch the motor control to "down". When the buret is full, place the control onto "remote" and rinse into the waste beaker. Place tube and electrode back into the solution and press **return** to continue the titration.

3.4.3A Speeding up the titration

The time in which a titration is performed depends on the method used, each with its own advantages and disadvantages. Most of the time that the titration uses is in waiting for proper mixing and taking a stable pH reading. The method to shorten this time is to either take less readings or shorten the time for a pH reading. In the following, (a) is the least desired choice.

a) Change the pH stability criteria; parameters "pH noise" and "pH max dev slope" and/or decrease the "mix time".

   If these criteria are made less stringent, the titration may speed up due to shorter data acquisition time, but the pH value may be less reliable.

b) Use a new pH electrode.

   A new electrode will have less noise and better response than an old electrode, assuming similar quality when each are new.

c) Optimize the mixing rate.

   Since waiting for stability is the major contributor to dead time, a more efficient method of mixing will shorten the titration time.

d) Change the "pH change" goal for each aliquot.

By increasing the "pH change", larger and fewer aliquots are delivered. If allowed to remain at an increased value for the entire titration, fewer data values may be taken around the endpoint and the endpoint will become less well defined. However, as the titration proceeds, it is possible to change the parameter back to a smaller value when deemed necessary (i.e., near an equivalence point).

e) Add reagent manually

This can be performed in two ways:

1) either add a quantity before the titration starts and record the amount and add it to your final volumes or:

2) After the titration has started, only when the LED on the front of the buret is on, switch the buret from "remote" to "up" until the desired amount has been delivered. In the latter case, the computer will keep track of the amount delivered even though it has turned off the delivery relay. Switching the knob must occur when the LED is on, or up to "mix time" (parameter: default values is) 2 seconds after it turns off. After the computer starts delivery of an aliquot, it keeps track of the count until the motor has stopped moving. If you have switched the knob at the wrong time (i.e., a time that the computer is not monitoring the buret movement), then you will see the message:

ERROR - buret is moving by itself or there is noise on ST1 input:...Counts

If the message occurs and you do not know the quantity that was delivered, then the experiment is ruined.

f) Press the tilde ~

This will cause the next delivery to output the maximum volume allowed by the program, in parameter "maximum aliquot"; the default is 2 ml, but this value may be changed, if desired.

A parameter may be changed before the titration has begun by selecting "change parameter" when given the choice. When a titration is in progress, a parameter may be changed by pressing the pound key (#). After the pound key is pressed, you will be prompted for the parameter change after the next aliquot.

4.0A Equations

4.1A Numerical Derivatives and Calculations

This section discusses how the numerical derivatives are defined and how they are calculated in both the automatic titration program **TIGHTC** and in the curve fitting program, **ENDPOINT.**

The term used in this thesis, "numerical derivative", is not a continuous derivative; it is from discrete values and defined in equation 5.1A.

$$\text{Numerical derivative} = \frac{\Delta Y}{\Delta X} = \frac{Y_{n+1} - Y_n}{X_{n+1} - X_n} \qquad (5.1A)$$

If we take two adjacent data points and connect a straight line between them, equation 5.1A is the value of the slope of the line.

The first numerical derivative of a pH volumetric titration curve is calculated from equation 5.2A.

$$\text{Numerical derivative} = \left(\frac{\Delta pH}{\Delta V}\right)_{(n,n+1)} = \frac{pH_{n+1} - pH_n}{V_{n+1} - V_n} \qquad (5.2A)$$

The volume at this derivative is somewhere between V_{n+1} and V_n, as defined by equation 5.3A:

$$V1d = V_{\left(\frac{\Delta pH}{\Delta V}\right)_{(n,n+1)}} = 0.5 * (V_{n+1} + V_n) \qquad (5.3A)$$

Where:

V1d is the volume at the first numerical derivative

Therefore, we cannot really say that the first numerical derivative is at a specific data point (except for convenience); it is between the two data points.

The second numerical derivative may be calculated from the first derivative in equation 5.4A.

$$\left(\frac{\Delta^2 pH}{\Delta V^2}\right)_{(n+2,n+1,n)} = \frac{\dfrac{pH_{n+2} - pH_{n+1}}{V_{n+2} - V_{n+1}} - \dfrac{pH_{n+1} - pH_n}{V_{n+1} - V_n}}{0.5 * ((V_{n+2} - V_n))} \quad (5.4A)$$

Where

The numerator is the difference in first derivatives

The denominator is the volume at these first derivatives,

as described in equation 5.3A.

Using a similar method as equation 5.17, the volume at the second derivative is equation 5.5A.

$$V_{\left(\frac{\Delta^2 pH}{\Delta V^2}\right)_{(n+2,n+1,n)}} = 0.25 * (V_{n+2} + V_{n+1} + V_{n+1} + V_n) \quad (5.5A)$$

Again, we cannot really say that the second numerical derivative is at one specific data point or even two for that matter, it is between and involves three data points, with weight given to the middle point (note that V_{n+1} appears twice).

To calculate the pH and Volume at the point where the second derivative is zero, the curve joining the second derivatives is assumed to approximate a straight line. Then, these values are interpolated as in equation 5.6A .

$$V_{\left(\frac{\Delta^2 pH}{\Delta V^2} = 0\right)} = \frac{(Y_1 * X_2) - (Y_2 * X_1)}{(Y_1 - Y_2)} \quad (5.6A)$$

Where:

X is volume as defined in equation 5.5A

X_1 is evaluated at the (n+2, n+1,n) second numerical derivative

X_2 is evaluated at the (n+3, n+2, n+1) second numerical derivative

Y is a second numerical derivative as defined in equation 5.4A

Y_1 is the (n+2, n+1,n) second numerical derivative

Y_2 is the (n+3, n+2, n+1) second numerical derivative

4.2A Equations to Calculate Molarity & Composition

The following is a list of equations and values used to calculate molarity of HCl and percent composition of the various samples.

Formula weights (g/M):

Na_2CO_3	105.9887	$Na_3HCO_3{\cdot}CO_3{\cdot}2H_2O$	226.02601
$NaHCO_3$	84.00687	H_2O	18.012245
$Na_3HCO_3{\cdot}CO_3{\cdot}2H_2O$	226.02601	TRIS	121.14

Sesquicarbonate consists of 46.89% carbonate and 37.17% bicarbonate.

TRIS Standardization of HCl :

$$M_{HCl} = \frac{\text{TRIS weight}}{\text{TRIS Molecular Weight} * \text{Endpoint Volume}} \qquad (5.7A)$$

$$M_{HCl} = \frac{\text{TRIS weight}}{121.14 * \text{Endpoint Volume}}$$

Pure Sodium Carbonate Sample:

$$\%\ \text{Carbonate} = \frac{\text{Titrant Molarity} * \text{Endpoint} * \text{Formula Weight}}{\text{Sample Weight}} * 10 \qquad (5.8A)$$

$$\%\ \text{Carbonate} = \frac{0.12246 * \text{Endpoint} * 105.9887}{\text{Sample Weight}} * 100$$

Mixtures of Sesquicarbonate and Bicarbonate:

$$\%\ \text{Sesquicarbonate} = \frac{\text{Titrant Molarity} * \text{Endpoint 1} * \text{Formula Weight}}{\text{Sample Weight}} \qquad (5.9A)$$

$$\%\ \text{Sesquicarbonate} = \frac{0.12554 * \text{Endpoint 1} * 226.02601}{\text{Sample Weight}}$$

$$\%\ \text{Bicarbonate} = 100 * \text{Titrant Molarity} * \frac{(\text{Endpoint2} - 3 * \text{Endpoint1}) * \text{Formula Weight}}{\text{Sample Weight}} \qquad (5.10A)$$

$$\%\ \text{Bicarbonate} = \frac{0.12554^* (\text{Endpoint2} - 3 * \text{Endpoint1}) * 84.00687}{\text{Sample Weight}} * 100$$

5.0A Modeling for Inflection Points and K Values

5.1A Program for Modeling and Manipulating Data

A program, **ENDPOINT**, was written to perform some of the analysis of the titration data. With this program, data files created with the **TIGHTC** titration program (as well as "ASCII" files with two columns of numbers) can be read in and manipulated in many ways. This includes extracting a section or range of channels of the data for manipulation, smoothing data, removing glitches by averaging adjacent points, plotting and graphics with cursors and fitting the data using both linear and non-linear methods. It is meant to run on a Vax UNIX system, but it should not be difficult to adapt it to other computers and operating systems. It has a single menu with the functions listed in Table 1.3A.

Command	Description
Help	help for each command
UNIX	go into UNIX shell without stopping the program
example	gives examples on how to manipulate the data
list	lists all data as well as first and second numerical derivatives
channel	moves cursor to channel # specified
find	moves cursor to a channel where the second derivative changed sign
plot	plots data on paper
range	marks the subset of data to be examined: as a number of point s around the cursor
fit	fit the data that was marked
refit	perform fit again, starting with x% of previous parameters determined as a starting point
new	read in new data
-	move cursor left
+	move cursor right
percent	marks the subset of data to be examined: this is the consecutive data on both sides of the cursor position whose first derivative is x% to 100% of the current cursor position
smooth	The current data point is replaced by a "smoothed" point which is interpolated by the first derivative of the points around it.
glitch	glitch smooths noisy points in the selected subset, except for the cursor channel which for most cases should be on the point closest to the inflection.
max	Max moves the cursor to the channel in the selected subset whose first derivative is maximum
rchannel	marks a subset of data by channels which are entered
store	store the selected subset into a file
sfile	set up a default directory path
show	re-lists fit information and statistics
quit	Exit the program and return to the monitor

Table 1.3A List of commands available for the ENDPOINT program

5.2A Modeling Technique for the Inflection Point

The data was modeled using equation 6.1A described in Appendix 5.3A to determine inflection points. The program used to fit the data is called **ENDPOINT**. The fitting method used in **ENDPOINT** is a non linear technique known by many names: Nelder Mead Algorithm, Simplex, Downhill Simplex Method and Amoeba[33-39]. This method is slow and the amount of computer memory required is N^2+5*N (where N is the number of data points to be fit), but it is robust and the program code itself is compact and easy to use, alter, and implement. It does not diverge as easily as other methods. It performs the fit by using initial guesses for the equation parameters, creating a simplex form whose vertices are these parameters and then varying them by moving the vertices of the simplex through error space via reflection, contraction and expansion until a minimum criteria is met. The minimum found is when a combination of the equation parameters minimize the function. This minimum may or may not be the lowest value for the function as the error space may contain local minimums. If the simplex goes into one of these local minimums, a better value may sometimes be found by restarting the program with the parameters that it returned, but offset by a small constant. The routine will continue and try to find a lower minimum if there is one. Sometimes the local minimum may be deep and it is difficult to move the simplex enough to find the lower minimum.

The simplex program is a subroutine which requires a controlling program which calls it, passing arrays, convergence criteria, initial guesses, and a function which, when given an array of parameter guesses that the simplex passes, can calculate an error between the data and the model equation using the parameter guesses. The error used in this thesis is the sum of residuals. The program has been published in several places[34,35] as well as functions for specific problems[35]. A program found in the book, Numerical Recipes,[36] is most up to date in terms of structure and programming technique but lacks construction of the initial simplex which may be found in the Olsson paper[35].

The simplex program used was adapted from these sources. Some features added by Kim[37] were also incorporated, such as improved error reporting and debugging by optional listing of the results of each iteration. I added additional modularity and error reporting to the simplex routine and an aid to convergence in the function, which is described in Appendix 5.6A .

5.3A The Model Used to Determine Inflection Points

In **ENDPOINT**, the model equation used to determine inflection points of the data was of the following form:

F(V) = nonlinear sigmoid + linear

$$F(V) = pH = \frac{A}{1 + e^{(B + C \cdot V)}} + D \cdot V + E \qquad (6.1A)$$

Where:

A, B, C, D and E are the parameters of the equation

V is Volume of reagent added at any instant of the titration

This semi-empirical equation was used because it is in the form of a sigmoid, which is the form of a titration curve, and it fit the experimental data very well. It is similar to an equation which can be derived from the relationship between pH and pK[4],

$$pH = pK + \log\left(\frac{[A]}{[HA]}\right) \qquad (6.2A)$$

to form:

$$Y = \frac{Ymax}{1 + e^{(a \cdot (z - z_o))}} \qquad (6.3A)$$

but it has a linear part (D · V + E) added.

The equation may be understood as consisting of two parts, illustrated in equation 6.1A. The parameters in equation 6.1A: A, B and C are in the non-linear part and D and E are in the linear part of the equation. Each parameter is nondegenerate and each has properties which can vary the curve. The linear term was added because in titrametric data, the sigmoid is rotated somewhat (D*V term) and the position of the curve or pH values

can be translated up or down (the E term). In the non-linear term, the parameter A controls the height of the curve, i.e., from equation 6.1A:

$$F(V)_{\text{limit } V\rightarrow \infty} = 0 + D \cdot V + E$$

or:

$$F(V)_{\text{limit } V\rightarrow -\infty} = A + D \cdot V + E \qquad (6.4A)$$

the limiting values of the sigmoid is between 0 and A. From observation of the curve at various values of the parameters, the parameter C changes the slope and the B term translates the curve left or right. This description of the parameters helps decide initial parameter guesses when fitting the data. This is discussed in Appendix 5.9A and in Table 1.4A.

Equation 6.1A can only be used to describe a single sigmoidal shape. In the case of multiple endpoints, only data about a single endpoint can be used. Even though this is an empirical model, the fit is exceptionally good, as Table 1.5 shows. The statistics used are described and discussed in Appendix 5.10A .

The result of interest in the fit of data to equation 6.1A is the volume at the point that the second derivative is zero. Equation 6.1A is easy to differentiate, and the result is equation 6.5A:

$$\frac{\partial F}{\partial V} = \frac{-A \cdot C \cdot e^{(B + C \cdot V)}}{\left(e^{(B + C \cdot V)} + 1\right)^2} + D \qquad (6.5A)$$

Taking the second derivative results in equation 6.6A.

$$\frac{\partial^2 F}{\partial V^2} = \frac{2A \cdot C^2 \cdot e^{2(B + C \cdot V)}}{\left(e^{(B + C \cdot V)} + 1\right)^3} - \frac{A \cdot C^2 \cdot e^{(B + C \cdot V)}}{\left(e^{(B + C \cdot V)} + 1\right)^2} \qquad (6.6A)$$

Equation 6.6A can be zero, $\frac{\partial^2 F}{\partial V^2} = 0$, if the value in the exponent is zero or if A or C is zero. Since the latter yields no information, the nontrivial result is equation 6.7A:

$$C \cdot V + B = 0 \qquad (6.7A)$$

From Table 1.4A, If A is zero, then the range of data values must be zero. If C is zero, then the slope of the curve is zero. Therefore the only non trivial value is when equation 6.7A is zero, and thus the volume when the second derivative is zero is given simply by equation 6.8A.

$$V = -\frac{B}{C} \qquad (6.8A)$$

Thus, the parameters of greatest interest from equation 6.1A is B and C.

5.4A The Model for Determining Dissociation Constants

The expression for volume of reagent as a function of pH is derived in the following text for the titration of sodium carbonate / sodium bicarbonate / sodium sesquicarbonate mixtures with HCl. The form of the derived equation is volume as a function of pH, that is, V(pH), even though experimentally pH is a function of titrant volume. The expression of pH(V) is more mathematically complex than V(pH) and both forms, for this titration, are single valued functions. By using an iterative method with modern computers, it is simple to find a value of pH at a given volume. The simplex program which uses this equation to fit the experimental data does not care about the form or type of function. The chemical equations are:

$$2\,Na^+ + CO_3^{--} + H^+ + Cl^- \rightleftarrows HCO_3^- + Cl^- + 2\,Na^+ \qquad (6.10A)$$

$$Na^+ + HCO_3^- + H^+ + Cl^- \rightleftarrows Na^+ + Cl^- + H_2CO_3 \qquad (6.11A)$$

$$H_2CO_3 \rightleftarrows CO_{2(aq,gas)} + H_20 \qquad (6.12A)$$

$$H_2O \rightleftarrows H^+ + OH^- \qquad (6.13A)$$

The corresponding equilibria are:

$$K_2 = \frac{[H^+]\,[CO_3^{--}]\,\gamma_{II}\,\gamma_{CO_3}}{[HCO_3^-]\quad\gamma_{HCO_3}} \qquad (6.14A)$$

$$K_1 = \frac{[H^+]\;[HCO_3^-]\;\gamma_H\;\gamma_{HCO_3}}{[\,H_2CO_3\,]\quad\gamma_{H_2CO_3}} \qquad (6.15A)$$

$$K_h = \frac{[\,CO_2\,]\;\gamma_{CO_2}}{[\,H_2CO_3\,]\;\gamma_{H_2CO_3}} \qquad (6.16A)$$

$$K_w = [H^+]\;\gamma_{II} \cdot [OH^-]\,\gamma_{OII} \qquad (6.17A)$$

From this point, it will be assumed that γ_{CO_2} and $\gamma_{H_2CO_3}$ are unity. The amount of sodium is constant, since its only source is the initial concentration of carbonate salts:

$$[Na^+] = \frac{2 \cdot M^o_{CO_3^-} + M^o_{HCO_3^-}}{V_{HCl} + V^o} = \frac{M^o_{T2}}{V_{HCl} + V^o} \qquad (6.18A)$$

Where:

$M^o_{CO_3^-}$ is the initial quantity of moles of carbonate

$M^o_{HCO_3^-}$ is the initial quantity of moles of bicarbonate

M^o_{T2} is the total initial quantity of bicarbonate and twice that of carbonate ($2 \cdot M^o_{CO_3^-} + M^o_{HCO_3^-}$)

V_{HCl} is the volume of HCl added at any point in titration

V^o is the initial volume in which the material is dissolved

When sesquicarbonate is present, one mole of sesquicarbonate will add one mole to each $M^o_{CO_3^-}$ and $M^o_{HCO_3^-}$ or three moles to M^o_{T2}.

Equation 6.18A takes into account the sodium contribution from the carbonate and bicarbonate, as well as it compensates for volume changes due to addition of reagent. The concentration of Cl^- present depends upon the quantity of HCl added and total volume of solvent:

$$[Cl^-] = \frac{C_{HCl} V_{HCl}}{V_{HCl} + V^o} \qquad (6.19A)$$

Where:

C_{HCl} is the molarity of HCl

From equations 6.10A, 6.11A, and 6.13A, the charge balance condition is:

$$[H^+] + [Na^+] = [Cl^-] + [OH^-] + 2[CO_3^{--}] + [HCO_3^-] \qquad (6.20A)$$

Substituting for Na^+ (6.18A), Cl^- (6.19A), OH^- (6.17A), and CO_3^{--} (6.14A):

$$[H^+] + \frac{M^o_{T2}}{V_{HCl} + V^o} =$$

$$\frac{C_{HCl}\, V_{HCl}}{V_{HCl} + V^o} + \frac{K_w}{[H^+]\ \gamma_H \gamma_{OH}} + \left(\frac{2 \cdot K_2\ \gamma_{HCO_3}}{[H^+]\ \ \gamma_H\, \gamma_{CO_3}} + 1 \right) \cdot [HCO_3^-] \quad (6.21A)$$

and solving for $[HCO_3^-]$,

$$[HCO_3^-] = \frac{[H^+] + \frac{M^o_{T2}}{V_{HCl} + V^o} - \frac{C_{HCl}\, V_{HCl}}{V_{HCl} + V^o} - \frac{K_w}{[H^+]\ \gamma_H \gamma_{OH}}}{\frac{2\, K_2\ \gamma_{HCO_3}}{[H^+]\ \ \gamma_H\, \gamma_{CO_3}} + 1} \quad (6.22A)$$

All of the carbon is due to the initial concentration of bicarbonate and carbonate. From equations 6.10A , 6.11A, and 6.13A, the carbon mass balance is:

$$\frac{M^o_T}{V_{HCl} + V^o} = [CO_3^{--}] + [HCO_3^-] + [H_2CO_3] + [CO_2] \quad (6.23A)$$

Where:

M^o_T = the total initial moles of carbonate and bicarbonate or $M^o_{CO_3^-} + M^o_{HCO_3^-}$

Each mole of sesquicarbonate is added twice, since it contains one each of bicarbonate and carbonate.

This equation neglects the CO_2 lost from the system after saturation of CO_2 occurs, around ca. pH 6. Substituting equation 6.16A into 6.23A to eliminate the $[CO_2]$ term,

$$\frac{M^o_T}{V_{HCl} + V^o} = [CO_3^{--}] + [HCO_3^-] + (1 + K_h) \cdot [H_2CO_3] \quad (6.24A)$$

Taking equations 6.15A, 6.14A, and substituting into 6.24A, to put everything in terms of $[HCO_3^-]$,

$$\frac{M_T^o}{V_{HCl} + V^o} = \left(\frac{K_2 \ \gamma_{HCO_3}}{[H^+] \ \gamma_H \gamma_{CO_3}} + 1 + (1 + K_h) \frac{[H^+] \ \gamma_H \ \gamma_{HCO_3}}{K_1} \right) [HCO_3^-] \quad (6.25A)$$

Substituting 6.22A for $[HCO_3^-]$ into 6.25A for the $[HCO_3^-]$ term and rearranging and multiplying both sides by the denominator of 6.22A gives:

$$\frac{M_{T2}^o - C_{HCl} . V_{HCl}}{V_{HCl} + V^o} =$$

$$\frac{K_w}{[H^+] \ \gamma_H \gamma_{OH}} - [H^+] + \frac{\frac{M_T^o}{V_{HCl} + V^o} \cdot \left(\frac{2 \ K_2 \ \gamma_{HCO_3}}{[H^+] \ \gamma_H \gamma_{CO_3}} + 1 \right)}{\frac{K_2 \ \gamma_{HCO_3}}{[H^+] \ \gamma_H \gamma_{CO_3}} + 1 + (1 + K_h) \cdot \frac{[H^+] \ \gamma_H \ \gamma_{HCO_3}}{K_1}} \quad (6.26A)$$

and separating out the terms involving $V_{HCl} + V^o$ and then bringing V^o to the right side:

$$V_{HCl} =$$

$$\frac{- M_{T2}^o + C_{HCl} . V_{HCl} + \frac{(M_T^o) \cdot \left(\frac{2 \ K_2 \ \gamma_{HCO_3}}{[H^+] \ \gamma_H \gamma_{CO_3}} + 1 \right)}{\frac{K_2 \ \gamma_{HCO_3}}{[H^+] \ \gamma_H \gamma_{CO_3}} + 1 + (1 + K_h) \cdot \frac{[H^+] \ \gamma_H \ \gamma_{HCO_3}}{K_1}}}{\frac{- K_w}{[H^+] \ \gamma_H \gamma_{OH}} + [H^+]} - V^o \quad (6.27A)$$

Bringing the $C_{HCl} . V_{HCl}$ term to the left and solving for V_{HCl}, we have separated pH and volume:

$$V_{HCl} = \frac{\left(- M^{\circ}_{T2} + \frac{M^{\circ}_{T} \cdot \left(\frac{2 K_2 \gamma_{HCO_3}}{[H^+] \gamma_H \gamma_{CO_3}} + 1 \right)}{\frac{K_2 \gamma_{HCO_3}}{[H^+] \gamma_H \gamma_{CO_3}} + 1 + (1 + K_h) \cdot \frac{[H^+] \gamma_H \gamma_{HCO_3}}{K_1}} \right) - V^{\circ}}{1 - \frac{C_{HCl}}{\frac{- K_w}{[H^+] \gamma_H \gamma_{OH}} + [H^+]}} \qquad (6.28A)$$

If activities are assumed to be near unity the relation is :

$$V_{HCl} = \frac{\left(- M^{\circ}_{T2} + \frac{M^{\circ}_{T} \cdot \left(\frac{2 K_2}{[H^+]} + 1 \right)}{\frac{K_2}{[H^+]} + 1 + (1 + K_h) \cdot \frac{[H^+]}{K_1}} \right) - V^{\circ}}{1 - \frac{C_{HCl}}{\frac{- K_w}{[H^+]} + [H^+]}} \qquad (6.29A)$$

5.5A Convergence Criteria

Whenever the simplex routine requires a value from error space, it calls a function which calculates the error between the parameters that are used by the guesses and the actual data. The equation used to calculate the error space is equation 6.30A. It calculates the sum of the square deviations.

$$\text{error} = \sum_{i=1}^{n} (F(V_i) - pH_i)^2 \qquad (6.30A)$$

Where:

$F(V_i)$ is the value of equation 6.1A or 6.29A with the parameters given by the calling routine at each experimental V_i

pH_i is the experimental pH at each V_i

The relative criteria for convergence of the simplex is shown in equation 6.31A. This value is based on the normalized smallest double precision number for a UNIX operating system, Vax 11/780 machine, f77 compiler. When the simplex vertices or value is less than this criteria, then a minimum for the parameters is considered to be found.

$$\text{Criteria} < 1000 * 2.8 * 10^{-17} \qquad (6.31A)$$

When this criteria has been reached, the squared deviation, equation 6.30A, is at a minimum for the region of the equation that the simplex is located.

5.6A Additions to Simplex Least Squares Error Function

To use the equilibrium constants model (equation 6.29A) required a trivial modification in the least square error function; the X and Y axis were switched. The model used for determining the inflection points, 6.1A, required two unique additions to aid convergence.

The first addition clipped parameters which were out of range. When the simplex is moving through error space, the parameters are changed in many directions. Numbers in the UNIX FORTRAN compiler used (f77) are limited to values whose magnitude is not greater than 10^{38} and not smaller

than 10^{39}. Since the model equation 6.1A has an exponential term, under-flow may occur if the exponential argument is too negative and overflow if it is too positive. The solution to this problem was to test the exponential parameter, and if it is greater than 70 or less than -70, it was replaced with 70 and -70 respectively. This prevents the exponential from getting larger in magnitude than 10^{30} and 10^{-31}. A flag is set every time that this is done in the event convergence was achieved in this invalid region.

The second addition involved lifting the error surface in regions where the second derivative is not expected to be zero. This was done to minimize convergence to local minimums, which happened with some sets of data. Sometimes simply restarting the simplex was not enough to get it out of the local minimum. It was apparent that the fit was not the final one, as the R^2 value was smaller than other fits, approximately 0.98.

Lifting of the error surface was accomplished by calculating a relative error based upon the numerical second derivative (equation 6.32A). The relative error between this value and the second derivative gives an estimate of the sum of square deviation (equation 6.30A) when equation 6.32A was greater than the criteria 0.1.

$$REPN = \left| \frac{pH_{guess} + \frac{-B}{C}}{pH_{guess}} \right| \qquad (6.32A)$$

$$error = \sum_{i=1}^{n} (F(V_i) - pH_i)^2 + \left| \frac{pH_{guess} + \frac{-B}{C}}{pH_{guess}} \right| \qquad (6.33A)$$

(for REPN > 0.2)

Where:

REPN	is the Relative Error between second derivative of the Parameters from the fit and the Numerical second derivative
B and C	are the parameters from equation 6.1A
pH_{guess}	is derived from the numerical derivative.

Lifting of the error surface was used to speed convergence and to help prevent local minimums. The technique used to lift the error surface assumes that the value of $\frac{-B}{C}$ is close to the numerical derivative. Lifting the error surface is a feature which can be switched on or off by specifying an error bias before the fitting process starts. In actual practice, the data was first fit with this feature on, and then the simplex was expanded about 10% and then fit again with the feature off.

5.7A How to Use ENDPOINT and its Commands

Most of the commands in **ENDPOINT** have default values, including the "fit" command which supplies reasonable criteria as well as initial guesses for the parameters of the fit. When a value or option is required, a prompt is given requesting the option and if a default value is available, then it is presented in curly brackets { }. Pressing **return** enters the default value.

An **ENDPOINT** command is activated by entering the command name as listed on the menu screen and pressing return, or by entering the command name and the parameters on the same line, separated by spaces. If you do not enter parameters, then you are prompted for them.

5.8A Procedure for Model Analysis of Data with Endpoint

When analyzing data, **ENDPOINT** was used as follows:

1) The data was read into program memory ("new" command).
2) An endpoint was selected using "find" and by viewing the data listing to determine whether the pointer is on the data with the maximum derivative.
3) The data subset whose first derivative was 30% of the value was marked by the "percent" command. This selects the data subset that will be used for fitting and other manipulations.

4) The cursor was moved to the maximum first derivative with the "max" command, and the data subset boundaries adjusted with the "percent" command again.
5) If there are places where the second derivative changes sign in the subset besides at the maximum first derivative, the "glitch" command was used. This command smooths the data at the first derivative.
6) The data was then fit with the "fit" command, which uses the empirical model in equation 6.1A and method described below.. The fit was set to lift the error surface ("error bias") in order to speed convergence. Then the simplex was expanded and "refit" again. Finally, the range of points in the data subset was expanded and contracted by a few data points on each end to see if the additional or fewer points would improve the fit. The fit usually did not change, but if it did, the fit with the best statistics was used.

5.9A Initial Guesses for Parameters of the Models

The simplex fitting routine requires that initial guesses for the parameters to be fitted are provided. The guesses need not be very close to the actual (usually unknown) values, but they should be a reasonable approximation. The word "reasonable" is subjective and depends upon the equation, but generally the guess should be within 100% of the value; sometimes within a few orders of magnitude is good enough. Poor guesses will cause the simplex to take more time and possibly provide more false convergence's due to local minimums, which require restarts. If the parameters are bounded (not allowed to have certain values), sometimes a poor guess will cause convergence to one of the parameter boundaries. Therefore, it is good to start with reasonable values for initial guesses.

ENDPOINT allows the user to either enter initial guesses or it can supply initial guesses . Equations for the empirical model are given in Table 1.4A. The initial guesses used for determining equilibrium constants were literature values, but it was found that any value within a several orders of magnitude would provide for a good guess.

Parameter	Guess used	Effect on Equation
A	$\lvert pH_{\text{data value range}} \rvert$	Height of curve
C	$\frac{-4 * S * \left\lvert \left[\frac{dpH}{dV}\right]_{max} \right\rvert}{A}$	Affects slope
B	$-V_{\frac{d^2pH}{dV^2}=0} * C$	translate curve left or right
D	$-V_{\frac{d^2pH}{dV^2}=0} - \frac{A}{2}$	Rotates curve
E	pH at numerical endpoint	translate curve up or down

Table 1.4A Parameters of equation 6.1A; initial guesses and each parameter's effect on the equation.

S is the sign of the average slope of the data subset

A,B,C,D,E are parameters from equation 5.1b

$\left[\frac{dpH}{dV}\right]_{max}$ is the maximum first derivative in data set

$pH_{\text{data value range}}$ is the range of pH data in subset

5.10A Statistics for Data Modeling

Several statistics were used to determine the error in fitting and determine the validity of the empirical model that was used. The statistics indicate the goodness of the fit[40,41]. These statistics are the R value and the relative model variance. The **ENDPOINT** program, described earlier, calculates, reports and stores these values after data is fit. They are reported as R, Variance, Residual, Model Variance and Relative Model Variance. These values, their significance, and experimental results are described below, and the values are listed in Table 1.5.

5.10.1A The R Value

The R value or sample correlation coefficient of the fit, is a measure of the goodness of the fit[40]. It has values between -1 , 0 and 1, with each extreme having its own meaning:

R value = 1.0: perfect correlation or linear relationship (fit)

R value = 0.0: no fit (worst case)

R value = -1.0: inverse correlation (e.g., R=-1 for a reflection of the data)

The R^2 value is used when data is modeled. The R^2 value is known as the coefficient of multiple correlation of the fit. The closer that it is to 1, the more "explanatory" power the model is said to have, or, in other words, the closer it is to 1, then the model used to fit the data describes the data in a better manner.

The mathematical definition of the R^2 value is given in equation 6.34A. This is the sum of squares explained by the fit divided by the product of the standard deviations of the data and of the fit values.

$$R = \frac{\sum_{i=1}^{n}(pH_i - \overline{pH})^2 * (F(V_i) - \overline{F(V)})^2}{\sqrt{\sum_{i=1}^{n}(pH_i - \overline{pH})^2} * \sqrt{\sum_{i=1}^{n}(F(V_i) - \overline{F(V)})^2}} \qquad (6.34A)$$

Where:

V = Volume

$F(V_i)$ = the fit pH at each V

pH = the experimental pH values

$\overline{F(V)}$ = the mean of the values of the fit

$\overline{pH}$ = the mean of the experimental pH values.

The denominator is the product of the separate square root of variance about the mean for the data and the model; the numerator is the sum of squares due to the model.

5.10.2A Variance

The "variance" is the variance of the data, defined in equation 6.35A.

$$\text{Variance} = \sum_{i=1}^{n}(pH_i - \overline{pH})^2 \qquad (6.35A)$$

Where:

pH_i is each value of experimental pH

$\overline{pH}$ is the mean of the experimental pH values

The variance is used in the calculation of relative model variance.

5.10.3A Residual or Estimate Error of the Fit

Another statistic is the residual, estimate of error of the fit or estimator about the model[40,41]. This is derived from the variance between the experimental pH and the fit pH. This indicates the variation of the fit about the model.

$$\text{residual of fit} = \sum_{i=1}^{n} (F(V_i) - pH_i)^2 \qquad (6.36A)$$

Where:

$F(V_i)$ = fit pH at each experimental point V, volume

pH_i = experimentally determined pH for each Volume

5.10.4A Model Correlation

Another measure of the goodness of the fit which was calculated is the model correlation[41]. This is determined from the variance between the fit pH and the average data pH. The definition of model correlation is given in equation 6.37A. This is the source of variance due to the model.

$$\text{model correlation} = \sum_{i=1}^{n} (F(V_i) - \overline{pH})^2 \qquad (6.37A)$$

Where:

$F(V_i)$ is the pH value of the fit

$\overline{pH}$ is the mean of the experimental values of pH

This value gives variance due to the model. This value is used to calculate the Relative Model Variance.

5.10.5A Relative Model Variance

The relative model variance is the model correlation from equation 6.37A divided by the data variance 6.35A resulting in equation 6.38A.

$$RMV = \frac{\sum_{i=1}^{n}(F(V_i) - \overline{pH})^2}{\sum_{i=1}^{n}(pH_i - \overline{pH})^2} \qquad (6.38A)$$

The sum of the Model Variance and the Estimate Error should be close to the Variance in a good model. Another indication of a good model is the relative model variance (RMV), which indicates how much of the total variance is due to the model. When the RMV value is a significant part of the variance, this indicates that the model accounts for a high portion of the variance and it indicates a very good model[41]. A model which is not good but is near the data points would have a small RMV.

Appendix B Voltammetric/HPLC Instrument
(for section 2)

1.0B Abbreviations and Symbols

1) Convention used to label parts in circuit diagrams

R.....Resistor, usually 5% tolerance
S.....Switch
C.....Capacitor
Q.....Transistor or other solid state device
LED.....Light Emitting Diode, default is a red color
T.....Transformer
t.....tie point
F.....Connection which is continued in another figure
D.....Diode
IC.....Integrated Circuit
IS.....Socket for integrated circuit
S.....Switch
DPTT.....Double Pole Triple Throw (switch)
P.....Variable resistor (Potentiometer)
J.....Connector
N.C.....Not Connected
+5 V.....Positive 5 volt DC power
-15 V.....Negative 15 volt DC power
+15 V.....Positive 15 volt DC power
TTL.....Transistor Transistor Logic level electrical signal or circuit
NO.....Normally Open connection or path
NC.....Normally Closed connection or path
BNC.....Bayonet type coaxial connector for RG-58 cable

2) Other Abbreviations

MME.....Metrohm Multi-Mode mercury Electrode
SME.....Static Mercury Electrode
DME.....Dropping Mercury Electrode
HME.....Hanging Mercury drop Electrode
HPLC-EC..High Performance Liquid Chromatography with ElectroChemical detection
FIA.....Flow Injection Analysis
SCE.....Saturated Calomel reference Electrode
WJ.....Wall Jet electrode
WT.....Wall Tube electrode
AVC.....Air Valve Control
A/D.....Analog to Digital Converter
D/A.....Digital to Analog Converter
I/V.....Current to Voltage converter or conversion value
clk.....Clock (frequency) connection
HSWAVE....The name of the program which drives the electrochemical instrument
DEC.....Digital Equipment Corporation
PARC.....Princeton Applied Research Corporation
BAS.....BioAnalytical Systems

2.0B General Operation

2.1B Analyzing Data for Area, Plates, Peak, etc.

Selected portions of data can be analyzed for background, range, area between the background and the curve, the retention time of a peak, the half width of a peak, and the number of theoretical plates. To obtain this analysis, the **Display** menu **I(T)** command "I" is used. The data analyzed are selected according to the display mode: summed pairs, differential pairs, or normal mode. There are three additional modes related to normal mode data. They are: all data, which is simply the data from all of the channels; curve 1 which is data from every odd channel; and curve 2 which is data from every even channel.

Before the "I" command can be executed, the region of data to be analyzed has to be defined. This is performed by using the "**M**" mark command. The region of interest is marked by first moving the cursor to the left of the region and marking by typing **M1** ; then to the rightmost and entering **M2**. Then, the area which defines the background signal must be marked by using **M3** for the left of the background region and **M4** for the right of this region. The background is of the form:

$$I = m * T + b \qquad (1B)$$

Where:

I is current

T is Time

m and b are parameters of the background signal equation

The background region is selected depending upon how it is to be defined. This region can either be 1) a set of data which will be averaged or 2) two points with a straight line between them (this is a background with b and m defined). The method of obtaining the background is determined by the experimenter. The default is a line fit (1), with **M3** at the same point as **M1** and **M4** at the same point as **M2**.

After L is pressed, the method of determining the background signal is prompted for. If a fit background is selected, the boundaries of the data region are the same as the boundaries for the background region. On the following page is an example of the output. the listing shows the file name of the data along with the number of data points and ID number of the waveform. Then listed are comments for the data, the channel number for data region and background region, current minimums and maximums, and the area under the curve.

The area under the curve is calculated by estimating the integral using a numerical summation. The area between data points is estimated to be the trapezoid formed by each pair of data points and the background. These values are then summed to estimate the area.

$$\text{Area} = 0.5 * \sum_{i=M1}^{M2-1} (T_{i+1} - T_i) * (I_{i+1} - B_{i+1} + I_i - B_i) \qquad (2B)$$

Where:

T	is time value
I	is value of current
B	is current calculated from background
M1, M2	are the boundaries of the data set

The background equation 1B is presented, which should be briefly examined for any oddities. Next comes Area Weighed Retention Time (AWRT), where the time of each data point in the data region is given a weight according to its current value and then summed according to the following equation:

$$\text{AWRT} = \frac{\sum_{i=M1}^{M2} T_i * I_i}{\sum_{i=M1}^{M2} I_i} \qquad (3B)$$

a line fit Following the AWRT is the retention time based solely on the time value of the maximum . The channel number when this occurs is also given,

and the half width is calculated from the half height of the peak. Then the number of theoretical plates are given, which is calculated by:

$$N = 5.54 * \left(\frac{\text{Retention\_Time}}{\text{half\_width}} \right)^2 \qquad (4B)$$

and finally, the current at the half peak, relative to the background is calculated. An example of the printout follows:

```
Press  F or A  Fit line or Average between marks 3,4 {F} F

Data  0NOV12.003  : 274 points   52  wave type

ABCD
niacin 5*10-5 M  2000 PSI

Marks between:     221     256 back:     221     256        Channel # for marks

Current Min & Max   -0.619201183    µA  -0.156017780    µA
Background Equation subtracted:  -0.459839395    µA    0.442960300E-07 µA
Area,  Background eqn. offset     -0.494456223E-01µA * Min

Background equation:  I =   -0.278664E-03 * channel# +   -0.944332E-01
Area Weighted Retention Time      1.96961578     Min
Retention Time                    1.94916666     Min     233# channel

Time at each half width           1.90666664     Min    2.00162005     Min
Distance between halves           0.949533358E-01 Min

      N = 5.54 * (   1.949167    /  0.9495334E-01) **2

Number of theoretic plates =      2334.464

( Between channels   227   229 and    239   241:
 Relative half peak =          -0.229919697    µA )
 Press return....
```

2.2B Short Summary of the Data, HSSUMA

There is a utility program, **HSSUMA** which furnishes a short summary of data stored in files that are labeled with the same name and that have sequential numeric extensions (e.g., NIACIN.004, NIACIN.005, NIACIN.006, NIACIN.007, etc.). The summary is presented on the terminal screen or in a file on disk. The default for the file name is the name of the data with the extension .SSM (example: for the NIACIN set in the previous example, the summary would be named NIACIN.SSM).

The summary consists of a header and, for each file, four lines of information. It is very terse and intended that way so that the experimenter can have a quick reference to the data set. If a more verbose and descriptive summary is needed, the program **HSSUM** should be used.

The summary consists of the following:

1) The first line contains, in order, the file name, the waveform type (SQW for square wave, etc.), how the drop knocker was set, cycle type (cyclical or normal), the current gain, the current to voltage conversion factor, the current offset, number of complete scans and total number of data points.
2) The second line consists of a list of all the parameters used for the waveform, in the same order as it would appear in the menu.
3) The third line contains the information given in the waveform;s menu screen; for example, scan rate, time for each scan, etc.
4) The fourth line gives the comments entered by the experimenter.

Here is an example of a summary produced by **HSSUMA**:

HSWAVE version short summary 2.020 Summary report starting with DY1:0AUG31.001

Info for each file, in order:

Name Wave Knock Cycle Gain I/V:μA/V I.offset # scans # data
Waveform parameters are listed here
Comments are listed here, if any

0AUG31.001 SQW scan norm 10. 100.0000 -98.00000 1 848
-99.985 -4.997 -49.935 -2200.008 0.000 25.000 300.000
Rate -0.9994629E-01V/s; 848 points; -99.9849 to-2197.7188 mV; 21.500 sec
Square Wave of Background buffer; pump is on

0AUG31.002 SQW scan norm 10. 10.00000 -9.800000 1 848
-99.985 -4.997 -49.935 -2200.008 0.000 25.000 300.000
Rate -0.9994629E-01V/s; 848 points; -99.9849 to-2197.7188 mV; 21.500 sec
Background, greater sensitivity (10uA F.S.) sqw

0AUG31.003 SQW scan norm 10. 100.0000 -98.00000 1 848
-99.985 -4.997 -49.935 -2200.008 0.000 25.000 300.000
Rate -0.9994629E-01V/s; 848 points; -99.9849 to-2197.7188 mV; 21.500 sec
10-5 M thiamine sqw

0AUG31.004 DDP cycle norm 10. 100.0000 -98.00000 1 444
-999.962 -4.997 -99.985 -2099.985 0.000 700.000 50.000 300.000
Rate -0.7139021E-02V/s; 444 points; -999.9619 to-2095.9412 mV; 222.00 sec
10-5 M thiamine diff pulse

2.3B Long Summary of Data, HSSUM

The utility program, **HSSUM** produces a long summary of data stored in files which have the same name and sequential numeric extensions (e.g., NIACIN.004, NIACIN.005, NIACIN.006, ..NIACIN.007, etc.). The summary is presented on the terminal screen or in a file on disk. The default for the file name is the name of the data with the extension .SUM (example: for the previous example, the NIACIN set , the summary would be named NIACIN.SUM).

The summary consists of all of the parameters that the data was run under, with a descriptive label for each. Included are the range of voltages used, current range measured and first and last data point time.

Here is an example of a summary of one file produced by HSSUM:

```
HSWAVE version   2.000000Summary report starting with DY1:0AUG31.002

DY1:0AUG31.002  file size =       1536 words
Data stored under version    1.400
    848 Data points;                    1  scans of    848 points each

Scan   4 cycles/scan =   424;  rev cycles/scan =         0;  data/cycle =    2
Data comment:
Background,  greater sensitivity (10uA  F.S.) sqw
Scan    4;  type = normal
    848 points;                    848 points/scan;          1 scans
Knocker was set to knock: scan
Air valve was on for time    300.0000ms
No initial Potential: start reference =   -99.98486    mV
Current display mode DIFF
 I/V conversion    10.0000 µA/V;  A/D gain    10.00000    offset 0.000
 A/D res   4095.000    ;  A/D channel #       5
 Time base   0.1000000          ms/Tick

A/D Calibration:
    1.019000mV <=>  1839.# ;          0.000000mV <=>  2048.# ;
D/A resolution    65535.00    ;  D/A Calibration:
    2500.000000mV <=> 65535.# ;  -2500.000000mV <=>      0.# ;
ioff     2;  strip chart offset  0.0000000    ;  display   2048
diff/sum strip chart magnification       0
Buffer size   4096;  status:                1      0

scan =    4 general parameter file SY:HSSQW.WAV

initial potential                -99.98486
step potential                    -4.997314
pulse potential                  -49.93530
final potential                 -2200.008
t0  initial time                    0.0000000
t1  pulse                          25.00000
tair  SME time 0=off              300.0000
max scans;0=nonstop                 1.000000
knocker                             3.000000
cycle type                          0.0000000
go                                  0.0000000
save and go                         0.0000000

Voltage from   -2247.692          to   -50.01147
Time of last point =   0.3583333Min ;  Ave current  0.9670705
Current mins =   -9.7513199E-03 -9.7513199E-03 µA
Current maxs =    12.14512             12.65705       µA
Time of current min,  max   5.4166666E-03  0.3004167    Minutes
# off scale values: low =                  0;  high =      118
```

2.4B List of Global Commands for HSWAVE

Global commands in **HSWAVE** are commands which are active in any menu (i.e., at any time) except when the graphics screen is displayed. This section presents a list of the commands and what they do.

REDRAW redraw the menu screen.

STOP Stop the current scan immediately.

HELP Give help. If an argument is used, help is given for the argument. The argument should be either a function from the current menu or a global command. Example: **HELP PULSE**

? Short form of help. The same as help but produces a summary instead of a long form. Example: **? PULSE**

?? Lists the global command names.

MONITOR Arguments: **ON OFF** Turns the data information monitor on or off. This shows the status of a scan. Example: Use **MON OFF** for fast scans.

OUTPUT Argument is a voltage number. Forces the D/A to output a specific voltage when the scan is off. Example: **OUT 200** will immediately apply 200 mV at the output of the D/A.

Input Reads and displays a value from the A/D converter (current) when the scan is off.

DISPLAY The number of complete scans to be displayed on the real time display (oscilloscope). Example: **DIS 20** If no argument is given, a reasonable value is used depending upon the scan type and number of scans chosen.

ROFF Provide an offset for the strip chart output.

MAGNIFY Magnify the output to the Strip chart recorder by 2

GAIN Change the gain of the A/D converter. Arguments are 1, 10, 100, 1000. Example: **GAIN 10** sets the A/D gain to 10. See Tables 2.5 and 2.6 (in main text) for information on gain.

ALARM Arguments: **ON OFF** This alarm is used when scanning. If the current goes off scale for the A/D, the terminal bell will ring. Example: **ALARM ON** will turn the alarm on.

OFFSET Argument is a number for current in mA. This value should be the offset of the current value due to the I/V converter (electrometer), and the Current Offset device. See Appendix 4.7B for more information on the current offset device. Example: **OFF 0.1** tells the computer that the actual value for the current is 0.1 mA greater than what it measures. Whenever a waveform is started, a prompt is automatically given for entering this value. This command is used if the offset needs to be changed.

The following affect the terminal, pen plotter and real time display:

DIFF Switch display mode to differential pairs of data.

NORM Switch display mode to normal.

SUM Switch display mode to summed pairs of data.

2.5B How to Get Help

On-line help is available for just about any command. To get help for menu commands listed on the screen, enter

HELP *command*

where *command* is the name of the command (e.g., **HELP LIST** will give help for the list command). If all that is needed is a short form of help, enter **?** *command* where a question mark **?** is used instead of the word help.

If a list of the global commands is required, enter **??**, two question marks or to get help for each one, enter **HELP** *global command* where *global command* is the name of the command.

2.6B How to Abort a Command or Function

Some commands may be aborted before they start. If a message appears, "press return" , pressing the **esc** key or, the **esc** key and then **return** will abort the process.

Another case is when displaying data or plotting. Pressing the **esc** key before the plot is done will abort the plot.

When a scan is finished, and it was started with "**Save**", and the program prompts for entering a filename, the save may be aborted by entering the esc key instead of a file name.

The **esc** key will not abort a scan. The only way to abort or end a scan is to enter the global **STOP** command.

The program may be ended by activating the "Exit Program" in the main menu. When the program ends, the RT-11 monitor is started. To restart the program, enter :

@VM:HSWAVE

3.0B Example of Displays on the Terminal Screen

3.1B Start up screens

The following is an example of what appears on the terminal screen when the instrument is started. Information in italics does not appear on the screen. Items with a dotted underline are entered by the user.

HSWAVE startup disk for 11/73
Electrochemical Waveform Generator and Data Acquisition for HPLC
There should be a formatted and initialized disk in DY1:
(Top disk drive) for storing data.
Do not remove the startup disk in DY0:

This disk will erase and format VM: and copy necessary files
to VM: to run HSWAVE.

If the computer asks you to enter the date, enter the correct date.
An example of the format of the date is as follows: 28-Mar-95

Please enter
Today's date: 21-aug-93
Current time: 5:00

At this point, if the computer does not have the date and time, you will be prompted for this information. Use the correct date.

Please wait a moment while VM: is being setup.
HSWAVE will start after everything has been set up.

There are other programs which can be run when HSWAVE is exited. They are:
HS3D Display HSWAVE data as 3 - D surface plot
HSSUM, HSSUMA gives you a summary file about a set of data files
(if they were stored with number extensions)
The virtual disk, VM: which resides in memory is now being setup. This takes about 2 minutes.
When VM: is ready, you will see the following which indicates that VM: is being booted:

HSWAVE
Electrochemical Waveform Generator and Data Acquisition for HPLC
To restart, enter @VM:HSWAVE

There should be a formatted and initialized disk in DY1:
(Top disk drive) for storing data.
Do not remove the startup disk in DY0:

Hswave error: if when trying to save data, it says "delete error",
make sure that HSWAVE.DAT on the data disk (DY1:) is unprotected
(and that there is a disk in DY1: !)

When the HSWAVE command file starts, you will see:

21-Aug-91
05:01:48 *When the program starts, you will see:*
11/73 system *and then the first screen is presented:*

Main menu commands:

A pulse voltammetry
B simple pulse
C other functions
D Internal values
E List Data
F Plot Data
G Storage
H Display
I Cursor functions
J Help
Z exit program

Hswave v 1.43 -main menu command :

3.2B Example Menu Screens

3.2.1B Scanning Waveform; Differential Pulse

This section presents an example of the scanning waveform menu for differential pulse. The first line of the menu is the header which presents the waveform name and internal number (differential pulse is waveform #3). The column on the left consists of single capital letters which may be used to select the command or parameter. The second column consists of parameters or commands which describe each function and may be used in lieu of single letters: e.g., to start a scan, enter either **I** or **Go**. The third column consists of parameters for many of the functions. The last column contains units of the parameter.

Numerical parameters which are entered are converted to the nearest value that the instrument will use. For example, if a value of -1000 was entered for the function **A Initial Potential**, it is then changed to -999.962 mV, which is the nearest quantity to -1000 that the D/A converter can actually output. Time parameters have the same property, where values are rounded to the nearest 0.1 ms.

Parameter **I Max Scans; 0=nonstop** determines the number of times that a scan will be run. If zero is in this place, the scan will continually repeat until the user enters the command **STOP.**

Parameters **Knocker** and **Cycle** use words as their parameters. To discover what their parameters are, enter the command and a list of values will be given.

Under the menu is an information line, which gives additional information concerning the scan with the parameters as listed in the menu. In this example, the scan rate is $-0.769 \cdot 10^{-2}$ V/s, the total number of data points is 384 (or 192 differential points); the exact starting potential is -999.9619 mV; the exact final potential is -1947.1656 mV and the time for one complete scan is 182.4 seconds. When the global command **MON** is set **ON**, the line below the information line will contain the status of the current scan. Underneath that is the prompt for the commands.

3) Diff Pulse Menu commands:

A	initial potential	-999.962	mV
B	step potential	-4.997	mV
C	pulse potential	-74.960	mV
D	final potential	-1949.989	mV
E	t0 initial time	0.000	ms
F	t1 time each step	650.000	ms
G	t2 pulse time	50.500	ms
H	tair SME time 0=off	300.000	ms
I	max scans;0=nonstop	1.000	
J	knocker	cycle	
K	cycle type	normal	
L	go		
M	save and go		
W	Wait/save		
Z	exit menu		

Rate -0.76882E-02V/s; 384 points; -999.9619 to-1947.1656 mV; 182.400 sec

Hswave v 1.43 -Diff pulse command:

3.2.2B The Plot Screen

The screen for plotting data may be entered either through the main menu **Plot** or through any of the **Display** screens. The options **A** through **L** affect both Tektronix (terminal) screen plot as well as the plot output from the Hewlett-Packard 7475 plotter.

Plot parameters for I(T)

A	Xlimit	autoscale						
B	Ylimit	autoscale						
C	Axis	autospace						
D	Connectivity	on						
E	XReverse	off						
F	YReverse	on						
G	Significant figures	5.000						
H	x size	17.000						
I	y size	10.000						
J	Time Label unit	min						
K	XY limits change							
L	Channels change							
M	Pens	Line 1	Axis 2	Title 3	Label 4	File 5	Comm 6	
N	Quadrant	0.000						
P	Plot							
Z	exit menu							

X = 0.01642 to 3.48333min; Y = -7.86922 to -2.94974uA
channels = 1 to 418 total #scans = 209 each scan = 2

4.0B Maintenance, Operation and Circuits

This section discusses the instrument: maintenance, utilization and the circuitry developed for it. Schematics and diagrams are located at the end of this section.

4.1B Air Valve and the Control Circuit (AVC)

The Air Valve is used to control pressure to the MME. The valve has three connections: common, normally open (NO), normally closed (NC). When the valve is off, common is open to (NO). When the valve is energized, the air path from common to (NO) is shut and the air path from common to (NC) is open. The airway above the diaphragm, the MME needle control, is connected to common and the (NO) connection is attached to the main pressure.

When the valve (or the instrument) is off, pressure is applied to the needle control which causes the needle to seat and the mercury flow is cut off (section 2, Figure 12). When the air valve is energized, the (NO) section is shut off, and the needle control is vented to atmospheric pressure and there is mercury flow (main text Figure 13). Since the needle control is a diaphragm on the other side of the mercury chamber, there is no possibility of venting mercury fumes to the air, unless the diaphragm is broken.

The Air Valve Control (AVC)

This electronic device provides for manual on and off for the air valve which controls the MME and it acts as an amplifier so that the digital output TTL level signal from the computer can control the air valve. It also has a light which indicates when the air valve is in a position that would allow mercury to flow. The schematic for this device is shown in Figure 1B.

Features

The AVC features an on/off switch, a manual mode switch, and an indicator which lights when power is applied to the air valve (and thus when mercury is flowing).

The heart of the circuit is an Angar Scientific #339V6H8 two way air valve. When unpowered, the air pathway is open between common and NO; when energized, the air path is open between common and NC (and NO is closed). All the other circuitry is used to power the valve, provide a voltage interface to the computer, and provide a way to switch from manual to automatic.

Operation

The transformer box must be plugged into a 60 Hz 110 VAC power source. The switch S1 ("power") turns the low power on and off. The switch S2 ("manual") will turn on the output to the air valve if the remote signal is not overriding this switch. To manually turn the air valve on and off and bypass the remote signal , set switch S2 to manual and use S1 to switch on and off. LED1, a red light, indicates the state of the signal to the air valve.

Circuit description

The power is derived from a nominal 9 volt DC wall transformer. It goes through on/off switch S1 and then to D1. Diode D1 prevents the circuit from blowing out if reverse polarity or AC is applied. C1,C2, D2 D3 and Q3 make up the voltage regulator circuit. A voltage between 6 and 7 volts is required for this circuit. D2 and D3 brings the ground of the integrated five volt regulator, Q3 up by 1.5 volts, making the output 6.5 volts. This voltage is used to power the rest of the circuit. Q3, D2 and D3 may be substituted with a 6 volt regulator such as an LM7806.

When Switch S2 is off ("auto mode"), the output is on when a TTL low signal (0) is applied. When the output is on, the air valve is on.

The control signal input has a reversed biased diode, D5, to prevent signal undershoot. D4, R1 and R2 form an impedance termination to minimize signal reflection. Both transistors Q1 and Q2 are configured as

switches, with the input through their bases. Either R3 or R6 drive the base of transistor Q1. The signal through R3 is from the control signal. R6 is connected to S2, which when switched to ground, will override a TTL signal at R3 and turn the output on. When Q1 is on, the base current for Q2 , the output transistor, which comes from resistor R4 is effectively shunted to ground and Q2 is thus off. When Q1 is off, the current going through R4 goes through the base of Q2 and thus the output is on. LED1 indicates with a red light when the output is on. Reversed biased D6 prevents inductive spikes from the coil of the air valve from damaging the circuit.

The Pressure Router

The Pressure Router is a connector for the air valve, gas, and MME gas lines. It is a Plexiglas™ block machined to fit the connectors which come with the MME as well as the air valve (Figures 11-14). The pressure is applied at the Gas In end. It splits to two connections. One connects to the mercury reservoir. The other goes to the (NC) connection of the air valve. The (NO) connection of the air valve connects to a path to the needle control.

4.2B Drop Knocker & Interface

The interface to the drop knocker allows the computer (or TTL level pulse generator) to control the Princeton Applied Research Model 174/70 Drop Timer. The schematics for the circuitry is given in Figure 2B. The circuit is built into the body of the drop knocker. There are two holes which were drilled on the top of the knocker to access the two 20 turn potentiometer controls. One control determines the strength of the knocker's strike and the other establishes the swiftness of the strike. The timing is adjustable from 1ms to 80 ms. This time refers to the signal applied to the knocker by this circuit. It is best to adjust the adjustment controls with the instrument set to knock at a reasonable rate, such as once every half second.

There are two connections to the drop knocker. One is a cable with two connectors which are connected to the power supply. The power supply consists of a wall transformer. Near the transformer are the capacitors C2

and C4 and the five volt regulator, Q2. The other cable is a shielded twisted pair cable (the shield connected to ground only at one side) which connect to the digital output of the computer. The black goes to ground and the lighter color connector is the signal.

Operation

The transformer box must be plugged into a 60 Hz 110 VAC power source. There is no power switch; either unplug it or connect it to a power strip which is turned off when the system is off. There is no direct manual method to fire the drop knocker. If it is necessary to manual fire the knocker, unplug the signal connector and temporarily ground it.

Circuit description

The power is derived from a nominal 9 volt DC wall transformer which is connected to a five volt regulator, described above. Inside the drop knocker, and at the signal input, resistors R1 and R2 form a divider which serves as both a reference voltage when the input is unconnected and as a signal terminator which helps to prevent reflections of the input signal. The signal then goes into the Schmitt trigger input of the monostable multivibrator integrated circuit , 74LS221. This circuit outputs a pulse signal (at the pin used, pin 4, high to low back to high) which is precisely controlled by the capacitor C2 and the sum of R4 and potentiometer P1 by $t = C2 * (R4+P1) * ln(2)$. Once this circuit has been triggered, any additional signals from any source are ignored until the output returns high. The output is coupled to transistor Q1 through resistor R6. The output of Q1 is normally low and goes to the input of the drop knocker. This signal goes high for the duration of the 74LS221 output pulse. The input to the knocker is to the base of an NPN transistor. Q1 provides a current to the knocker circuitry whose value depends upon resistor R7 and Potentiometer P2. This potentiometer controls the strength of the knocker blow. The PARC drop knocker circuitry consists of NPN transistors in a Darlington configuration and two solenoids in opposition. One solenoid is always energized; when the

transistors are energized, charge from a large capacitor is dumped across the second solenoid and the knocker arm is moved.

Resistor R3 provides a reference level for the active part of 74LS221, capacitor C1 is for power reserve and acts as a noise absorber for the integrated circuit, and resistor R5 provides a reference level for the unused section of the integrated circuit..

4.3B Digital to Analog Converter Circuit (D/A)

The D/A converts a digital signal from the computer into an analog output signal. The D/A converter consists of a Burr-Brown 729K 18 bit Digital to Analog converter with some support circuitry. It was carefully designed and constructed to prevent noise and current loops as per the specification sheet. Important connections are kept as short as possible, are shielded whenever possible, and the analog and digital grounds are connected at only one point. Capacitors are provided to both provide reserve power and as decoupling capacitors to reduce noise. The schematics are in Figures 3B-6B.

Features

The default output is ± 2.5 volts. By jumpers on the circuit board, the output range may be changed to ± 5 volts or ± 10 volts, as indicated in the circuit diagram, Figure 3B. The D/A converter chassis has two LEDs. The green LED indicates (5 volt) power on and the red LED flashes whenever data comes in and serves as an indicator that the data cable is loose (the red LED is on when the cable is loose). There are three connectors, illustrated in Figure 6B. The DB-25 (25 pin) cable goes to the computer I/O port, the isolated BNC is the digital output and the Molex 6 pin connector goes to the regulated power supply. The support circuitry includes noise reducing capacitors, a data latch to minimize noise due to a slow rising or uncoordinated data signal, and a pulse conditioner.

Circuit description

All integrated circuits are placed in gold plated machined sockets. The D/A 729K was designed according to examples in the specification sheets. The schematics are in Figures 3B to 6B. Digital and analog grounds are kept separate, and are connected only at the power supply. All capacitors are used for power reserve and noise reduction. All resistors are used to provide a voltage reference except R3 and R4 which are current limiters for the LEDs.

In operation, 16 bits of data are presented by the computer on connector J3 (pins 2-9, 15-22) to IC3 and IC4 edge triggered octal data flip flops (Figure 5B). The outputs of the flip flops remain at their previous value. Then, after data is stable, a high to low transition should be applied by the computer on J3 pin 12 which is connected (Figure 4B) through diodes D1 and D2 (which clamp the signal if it overshoots) and goes into the input of IC4, a Schmitt trigger inverter. The output at pin 12 of IC4 is connected to another inverter on the same chip, which drives an LED which indicates when data has come in (if the data cable comes loose, the input of the inverter will be high, output low, and the second inverter will bring it high again providing power and lighting the LED). The output at pin 12 is also connected to the clock input of the flip flops. The computer signifies data ready with a high to low transition; the inverter cleans the signal and changes it to a low to high transition which causes the flip flops to change their output to reflect their inputs. The outputs of the flip flops are directly connected to the D/A 729K inputs (Figure 3B). The D/A converter changes its analog output according to the digital inputs. This is the reason for using flip flops to latch the signal, as capacitance from a long cable may cause some of the data lines to change level at different speed than other lines and a slow rise time may cause superfluous analog outputs from the converter. The output of the converter is brought to an isolated BNC connector. The posts near the D/A 729K which can be jumpered to change the output range are labeled t23,t24,t25 and t27 on the diagram. For a ± 2.5 volt output, t23 is shorted to t27 and t24 is shorted to t25. Other ranges are described on the schematic. Short length jumpers should be used.

4.4B Connections

This section describes the connections to the instrument. In general, if a cable has a shield which is not used to carry current, the shield should be connected to ground only at one side of the cable, and the other side of the shield should be left unconnected. Usually a BNC cable does not follow this rule as the shield is used to carry current to ground.

4.4.1B Connections to the computer

1) 25 pin connector from serial port 0 to terminal
2) 25 pin connector from port 3 to pen plotter
3) BNC cable from channel 5 (or 2) to output of Keithley 614 electrometer (rear of electrometer)
4) Wire or shielded cable with pin jack on one end from analog ground to Keithley electrometer ground (rear, non insulated post)
5) Optional: BNC from D/A #0 to scope input
6) Optional: BNC from D/A #1 to strip chart recorder
7) Digital Out #0 banana cable to Air Valve Control
8) Digital Out #1 banana cable to Drop Knocker
9) Digital output to external 16 bit D/A converter

4.4.2B Electrometer connections

The input is connected to a "T" triaxial connector. On one end of the "T" is the current offset box. In parallel, on the other end of the "T" connector is the triaxial cable supplied with the electrometer, with alligator clips on one end. The red alligator clip is connected to the working electrode, the black clip to the potentiostat "working electrode" connection and the green clip to the potentiostat common.

The back of the electrometer has a connector with voltage proportional to the input current and the current range selected. The output goes to Channel 5 or 2 on the computer via a BNC connector. The bare connector goes to the computer with a wire or shielded wire to the computer analog ground.

4.4.3B Air Control Valve Connections

The output of the AVC is connected to the air valve, which should have twisted wires which are covered with a shield. The wires go to the output of the AVC (there is no polarity). The shield should connect to the ground of the input signal. The input connection goes to the computer Digital Out 0 (middle connector).

4.4.4B 16 bit D/A converter

The output is connected via a BNC cable to the "control potential" input of the potentiostat. The ground of the D/A output (shield of cable) is connected to the potentiostat common.

The 25 pin connector goes to the Digital output of the computer, below the banana jacks. Do not plug the 25 pin connector into the serial port. The Molex connector goes to the D/A power supply in a separate case.

4.5B Real Time Display

An analog output board with two 12 bit D/A converters provide outputs for two **HSWAVE** functions that output information during and after an experiment. The outputs are connected to BNC connectors which are mounted (and electrically isolated from) on the rear of the computer.

The output connected to D/A #0, is a real time display output. Current values from a range of data channels are continually output, with a synchronization pulse at the beginning of the output. The range may be specified at any time by the user via a software command. As new data comes in, the new points are output. If the number of data points exceed the size of the specified range, then the data is displayed as a window, which shifts as new data comes in. This display output goes on even after a scan is finished. This output is intended to be connected to an oscilloscope with a time base and trigger.

The other output, connected to D/A #1, provides a voltage output which is proportional to current and changes at the same time that new data

comes in. This output is intended for connection to a data recorder, which may be a strip chart recorder.

Both outputs display data as differential pairs, summed pairs, or raw data depending upon the display mode selected (**DIFF, SUM, NORM**) commands. The display mode is reset to the default display mode of the waveform each time a new waveform is run). The connector grounds for the D/A converter are isolated from the computer chassis to prevent current loops.

4.6B Monitor, Printer and Plotter

There is a serial board with four outputs which are each connected to DB-25 connectors on the rear of the computer. Two are used for this instrument. The serial output on the bottom is the console and it is used for driving a Tektronix 4014 compatible graphics terminal (which may be another computer running a communication program) for communication with the user and display of data after an experiment. Another output is used to drive a Hewlett-Packard 7475 (or equivalent) series pen plotter for printed output after an experiment.

Other outputs may be used and may be changed by the monitor; **@PORT1 , @PORT2** or **@PORT3** commands which are described in Appendix 5.4B. Some of the DB-25 connectors have switches which reverse pins 2 & 3 (transmit and receive). This is for compatibility with connections to peripherals as well as to other computers.

4.7B Current Offset Device

The Offset Device is used to add or subtract a constant current to the current coming from the working electrode. The purpose is to enable use of the maximum resolution of the A/D converter by utilizing the full range of input to the current-to-voltage converter or electrometer.

Sometimes current signals may be a few μA or nA on top of a background which is several orders of magnitude larger. The background may be due to charging current, a constant concentration of reducible

material in the bulk, a large electrode surface area, or from eluent flow in an HPLC detection method. If a scale is selected in which the signal plus background is on scale, the range of the voltage values in the A/D converter of the computer will be small and resolution will suffer. The electrometer has a limited resolution as well.

The Keithley electrometer has a built in offset which in the 20x and 200x scales can offset ± full scale and in the 2,000x scale (2k) offset one tenth of full scale. This is not sufficient to subtract background which is much larger than the signal. Therefore, this device was added. The circuit diagram is depicted in Figure 7B. It is simply a very stable voltage source (mercury or lithium batteries) connected to a voltage divider. The maximum current is determined by the output resistor R1. It is the total battery voltage divided by R1. The circuit must be shielded to minimize noise.

In use, it is connected to the electrometer via a triaxial T adapter in order that it be in parallel with the signal coming from the working electrode.

4.7.1B Offset Device Operation Instructions

1) Always move switch to center position when not used.
2) The absolute current output may change when reversing polarity.
3) Always unlock the dial before changing a setting.
4) Always use the lever on the dial to lock a setting.
5) Always measure the offset and use the **HSWAVE Offset** command to record this value together with the data.

Methods A or B are possible ways to set the current.

A) Method A: applying an offset of a desired value (the amount of offset required is known)

i) Unlock the potentiometer dial.

ii) Disconnect the connection from the working electrode at the meter; the only input to the meter should be the offset device. Turn the meter current offset to off.

iii) Set meter to I and the desired current offset range.

iv) Switch offset device to + or - .

v) Move dial to until reading is desired offset value.

vi) Wait for the reading to stabilize, then lock the dial. Read the meter and note the value; this plus the internal meter offset will be the total current that the experimental current is offset. Use the **Offset** command in **HSWAVE** to store with the data.

vii) Reconnect the electrode cable.

B) Method B: Offset a background current (offset is determined dynamically)

i) Unlock the dial.

ii) Use the **OUT** command of the **HSWAVE** program to output a desired voltage (e.g.., **OUT -200** will output 200 mV); or start the desired waveform.

iii) The meter should be set to **I** and to or near the scale that is desired.

iv) Switch to + or - and move dial until desired offset is realized. This is determined when the current is on scale in both the computer and the meter. The meter display should not be flashing and the values in the computer should not be clipped. To determine if the computer values are clipped, if a waveform is output, use the **ALARM ON** command. **MON ON** may be used as well, but the viewing mode should be set to **NORM.**

v) Lock the dial with care. Locking may shift the current slightly.

vi) Disconnect the connection from the working electrode at the meter; the only input to the meter should be the offset device. Switch the meter current offset to "off".

vii) Read the meter and note the value; this value plus the internal meter offset is the total current that the experimental current is offset. Use the **offset** command to store this value with the data.

viii) Reconnect the electrode cable.

4.8B Circuit Diagrams and Schematics

This section consists of circuit diagrams, used and discussed in the previous sections.

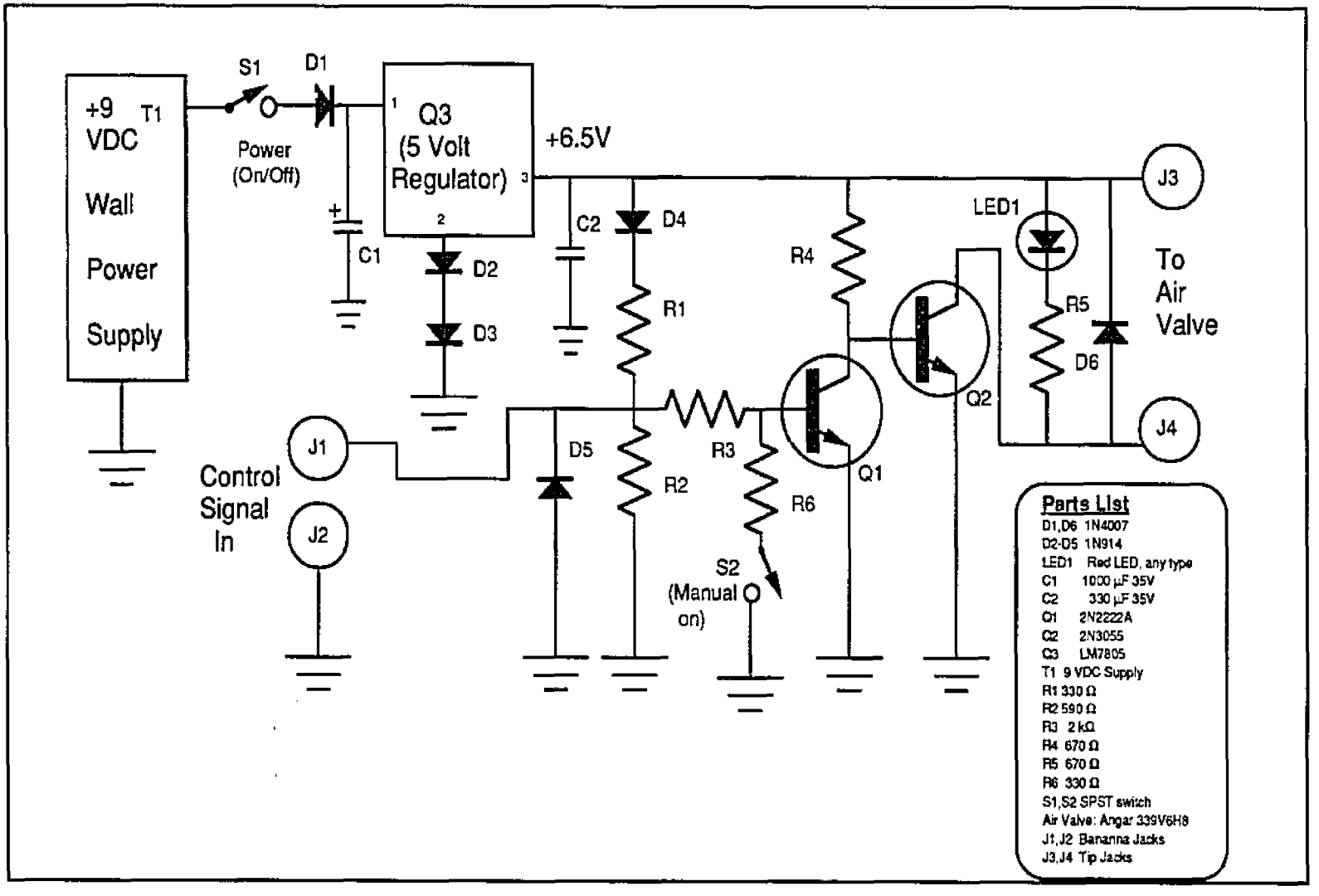

Figure 1B Air Valve Control Circuit Schematics

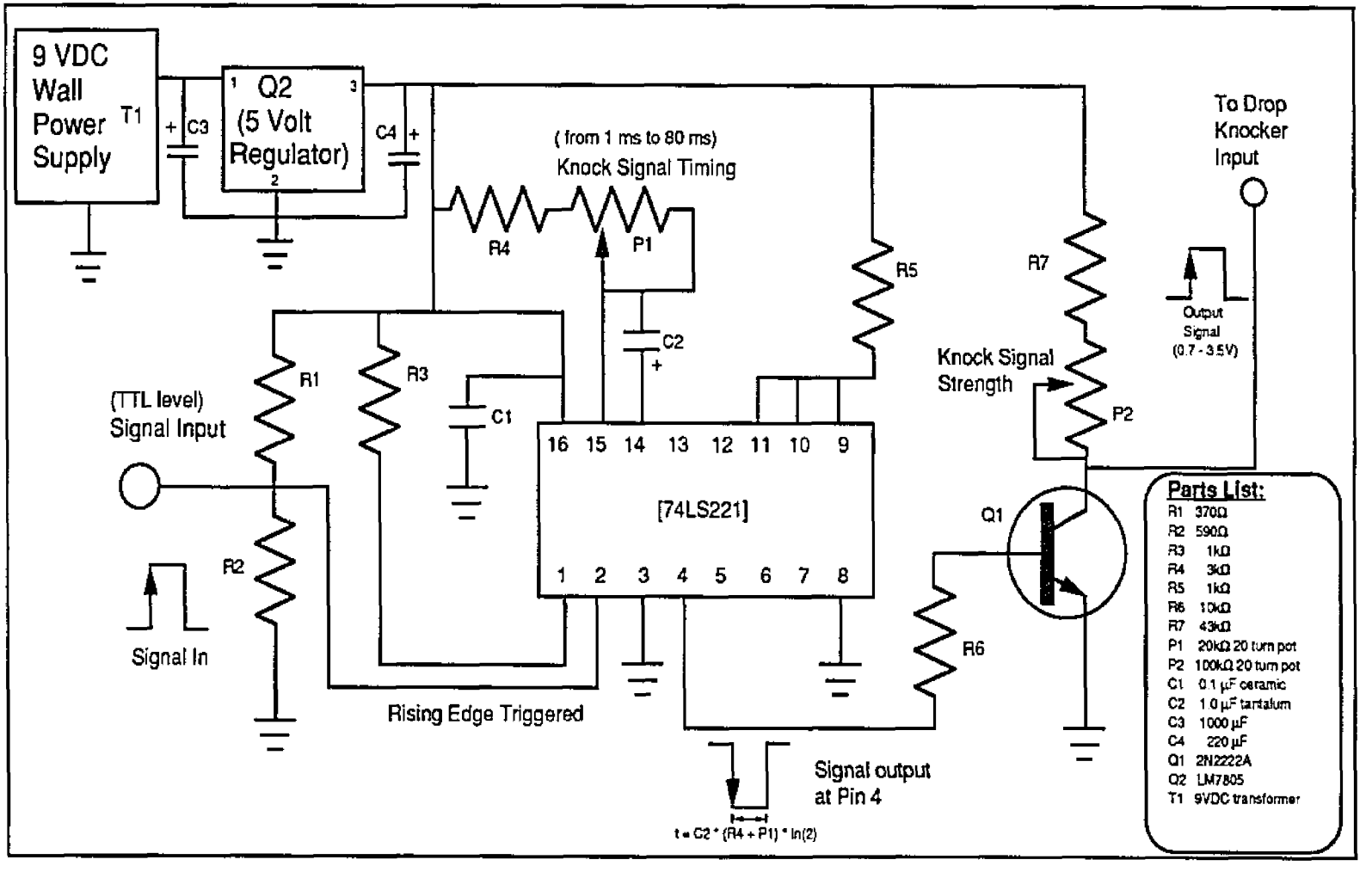

Figure 2B Drop Knocker Interface Circuit Schematics

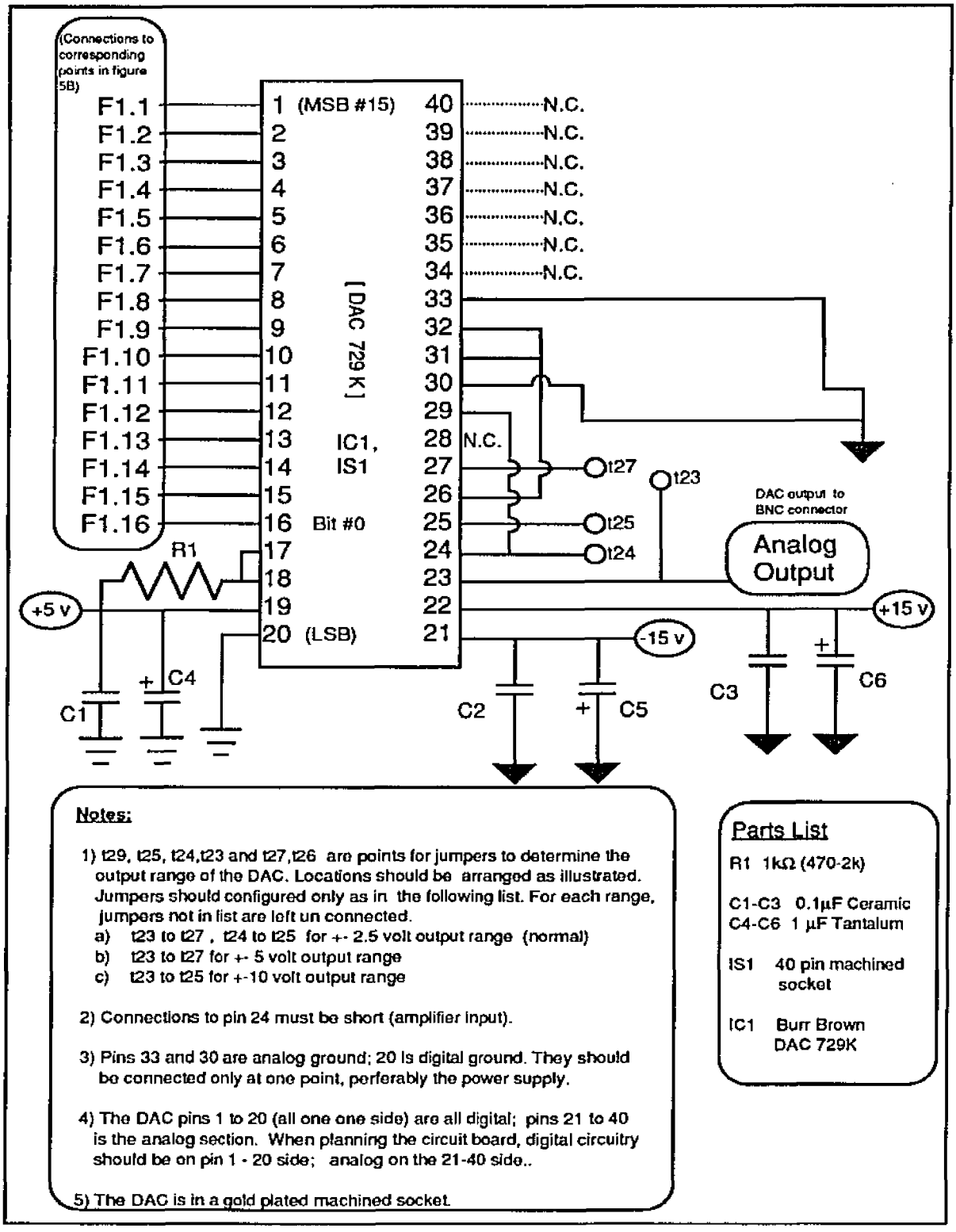

Figure 3B Digital to Analog Converter Circuit Schematics

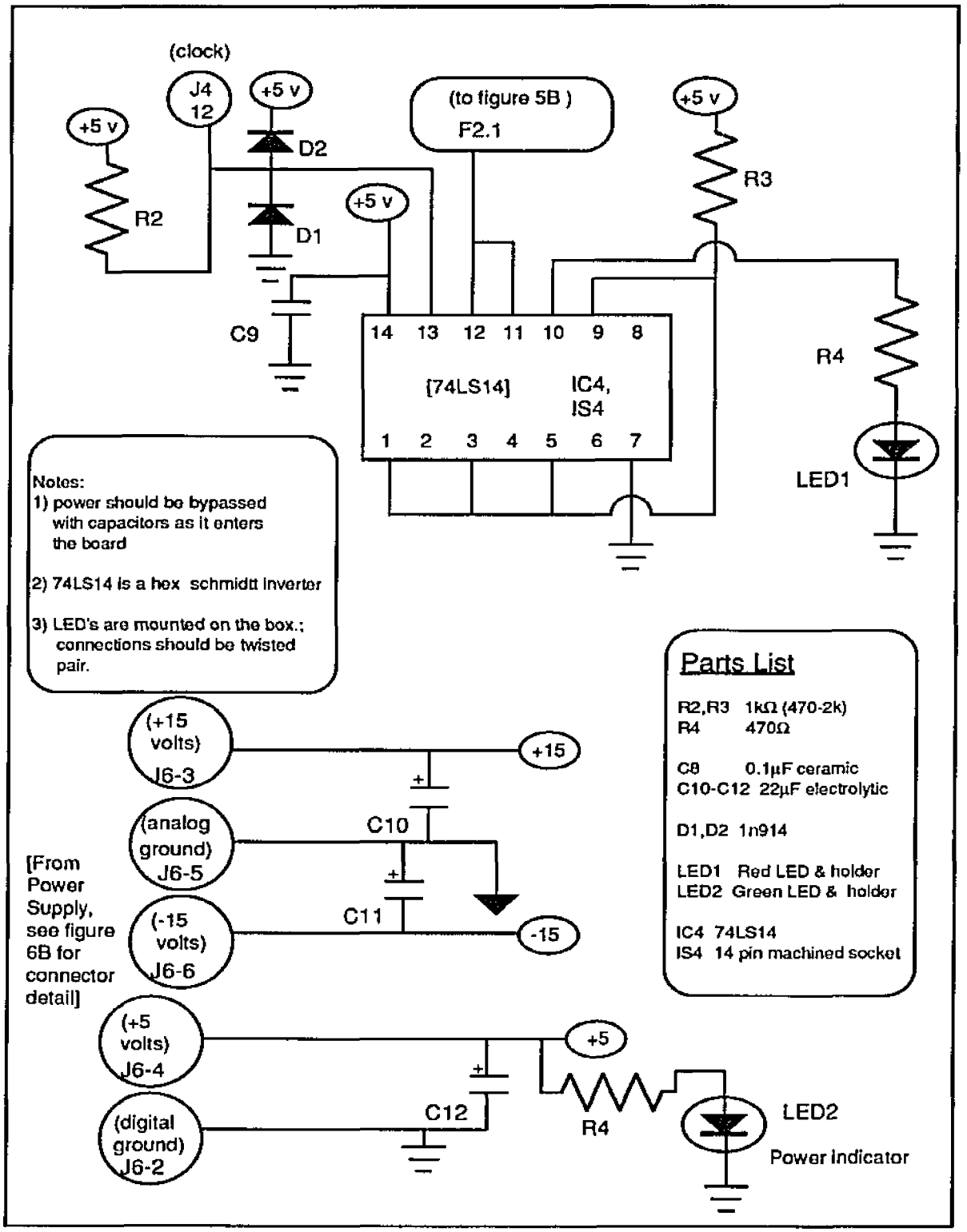

Figure 4B D/A Pulse Conditioner and Input From Power Supply

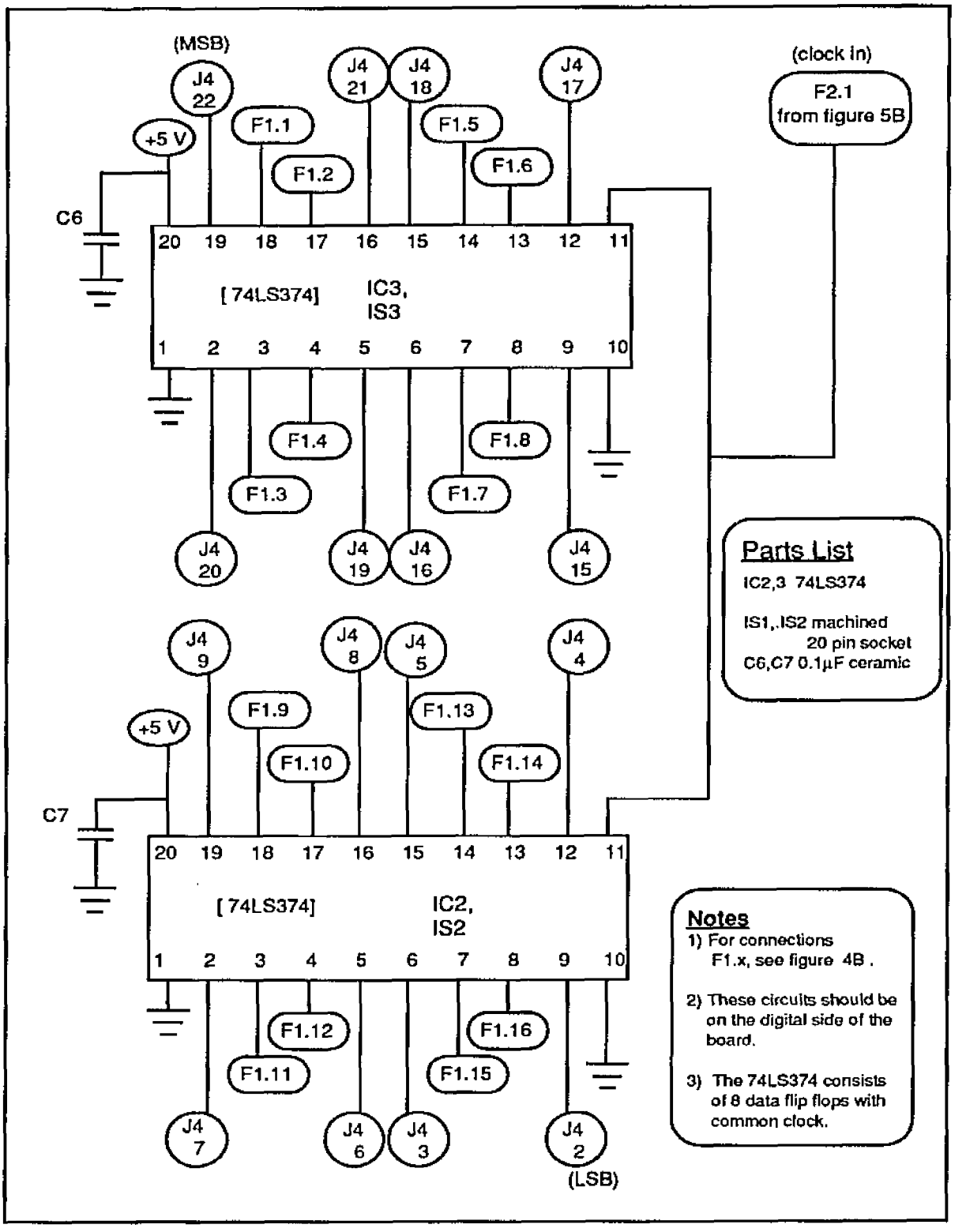

Figure 5B D/A Data Registers for Connection to Digital Output

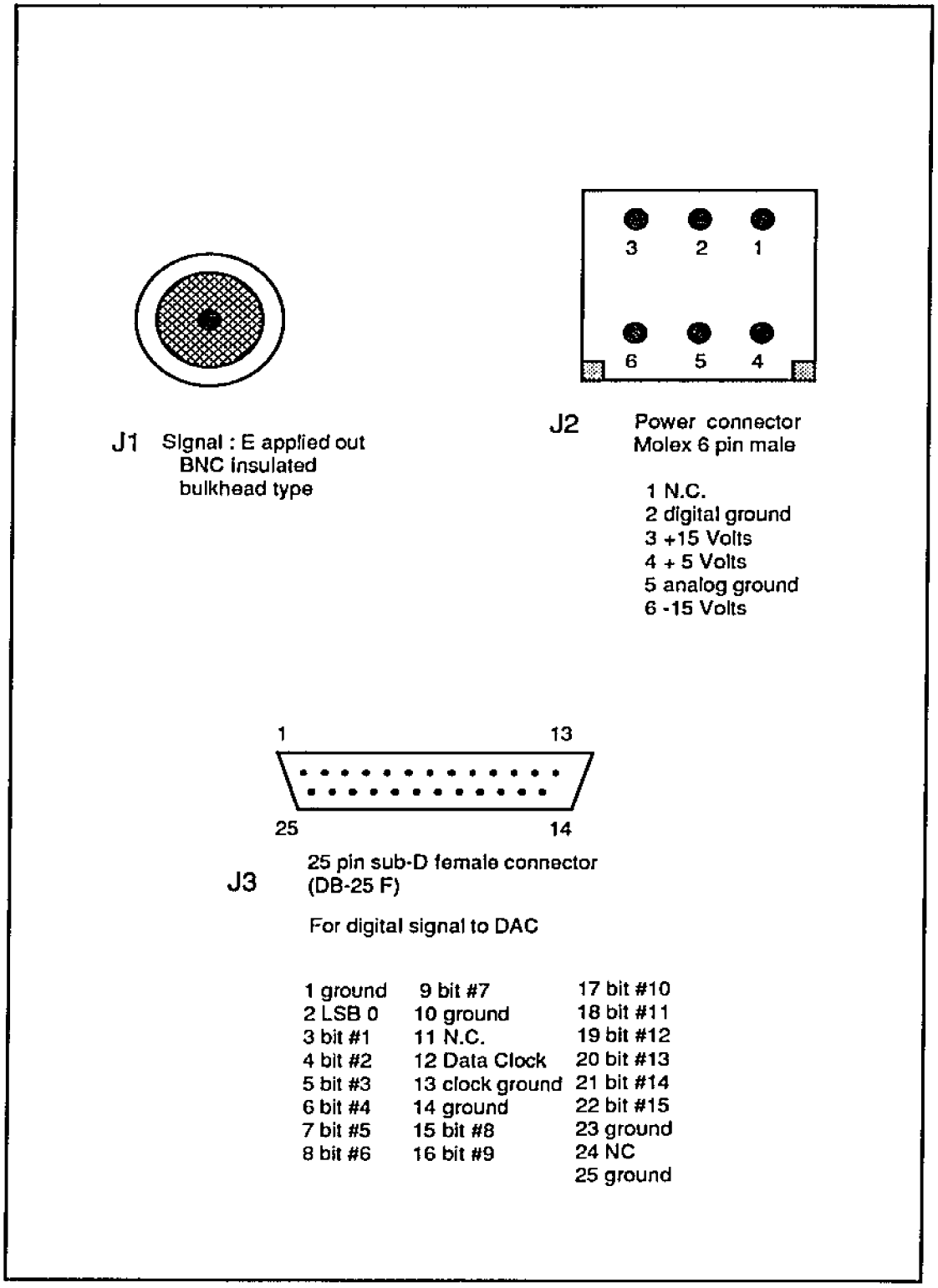

Figure 6B D/A Connector Detail (front view)

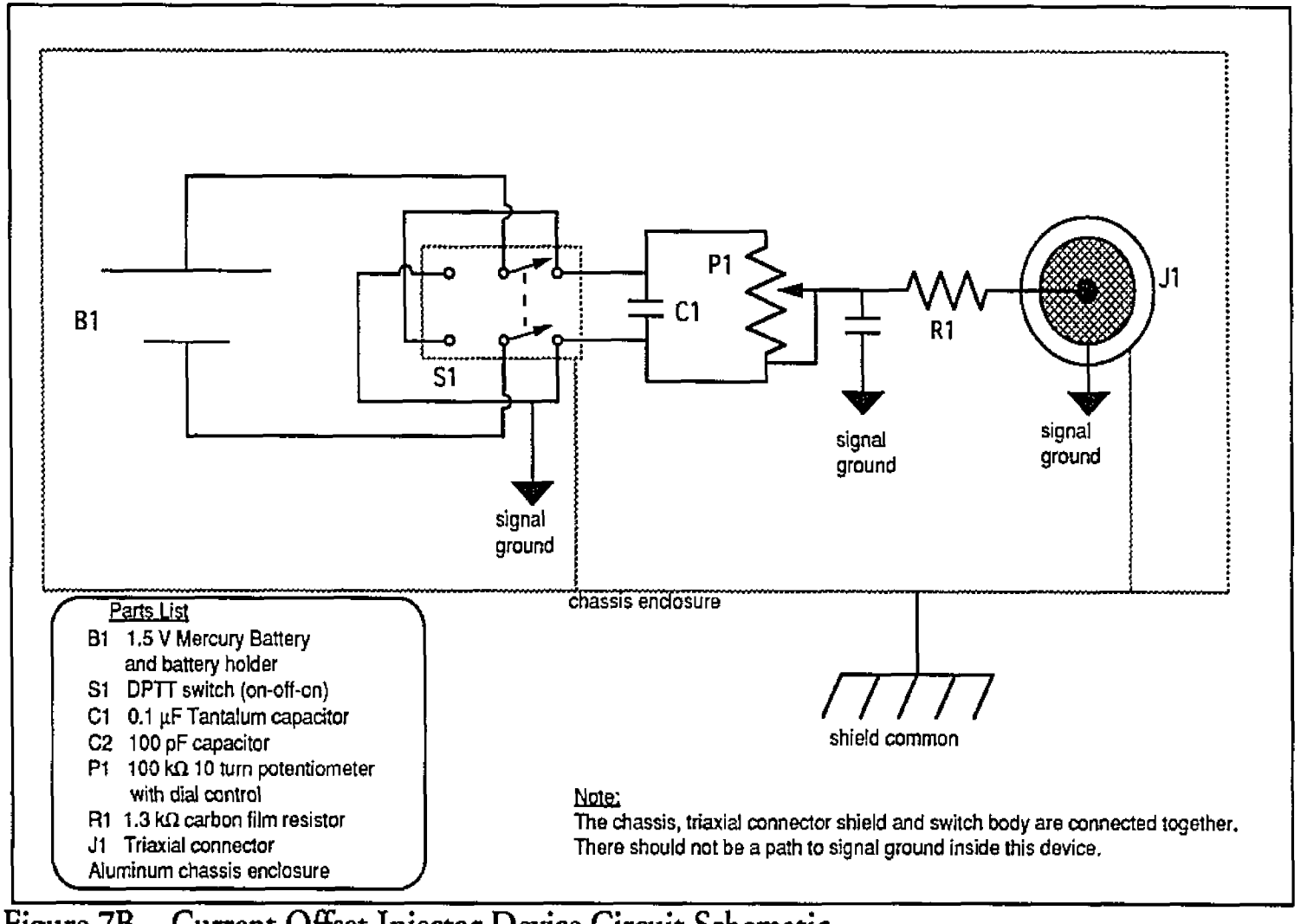

Figure 7B Current Offset Injector Device Circuit Schematic

5.0B HSWAVE Modules and Associated Programs

The name of the main waveform application is HSWAVE.SAV. It provides most of the functions of the instrument for performing electrochemistry and HPLC electrochemical detection. There are several files which it uses to store and recall parameters. These files give it the behavior of an instrument that keeps its settings from one day to another. There are also several support programs which provide utilities which are not contained in **HSWAVE** (due to memory size limitations). Finally, there are files and programs which are used to set up the environment in which **HSWAVE** operates. These files and programs are listed and described below.

5.1B HSWAVE Parameter Files

This section describes files which are required for **HSWAVE** to run. In most cases, except those noted, if the file does not exist, one will be automatically created. The new file will contain default parameters which were determined when the current program version was written and will most likely be usable for the current state of the program, but there may be cases when this is not true, such as when the A/D converter calibration has changed or if a select combination of waveform parameters were determined. In the latter case, if data was stored under these parameters, recalling the data can provide a list from which the parameters can be determined.

5.2B Waveform files

Each Waveform file contains a list of the parameters used the last time that the parameters were changed. **HSWAVE** uses these files whenever a waveform menu is entered and exited. By storing this information in files, the parameters will not change from day to day use unless desired. The waveform parameters are stored in a file bearing the name of the waveform (as well as could be fit into 6 letters; usually an abbreviation such as "HSDDP.WAV" for Differential Pulse), the extension is always ".WAV".
Each waveform also has a number associated with it. This is given in Table 2.1B. The parameter files are listed in Table 2.0B:

HSNAME.SET	This contains a list of waveform files in the first column and the associated number for each waveform in the second column. This file is not automatically created. The waveforms that each file is associated with is referenced in Table 2.1B.
HSWAVE.SET	This contains information used to setup HSWAVE parameters, such as D/A and A/D calibration. A list is found in Table 2.2B.
HSWDAT.SET	This contains the last data filename used. If an automatic name is used when storing data, HSWDAT.SET is consulted to determine the last automatic data filename used and is updated so that a sequential name is always used, even when the program is restarted.

Table 2.0B Setup files used by HSWAVE

Scanning Waveforms:			
number	Abbreviation	File Name	Waveform
1	STR	hsstr.wav	Staircase
2	NPV	hsnpv.wav	Normal Pulse
3	DDP	hsddp.wav	Differential Pulse
4	SQW	hssqw.wav	Square Wave
5	REC	hsrec.wav	Rectangle Wave
6	RNP	hsrnp.wav	Reverse Normal Pulse
Simple Pulse Waveforms:			
52	DUP	hsdup.wav	Dual Pulse
53	MUL2	hsmul2.wav	Dual Pair
54	MUL3	hsmul3.wav	Three Pair
55	MUL4	hsmul4.wav	Four Pair
56	MUL5	hsmul5.wav	Five Pair
51	DPT	hsdpt.wav	Dual Pulse Time Study

Table 2.1B Waveform parameter filenames, numbers and abbreviations

#	Description	Default
1	# lines on the Screen	24.
2	Resolution of Scope display	4095.
3	Resolution of A/D Converter	4095.
4	A/D Polarity (-1 = bipolar)	-1.
5	not used	1.
6	not used	1.
7	D/A Converter Resolution	65535.
8	D/A Polarity (-1 = Bipolar)	-1.
9	A/D Voltage Calibration point #1	1.019
10	A/D numeric value for point #1	1640.
11	A/D Voltage Calibration point #2	0.
12	A/D numeric value for point #2	2048.
13	D/A Voltage Calibration point #1	2500.
14	D/A numeric value for point #1	65535.
15	D/A Voltage Calibration point #2	-2500.
16	D/A numeric value for point #2	0.
17	Time offset	2.
18	Time Base	0.1
19	Time Base	4.
20	Default A/D Channel	5.

Table 2.2B Example of HSWAVE.SET file used in setting up HSWAVE. Only the Default values are contained in the file.

Initial Time	Tair	Potential applied at the beginning of each scan
= 0	= 0	First Potential
≠ 0	= 0	Initial Potential E0
= 0	≠ 0	First Potential
≠ 0	≠ 0	Initial Potential E0

Table 2.3B Effects of combinations of parameters Initial Time and Tair

5.3B Files in HSWAVE

The **HSWAVE** source program consists of subroutines and a main program contained in 95 separate files written in Macro assembly language for the PDP-11 and in FORTRAN. In addition there are some files which make it easy to recompile and link the programs and there are some utility and support programs. Also, there are support files which contain parameters discussed previously.

Recompiling and linking all these files is not as great a task as it might seem, for there are several command files which contain all the instructions to compile and link. The command files are:

HSCOMP.COM This compiles all the files

HSLNKT.COM This links the files together to create "H.SAV"

These files are in the subdirectory called "WORK.DIR" on the DL0: disk. They simply call the compilers and linkers and name the programs.. To run either of these files:

@HSCOMP

@HSLNKT

The source files must be in a directory named "LD5:". The virtual disk should be booted with the command file:

@HSGOES.COM

The contents of this command file is:

```
SET ERROR NONE
LOAD VM
MOUNT LD5: DL0:WORK
INITIALIZE/BAD/NOQ VM:
COP/SYS LD5:*.SYS VM:
COPY/BOOT VM:RT11SJ VM:
COPY SY:STARTZ.COM VM:STARTS.COM
COPY SY:(DUP,DIR,PIP).SAV VM:
COPY SY:(LINK,EXER,UCL).SAV VM:
COPY SY:(SYSMAC.SML,SYSLIB.OBJ,UCL.DAT,README.VM) VM:
COPY SY:(DATTIM,TECO).SAV VM:
COPY SY:TECO.INI VM:
COPY LD5:*.H VM:
COPY LD5:HSGLBL.MAC VM:
COPY LD5:HSLNKT VM:
BOOT VM:
```

These commands may be entered individually if HSGOES.COM does not exist. This command file was set up and used when editing and working on the **HSWAVE** program and utilities. Much effort was made to fit the entire program, which is over 250 kbytes, into a space of less than 56 kbytes. An overlay system was used which is supported by the linker. With this system, most of the program is on disk and is called in as needed. The problem with this system is that a subroutine cannot call another subroutine which is in the same overlay space. If it does, the return path for the first subroutine is lost as the overlay scheme does not preserve the memory image of the program. Therefore, great care had to be taken to make sure that routines have a defined path. The HSLNKT file shows this overlay scheme. A filename without a /O: is in the common memory area. The main program, as well as COMMON variables are contained in this space as well. Overlay regions are given numbers and can be seen as /O:1 for overlay 1, /O:2 for overlay 2, etc. Two filenames on a single line are placed into memory together. Each overlay region has a size which is the largest size of any of the subroutine(s) in that region.

The contents of HSLNKT is difficult to reproduce without detailed examination of all the program files. The contents of HSLNKT are as follows. Three columns are shown here to conserve space; the actual file uses one column.

```
R LINK
H,H=LD5:HSWAVE//
LD5:SHORT
LD5:F77NER
LD5:HSLINE
LD5:HSTATU
LD5:HSICOM
LD5:HSGTWV
LD5:HSSCRN
LD5:HSMFIL
LD5:HSIDLE
LD5:HSCPIN
LD5:HSTIMN
LD5:HSBOOL
LD5:HSDISP
LD5:TPCOOR
LD5:HSDSKS
LD5:HSREST
LD5:HSPDIS
LD5:HSMORE
LD5:HSDIN2/O:1
LD5:HSHELP/O:1
LD5:HSPLIM/O:1
LD5:HPLOTI/O:1
LD5:HPLABE/O:1
LD5:HPAXIS/O:1
LD5:HPLOTU/O:1
LD5:HPAXIZ/O:1
LD5:TPLOTI/O:1
LD5:TPLABE/O:1
LD5:TPAXIS/O:1
LD5:TPLOTU/O:1
LD5:TPAXIZ/O:1
LD5:HSGET/O:1
LD5:HSWAIT/O:1
LD5:HSEXTR/O:2
LD5:HSADAT/O:2
LD5:HPEXIT/O:2
LD5:TPEXIT/O:2
LD5:HSINFO/O:2
LD5:HSINF2/O:2
LD5:HSTDAT/O:3
LD5:HSMISC/O:3
LD5:HSPARS/O:3
LD5:HSWATC
LD5:HSTIMG/O:4
LD5:HSOTHG/O:4
LD5:HSTESG/O:4
LD5:HSSTRG/O:4
LD5:HSDDPG/O:4
LD5:HSDUPG/O:4
LD5:HSMULG/O:4
LD5:HSDPTG/O:4
LD5:HSSQWG/O:4
LD5:HSRNPG/O:4
LD5:HSNPVG/O:4
LD5:HSTPIT/O:4
LD5:HSTPET/O:4
LD5:HSTIET/O:4
LD5:HSTPIE/O:4
LD5:HSTP3D/O:4
LD5:HSTP32/O:4
LD5:HSHP32/O:4
LD5:HSHPIT/O:4
LD5:HSHPET/O:4
LD5:HSHPIE/O:4
LD5:HSHIET/O:4
LD5:HSDINT/O:4
LD5:HSDETC/O:4
LD5:HSOTHE/O:5
LD5:HSTES/O:5
LD5:HSDIT/O:5
LD5:HSD3D/O:5
LD5:HSDET/O:5
LD5:HSDIET/O:5
LD5:HSDIE/O:5
LD5:HSTPAR/O:5
LD5:HSCURS/O:5
LD5:HSPLOT/O:5
LD5:HSLIST/O:5
LD5:HSINTE/O:5
LD5:HSETUP,
LD5:HSDATE/O:5
LD5:HSMAIN/O:5
LD5:HSSCAN/O:5
LD5:HSSIMP/O:5
LD5:HSSTR/O:5
LD5:HSDDP/O:5
LD5:HSSQW/O:5
LD5:HSREC/O:5
LD5:HSDUP/O:5
LD5:HSMUL/O:5
LD5:HSDPT/O:5
LD5:HSRNP/O:5
LD5:HSNPV/O:5
LD5:HSSTOR/O:5
LD5:HSSTOP/O:6
LD5:HSEDAT/O:6
//
^C
```

The **HSWAVE** source files have been named to aid in recognition. The FORTRAN files have the extension .FOR and the Macro files have the extension .MAC. Most files start with the letters HS except for those files that directly control the Hewlett-Packard plotter (start with HP) and those that control the Tektronix graphics terminal (start with TP). Two files which are supplied with the FORTRAN 77 compiler are used to keep the executable as small as possible (end with .OBJ). A list of all files follow.

HSGLBL.MAC	Contains global variables for assembly routines
HSWAVE.FOR	Main Program
HSLINE.MAC	Gets characters from terminal
HSTATU.FOR	Puts status on terminal
HSICOM.MAC	Copies data header information
HSGTWV.FOR	Gets and stores waveform parameters
HSSCRN.FOR	Internal routines for cursor movements
HSMFIL.FOR	Internal: creates file names
HSIDLE.MAC	Internal: Idle routines, interrupt completion
HSCPIN.MAC	Copy data header
HSTIMN.FOR	Internal: converts time value to internal number
HSBOOL.MAC	Boolean operations for FORTRAN
HSDISP.MAC	Real time display (idle scope routine)
HSDSKS.MAC	Data storing routines for disk storage during interrupts
HSREST.MAC	Internal: reset routine
HSPDIS.FOR	Internal; determines scope display extent
HSMORE.FOR	Prints "enter return to continue"
HSDINT.FOR	Calculates area under curve, plates, etc.
HSDIN2.FOR	Part II of HSDINT
HSHELP.FOR	Help routines

The following drive a Hewlett-Packard pen plotter

HSPLIM.FOR	Determine axis limits, number increments, etc.
HPLOTI.FOR	Initialize plotter
HPLABE.FOR	Produce labels
HPAXIS.FOR	Draws axis part I
HPAXIZ.FOR	Draws axis part II
HPLOTU.FOR	Plots data points
HPEXIT.FOR	Close HP plotter and assorted routines

The following routines drive a Tektronix terminal

TPCOOR.FOR	Converts data to Tektronix screen coordinates
TPLOTI.FOR	Initialize graphics
TPLABE.FOR	Produce labels
TPAXIS.FOR	Draw axis part I
TPAXIZ.FOR	Draw axis part II
TPLOTU.FOR	Plot data
TPEXIT.FOR	Exit graphics
HSGET.FOR	Internal: routines which read in voltage, time, etc.
HSWAIT.MAC	Pause for specific time or until the esc key is pressed
HSEXTR.FOR	Interprets and executes global commands
HSADAT.FOR	Translates data into current
HSINFO.FOR	Lists data information
HSINF2.FOR	HSINFO part II
HSTDAT.FOR	Translates data into Time value
HSMISC.FOR	Internal character utilities
HSPARS.FOR	Takes command line and separates each component
HSWATC.FOR	Stop watch timer for "Other" menu
HSTIMG.MAC	Timing routines for HSWATC

The following contain the waveform producing routines

HSOTHG.MAC	Generates timed knocks, etc as per Other Menu
HSTESG.MAC	Test routine
HSSTRG.MAC	Staircase waveform
HSDDPG.MAC	Differential Pulse
HSDUPG.MAC	Dual Pulse
HSMULG.MAC	Multiple Pulse (2, 3, 4, and 5 pair)
HSDPTG.MAC	Time Study
HSSQWG.MAC	Square and rectangle wave
HSRNPG.MAC	Reverse Normal Pulse
HSNPVG.MAC	Normal Pulse

The following plot on the Tektronix

HSTPIT.FOR	Plot current vs. time
HSTPET.FOR	Plot Potential vs. Time
HSTIET.FOR	Plot Current vs. Potential at a specific Time

HSTPIE.FOR	Plot Current vs. Potential
HSTP3D.FOR	Plot 3 dimension, part I
HSTP32.FOR	Plot 3 dimension, part II

The following plot on the Hewlett-Packard pen plotter

HSHP32.FOR	Plot 3 dimension
HSHPIT.FOR	Plot current vs. time
HSHPET.FOR	Plot Potential vs. Time
HSHPIE.FOR	Plot Current vs. Potential
HSHIET.FOR	Plot Current vs. Potential at a specific Time
HSDETC.FOR	Utilities for display routines
HSOTHE.FOR	Other functions menu
HSTES.FOR	Test menu

The following are used to interpret commands and call the appropriate routines

HSDIT.FOR	Current vs. time plot
HSD3D.FOR	Three dimensional plot
HSDET.FOR	Potential vs. Time plot
HSDIET.FOR	Current vs. Potential at a specific Time plot
HSDIE.FOR	Current vs. Potential plot
HSTPAR.FOR	Plot parameter menu for HP and Tektronix plotter
HSCURS.FOR	Scope cursor routine
HSPLOT.FOR	Plot Menu
HSLIST.FOR	List Menu
HSINTE.FOR	Internal parameters menu
HSETUP.FOR	HSWAVE initialize routine
HSDATE.FOR	Get current date, convert to JAN-DEC and name
HSMAIN.FOR	Main Menu
HSSCAN.FOR	Scanning Waveform menu
HSSIMP.FOR	Non scanning waveform menu
HSSTR.FOR	Staircase Menu
HSDDP.FOR	Differential Pulse Menu
HSSQW.FOR	Square Wave Menu
HSREC.FOR	Rectangle Wave Menu
HSDUP.FOR	Dual Pulse Menu
HSMUL.FOR	Multiple Pulse Menu
HSDPT.FOR	Time Study Menu

HSRNP.FOR	Reverse Pulse Menu
HSNPV.FOR	Normal Pulse Menu
HSSTOR.FOR	Storage Menu
HSSTOP.FOR	Waveform stop completion routine
HSEDAT.FOR	Translate data into potential
SHORT.OBJ	Routines supplied with compiler which
F77NER.OBJ	Gives short error messages

5.4B HSWAVE Support Programs

This section describes programs which are useful utilities. They were written to make working with the instrument easier. To run any of these programs, **HSWAVE** must be exited; then enter the name of the program. The Macintosh programs are started by double clicking on the icon.

HS3D.SAV Takes stored data and plots it on the screen as a 3 dimensional surface plot with hidden line removal.

TIMER.SAV Stopwatch program, same as that built into **HSWAVE**.

HSSUM.SAV Takes a set of data that was named with number extension (such as the names given by automatic filenames) and produces a summary of the set. Included in the summary are comments, parameters, etc.

HSSUMA.SAV

Works like HSSUM.SAV, but it gives a short summary. It is strongly recommended that when a day's experiments have been completed, that either **HSSUMA** or **HSSUM** be run to produce a summary file and then print the file and keep with the notebook.

INITIZ.COM Formats and initializes a blank floppy disk. It is set up to create a large directory which is necessary when many files are stored on the disk.

PORT0.COM PORT1.COM PORT2.COM

These will set the serial port for the printer.

HSWAVE.COM

HSWAVE should be started from this command file, as it sets up many of the parameters for **HSWAVE**.

Skipper Apl This is a program on the Macintosh which can be useful in treating **HSWAVE** text data. It can transpose columns and rows, and convert sequentially stored data to several columns.

HSWAVE bin to text

This is a program on the Macintosh which will convert **HSWAVE** binary data into text format with tabs.

6.0B Waveform Generation, How HSWAVE Works

This section will deal with a general overview of the processes that occur when the waveform is generated.

Each waveform has two subroutines in **HSWAVE**. One, written in FORTRAN, displays the menu and reads in and sets up parameters for the waveform. The other is written in Assembly language and outputs the waveform as well as controls the air valve, drop knocker and acquires data through the A/D converter. This subroutine is interrupt driven. This means that once the waveform has started, the menus become active and are interrupted only when something has to be done with the waveform, such as collecting data or outputting a new voltage. The operator can use **HSWAVE** to perform other functions. Some functions might interfere with the waveform, either by affecting waveform parameters or might reside in the same area of memory. When a waveform is active, these functions are disabled until the waveform has completed.

Most of the time **HSWAVE** is waiting for a command. Rather than waste that time, an idle routine is running. Whenever **HSWAVE** waits for a command, in any menu, **HSWAVE** checks if something is typed on the terminal. If a key is pressed, the character is saved for the routine that requires it. Then, the idle loop is run once. Since the idle routine is written in assembly language, it is very fast and many iterations will pass between keystrokes, so it is transparent to the user. The idle routine checks for conditions, such as a beep command from an interrupt (e.g., if data is off scale and **ALARM** is **ON**, if the scan has finished, if a new scan has started, etc.). If the scan has finished, it tidies up appropriate values and stores data if enabled to do so; if **MONITOR** data is **ON** then idle checks if a new data point came in and if so, it prints the value on the terminal and then outputs scaled data through the D/A converter to provide a real time display of data. Data output to the strip chart recorder is performed via the interrupt routine, as well as storage of data to disk.

When the command for starting the waveform is given (**Go**, **Save**, **Wait**), a number of things occur before the waveform is actually started. When the command is given, a prompt may be given for entering a number of items. If **Wait** is commanded, the value for the delay time is prompted before the scan starts. If there is a scan currently in progress, a prompt is given as to whether the current scan should be abandoned and a new one started. In all cases, before the scan starts, a prompt is given for the Current to Voltage conversion factor (since the I/V converter does not communicate that information to the computer.). Before that prompt, if **Save** is commanded, a temporary file, HSWAVE.DAT is created (at the end of the scan this file may be renamed and made permanent). All of the time and voltage parameters are converted into a form that the I/O devices and the assembly program can directly use, and other parameters are optimized into values that the assembly program can quickly use. A copy of the waveform parameters as well as other information (such as A/D, D/A calibration, etc.) is made to describe the data. The purpose of this is have a complete set of conditions stored for each set of data, in the event that any conditions change in the future.

The computer performs a few tasks after the I/V value is entered and **return** is pressed which delay the start of the waveform by only a few milliseconds. Any remaining parameters are stored. If **Wait** is the command, the program will pause for the specified period of time (the keyboard is monitored for the **esc** key: if it is pressed during this time, the scan is canceled). The scope display is set up. Then the assembly subroutine is called. Some additional conversions are made, some variables are copied. The clock is set to its minimum time, the interrupt vectors are set to knock or start the waveform, and the first voltage is output and the clock is enabled. Then the routine returns control to the waveform menu and interrupts **HSWAVE** whenever the clock time is up. There are several places or vectors that the interrupt might go to. This is determined by the

knocker, air valve, cycle type parameters and the part of the waveform being output. The possibilities are:

knock (and air valve) off

Knock once

Knock at beginning of each cycle

Knock at beginning of each scan

combined with:

Air valve on (**Tair** ≠ 0)

Air Valve off (**Tair** = 0)

combined with:

normal cycle

cyclical

Flags are set up and as each part is performed, the vector for the next task is determined. So, if the air valve is enabled and the appropriate voltage is applied, the knocker will strike. Then the air valve will be turned on and the timing set for **Tair**. The vector is set to a routine which will turn the valve off and start the appropriate timing (for **Initial Potential** or the appropriate voltage). In another example, if the knocker is enabled and the valve is not, the voltage will be output and the knocker will strike and the timing will start.

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