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RAPID SCANNING SPECTROMETER: DESIGN, COMPUTERIZATION, AND APPLICATIONS

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

by

Dennis A. Yates, B.A.

* * * * *

The Ohio State University

1976

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A special appreciation is given to the author's wife and two sons.
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INTRODUCTION

In this laboratory we have been interested in developing or improving instrumentation or instrumental techniques to be used, primarily, in spectroelectrochemical studies.

We set forth the goals for this study of improving our capabilities for acquiring the spectra of electrochemically generated intermediates via rapid scanning spectroscopy (RSS), improving sensitivity for spectroelectrochemical measurements, and adapting a stopped-flow unit to rapid scanning spectrometer for kinetic studies of biocomponents. In accomplishing these we undertook the following studies: (1) examine, evaluate, and improve the galvanometer RSS developed earlier in this laboratory¹; (2) examine and evaluate alternative mechanisms of rapidly acquiring spectral information, particularly array detectors, for our specific needs; (3) examine and evaluate the signal processing technique, derivative spectroscopy; (4) develop and evaluate a real-time, dual terminal computer system for improving sensitivity via digital techniques (e.g., signal averaging, digital smoothing); and (5) adapt an Aminco-Morrow stopped-flow to the RSS.

The rapid scanning spectrometer¹ was based on an oscillating mirror in a galvanometer suspension and could rapidly sweep part of the ultraviolet (UV), visible (VIS), or near infrared (NIR)
spectrum by passing a linearly varying current through the galvanometer coils. This RSS has been successfully used in stopped-flow experiments, liquid chromatography, and emission spectroscopy. In this laboratory we have used the RSS for spectral measurements in indirect coulometric titrations, SO$_2$ concentration measurements, and detection of electrochemically generated short-lived intermediates.

The galvanometer RSS has undergone several modifications in optics and electronics and is presently in its third generation with improved resolution, speed, and sensitivity. The RSS monochromator is all reflecting optics and employs an improved light source housing. To understand the capabilities and limitations of the RSS we evaluated sensitivity, speed, and noise and examined the components of an RSS with respect to the effects on performance generated by each component and the interrelations between components. The results of the RSS work are presented in Chapter I.

Concurrently with the RSS study a computer system was developed and is currently being used in the following applications: (1) automation of the titrations of bio-components; (2) control of the Harrick (model RSS-B) Rapid Scanning Spectrometer; (3) acquisition of data and/or control of experiments in electrochemistry (chronoamperometry, cyclic voltammetry, chronocoulometry) and spectroscopy (rapid scanning spectroscopy, fixed wavelength spectroscopy); (4) digital simulations; and (5) maintenance of a literature data file for in-house literature searches.
In addition to standard computer peripherals we have three interfaces for data acquisition and experiment control containing the following components: (interface 1) a fast analog-to-digital convertor (12 bit, 200 KHz conversion rate), sample and hold amplifier (SH), a 12 bit programmable clock driven by a 10 MHz crystal clock, and two digital-to-analog (12 bit) convertors; (interface 2) a second ADC (12 bit, 77 KHz single channel conversion rate), 8 channel multiplexor, two sample and hold amplifiers and a 12 bit programmable clock; and (interface 3) four 12 bit digital output storage registers, two 12 bit DAC's, 12 bit presettable event counter, and two 16 bit digital input registers. The last two interfaces were constructed during this work. Both ADC's transfer data to computer memory via hardware control using the direct memory access channel which allows faster data transfer rates than is possible under software transfer.

The data acquisition programs are written in Fortran employing assembly language subroutines to control the interfaces. The programs execute under Data General's Real-Time Disk Operating System (RDOS) which also supports the peripherals. We find this approach extremely effective and simple since a user is not generally required to know assembly language.

RDOS also allows two programs to execute concurrently in memory using a teletype and a cathode ray terminal as individual consoles. This allows data acquisition from two experiments at the same time making more effective use of the computer resources. The computer
system and our philosophy of computer interfacing are described in Chapter II. The interface hardware and software developed are described in the appendices.

The ESS and the computer system are the basis of this work. In Chapters III through VII the application of the ESS and, if required, the computer system to various problems are described and discussed. The results indicate the types of problems which are solvable by ESS and will hopefully provide further impetus for research in this area.

The potential benefits of a signal processing technique based on wavelength modulation using the galvanometer ESS was first demonstrated by Kuwana and Hawkridge and showed promise for improved sensitivity. In the galvanometer ESS wavelength modulation was caused by superimposing a fast, small sinusoidal oscillation on the main drive wave. A lock-in amplifier was used to selectively amplify the modulation frequency which produced a derivative spectrum. This technique has distinct advantages in detecting overlapping bands and in elimination of periodic noise (i.e., 60 Hz line noise). The results of this work are presented in Chapter III.

To determine if one of the array detectors would be better suited to our needs we examined the one-dimensional linear diode array (LDA) and the two dimensional silicon vidicon tube (Vidicon). These detectors have inherent scanning capability, and, when placed at the exit slit of the ESS monochromator, serve as multiple (as many as 1024) exit slits/detectors. The data of Horlick and
Coddington on an LDA spectrometer and of Pardue on the Vidicon spectrometer indicated that both devices were yielding better sensitivity than our RSS but we felt that the rather sophisticated driving/processing electronics hindered their use. The recent availability of 1024 photodiode LDA containing all required circuits for operation prompted us to purchase and evaluate it. The simplicity and ease with which the LDA operated was significant but the measured sensitivity was a factor of 10 less than was hoped. The results of the LDA evaluation are presented in Chapter IV.

The RSS was applied to the determination of spectra of several oxidized electron rich substituted olefins using a platinum optically transparent electrode. Two hundred nanometer spectra were acquired in 5 msec during a 10 msec potential step and signal averaged by a computer to give a sensitivity of $10^{-4}$ absorbance unit (A) at 417 nm. This procedure and results of this technique, first demonstrated by Gruver and Kuwana in determining spectra of aromatic hydrocarbon intermediates generated during a several hundred msec potential step, are described, with particular emphasis on associated problems, in Chapter V.

The indirect coulometric titration of bio-components is employed in this laboratory to study the stoichiometry and energetics of cytochrome c and cytochrome oxidase. The experimental procedure requires extensive experimenter supervision involving, mainly, monitoring charge, controlling a magnetic stirrer, recording spectra,
and controlling relays and generally requires 3-4 hours per experiment. We felt that this experiment would be ideal to test and demonstrate the computer system. The automation procedure is unique in that we do not allow the computer to make intuitive decisions and is described in Chapter VI.

In Chapter VII a RSS stopped-flow instrument is described and tested by examining the oxidation of cytochrome c by 1,1'-bis-hydroxymethyl-ferrocinium (HMF+). The galvanometer RSS was fitted with an Aminco-Morrow stopped-flow for this purpose and the RSS served, in this study, as a fixed wavelength dual beam spectrometer. Cytochrome c is a low molecular weight (M.W. 12,500) enzyme with a single heme center and is found in the mammalian respiratory chain. The mechanism of the biological reactions of cytochrome c has been extensively studied but to date is still unknown. In addition to testing the instrument we hoped to gain mechanistic insight into cytochrome c reactions by employing the oxidant HMF+ while at the same time verifying some earlier results obtained on this reaction via fixed wavelength spectroelectrochemistry.

This dissertation is written in a modular fashion which allows for complete discussions of each subject in context. Although some redundancy occurs the discussions are clearer.
LIST OF REFERENCES


18. Reticon Corp., 450 E. Middlefield Road, Mountain View, Calif. 94043.
CHAPTER I

GALVANOMETER BASED RAPID SCANNING SPECTROMETER

The development of the galvanometer based rapid scanning spectrometer (RSS) by Strojek, Gruver, and Kuwana\(^1\) has led to renewed interest in RSS and extensive investigation into applications of RSS to chemical problems and techniques of obtaining rapidly scanned spectra.

The methods of obtaining rapid scan spectra in the UV-VIS-IR have been extremely varied from mechanical devices to solid state array detectors.

Multi-sided (2-6 sides) mirrors driven by a motor have been used\(^2-18\) where the mirror rotated at high speed and caused the dispersed spectrum to be swept across the exit slit. Speeds up to 20,000 scans/sec have been reported (used to study the decomposition of CF\(_3\)I:Ar mixtures\(^3\) and hydrogen plasmas\(^4,6,7\)). Dye et al.\(^15,17,18\) have recently computerized an RSS of this type for use with a stopped-flow device.

A modification of the rotating mirror was a wheel with several corner mirrors cemented on its edge\(^11\). This RSS has been used to study solid rocket propellant emissions\(^12\) and has been manufactured and sold commercially\(^13,14\).
Rotating mirror RSS have very high scan rate capabilities but they suffer from two major drawbacks. Firstly, the synchronization of the rotating mirror to the processing circuitry was difficult and suffered from motor speed variations, and secondly the spectral region being examined cannot easily be varied (i.e., the rotating mirror sweeps the entire dispersed spectrum across the exit slit).

The use of an oscillating mirror (galvanometer mirror) in place of a rotating mirror was originally developed by Niesel and redesigned by Strojek, et al. In this RSS the dispersed light from a grating was linearly swept across the exit slit of the monochromator by passing a linearly varying current through the galvanometer mirror coils. A 100 nm scan in 340 usec has been possible and has been applied to monitoring and characterizing transient intermediates produced during electrolysis at optically transparent electrodes, spectroelectrochemical studies of bio-components, as a wavelength modulated derivative spectroscopy, stopped flow studies, high performance liquid chromatography, and simultaneous multielement emission spectroscopy.

The galvanometer RSS has the desirable features of being able to vary the width of the wavelength region and the speed of scan simply by adjusting the magnitude and rate of change of the driving current.

There have been RSS's designed with no mechanical (i.e., no rotating or oscillating mirrors) parts. A RSS described by Greig and Cooper was based on varying the separation of a Fabrey-Perot Etalon and scanned 20 \( \mu \text{m} \) (in the visible region) in 300 nsec.
A vibrating refractor plate\textsuperscript{30} and electro-optic modulator\textsuperscript{31} have been used in RSS and were based on index of refraction changes to cause scanning. These devices have not seen extensive use.

An acousto-optic tunable filter has been described as a RSS.\textsuperscript{32,33} This filter, tunable from 4000 Å to 7000 Å, was based on strain-induced change in the refractive index of a single crystal of LiNBO\textsubscript{3}. An acoustic frequency of 428 MHz to 990 MHz, coupled to the LiNBO\textsubscript{3} crystal by an acoustic transducer, destructively interfered with all optical frequencies except one which passed through the crystal. Varying the acoustic frequency varied the wavelength of light (i.e., frequency) passing through the crystal. The acousto-optic filter functioned as the monochromator. Acousto-optic RSS's have not yet been extensively used due to some rather stringent optical restrictions (limited aperture, requiring polarized light) and high driving power requirements.

RSS's based on array detectors have gained increased acceptance. An array detector consists of a one dimensional series of photosensitive elements (linear diode array (LDA)) or a two dimensional array (silicon vidicon tube (Vidicon)). Both the LDA and Vidicon integrate the light incident on their surface.

Horlick and Codding\textsuperscript{34} described an RSS based on a 256 element LDA. The LDA was mounted in the spectrometer at the exit slit focal plane and sampled 128 Å. The spectrum was determined by sequentially sampling each photodiode element. This RSS has also been used in
the measurement of emission spectra,\textsuperscript{35} dye laser measurements,\textsuperscript{36} direct-current arc time studies,\textsuperscript{37} and multielement atomic absorption analysis.\textsuperscript{38} Dessey\textsuperscript{39} has described a computerized 128 element LDA for RSS applications. In this laboratory a self-contained LDA was investigated\textsuperscript{40} and was used for the detection of sulfur dioxide.\textsuperscript{41} The LDA acquired spectra in 10 msec although 10 usec scans were possible.

A Vidicon differs from a LDA in that a Vidicon has a two dimensional photosensitive surface equivalent to 1000 x 1000 photodiodes. A Vidicon is a vacuum tube whereas a LDA is solid state. In a Vidicon, a spectrum is determined by interrogating the photoactive surface with an electron beam.

Gloersen described a Vidicon RSS for time resolved spectra of flashtubes at submillisecond times. A similar RSS (capable of acquiring a 400 nm spectrum (300-700 nm) in 6 msec at a maximum repetition rate of 60 scans/sec) was used to study rapid changes in hemoglobin and cytochrome \textsuperscript{c} by Kimura.\textsuperscript{43}

Kosewski and Grabowski\textsuperscript{44,46} developed a RSS related to the Vidicon RSS that used a cathode ray tube phosphorescent screen (oscilloscope) as a light source, placed at the entrance slit plane of a prism monochromator. As the electron beam was swept across the phosphorescent screen the angle of light incidence on a prism changed and caused the wavelength of light at the exit slit to change. A scan of 470-580 nm was possible in 240 usec. A photomultiplier tube was used to detect the monochromatic light passing through the exit slit.
Since 1972 Vidicon ESS has seen increased use. Santini, et al. described a Vidicon ESS that scanned 400-700 nm at 250 scans/sec and have applied it to atomic emission spectrometry. Milano and Pardue used a UV sensitive ESS for the study of molecular absorption spectra. Such ESS has been applied to simultaneous multielement flame spectrometry and to astronomical photometry. Two Vidicon ESS's have been commercially available. The Vidicon, LDA, and galvanometer ESS will be discussed later for comparison purposes.

Modern techniques of ESS have been discussed by Santini, Milano, and Pardue and pre-1968 techniques of ESS have been discussed by Pimentel.

In this chapter a detailed discussion of the galvanometer based ESS will be undertaken for the following reasons: (1) evaluate and improve the ESS monochromator; (2) determine the optical and electronic factors which affect the instrument's performance as a ESS; and (3) examine possible alternative techniques of ESS and compare those with the galvanometer ESS.

A. Optical Configurations

This study involved the use of the following instruments:
(1) RSSI for use in the near IR region; (2) RSSII (a breadboard unit) for studies to evaluate optical characteristics of ESS; and (3) RSSIII (commercial) with optical configuration based on RSSII.

The optical configuration of RSSI is shown in Figure 1. A tungsten quartz iodide lamp was used to provide visible and near IR light (Norma iodide II 75w). The lamp was mounted about one cm. from
Figure 1

ESSI optical layout.
the entrance slit and no focusing lens was used. A lens, L1, (focal length (f1)=12 cm) focused the exit slit image approximately 10 cm behind the galvanometer mirror (Ga) (Bell and Howell). Ga reflected the beam back to the mirror (M1) (f1 = 15 cm). M1 focused an image of Ga on the grating (G). G defracted the light which was transferred to the exit slit by the mirror (M2). M1 also focused the entrance slit image on the exit slit because the entrance slit image occurred at a point between M1's radius (which was twice the focal length) and the focal length.

The final image distance can be calculated from the following relationship:

\[
\frac{1}{S_1} + \frac{1}{S_2} = \frac{1}{f_1}
\]

(1)

where f1 is the focal length of M1 (15 cm), S1 is the distance from the entrance slit image to M1, and S2 is the distance from M1 to the exit slit. If S1 was 20 cm and f1 was 15 cm then S2 was 60 cm and defines the point at which the exit slit should be located.

The optical gain (magnification) of the entrance slit image was the ratio of S2 to S1 and was approximately 3.

The grating (Bausch and Lomb) was a plane reflection grating, 300 l/mm blazed at 700 nm.

The beam splitter was a set of layer mirrors, ca. 2 mm thick arranged in an alternating fashion with their reflective faces reflecting the beam 90°.
The photomultiplier tubes (PMT) were Hamamatsu type 7102 with a S-1 photocathode response. A single H.V. power supply (Fluke model 412B) was used. A 500 K potentiometer divided and adjusted the voltage to each PMT. The dynode resistor divider network and PMT housings were supplied by Pacific Photomultiplier, Inc. The last three dynode resistors had capacitors in parallel to the dynode resistors to provide response linearity at high currents.\(^5^8\)

The optic components for RSSI were purchased from Harrick Scientific Corp. A spectrometer case about 60 x 70 x 25 cm was constructed from aluminum plate to enclose the optics. A baffle was used to separate the monochromator from the sample compartment and to eliminate stray light and dust.

It was felt that removal of II (Figure 1) would eliminate a source of chromatic aberration. The focal point of II was wavelength dependent\(^5^9\) which caused a series of wavelength dependent entrance slit images at the exit slit (i.e., the slit image moved at the exit slit dependent on the wavelength being examined) causing a deterioration of resolution.

In RSSII a mirror of approximately the same f1 replaced II. A beneficial side effect of this modification was a folding of the light path making the monochromator physically smaller. The RSSIII optical configuration was nearly identical to RSSII and is shown in Figure 2.

The source was a 75W tungsten lamp or a 75W Xenon arc (Illumination Industries, Inc.) powered by a PEK 401A short arc power supply. A General Scanning, Inc. model G-606 galvanometer was used. The
Figure 2

RSSII and RSSIII optical layout.
size of the galvanometer mirror was ca. 3 x 5 mm (in ESSIII the mirror was round; diameter = 5 mm). The General Scanning galvanometer does not have its coils fluid damped as the Bell and Howell does and has a much lower coil resistance (ca. 7 ohm versus 70 ohm for the Bell and Howell) requiring higher driving currents (+.5 amp). PMT's used in ESSII were EMI 9529B which had a S-ll cathode response. The grating was a Bausch and Lomb 300 l/mm blazed at 400 nm. The ESSII was used during a study of a linear diode array detector.

The optics in ESSII were mounted on a 30 x 60 cm aluminum plate which had .3 cm slots machined on 2.5 cm centers.

ESSIII was commercially produced (Harrick, RSS-B). Minor differences in the instrument were: (1) a lens was used to transfer the Xenon arc image to the entrance slit with an optical gain of two; (2) a new beam splitter consisting of a fine Al grid (.010 inch Al squares approximately .010 inch apart) was used; and (3) new RCA PMT's with a GaAs photocathode were used.

The Al grid eliminated beam splitter "flicker" noise seen with the earlier splitter. The ratio of split light was position dependent in the earlier splitter and small light position changes caused by minor optical misalignment or Xenon source inhomogeneity resulted in noise.

An important addition to ESSIII was a complete redesign of the Xenon arc housing. The alignment of the light source was experimentally found to be critical with respect to optimizing light throughput and baseline flatness.
The light housing was designed to provide adjustments in X, Y, and Z axes, sufficient mass and area to maintain the external temperature less than 50-60°C without any external cooling, and ease of replacing the source. A schematic drawing of the housing is shown in Figure 3. The housing was machined from Al plate and dovetail grooves were used as travel guides and 10-32 screws were used to adjust the various carriage positions. The housing was painted flat black and bolted on the entrance slit side of RSSIII via the adapter plate.

B. Electronics

The galvanometer drive circuit for RSSI was a constant current circuit\(^6\) (shown in Figure 4) with a unity gain current booster (Teledyne Philbrick model 2001) to insure that the circuit was not current limited.

A General Scanning sweep/drive module was modified by disconnecting the internal sweep generator and wiring an external one (Hewlett-Packard model 3300A-3302A) to the drive circuit. RSSIII was driven by circuitry supplied by Harrick Scientific Corp.

Since the RSS units were dual beam instruments, a logarithm amplifier (log-amp) could be used to produce absorbance. Absorbance (A) is defined as the negative logarithm of transmittance which is the ratio of the transmitted to incident radiant power. In the RSS, the incident radiant power is measured by the reference PMT. The radiant power striking the PMT is, in principle, directly proportional to the current produced by the PMT. The log-amp should
Figure 3

Drawing of RSSIII adjustable source mount.
Figure 4

RSSI drive circuit. CA1 and B were Teledyne-Philbrick 1009 and 2001 amplifiers, respectively.
be a bipolar (both + or-log produced) and have high band pass
(frequency). The log-amp chosen was the Teledyne-Philbrick model
4361, which operated in a current mode. The anodes of the two
PMT's were connected directly to the inputs of the 4361. The 4361
was rated to give $1.000 \pm 0.025$ V per decade difference in the
input currents, was linear in response over 6 decades, and had a
band pass greater than 50 KHz. RSSIII had an internal log-amp
and produced $1.00 \pm 0.02$ V per decade difference in PMT current.
It was linear in response over 6 decades and had a band pass greater
than 100 KHz.

C. Optical Alignment

The optics in RSSIII was designed to have the light beam
centered 1.5 inches above the base plate. The alignment was done
with a Xenon arc and a Ho$_2$O$_3$ filter.

The initial step was to verify the integrity of optical com-
ponents by checking the beam height. The galvanometer mirror image,
produced by M3 (Figure 2), on the grating surface should be
stationary. A minimal movement was expected since the galvanometer
oscillated and the relative area seen by M3 changes. The position
of M3 was adjusted for minimal movement. A magnifying glass was
useful for this purpose. Following this procedure for the initial
alignment, adjustments to optimize resolution and light throughput
could be done.
The \( \text{Ho}_2\text{O}_3 \) triplet centered about 450 nm is an extremely narrow band and reflected the instrument's resolution. While viewing the \( \text{Ho}_2\text{O}_3 \) spectrum, M2 (Figure 2) was adjusted to maximize the absorbance. Adjusting M2 moved the image of the entrance slit at the exit slit and maximum resolution occurred when the entrance slit was focused at the exit slit. The entrance and exit slit widths can be optimized, in a darkened room, by opening or closing the slits to maximize the absorbance. The light throughput was optimized while viewing one of the PMT emission envelopes and adjusting the light housing until maximum light throughput was obtained. A well adjusted RSS would have an absorbance of about 1.6 (using a 300 l/mm grating) for the most intense peak of the \( \text{Ho}_2\text{O}_3 \) triplet.

Several adjustments to insure that the entrance slit jaws were parallel to the grating grooves and to the exit slit were possible but were not usually required. The parallelism could be checked by adjusting the grating so that the zero order light passed through the exit slit and visually viewing the parallelism. The wavelength passing through the exit slit was adjusted by rotating the grating. Tilt adjustments in the beam splitter were possible but were not required.

D. RSS Performance

The absorbance and wavelength accuracy were verified through the use of filters and solutions of known absorbance. The operation of RSSI in the near IR (total wavelength range 550-900 nm) was
verified using a didymium filter. A typical spectrum of the didymium filter obtained from RSSI is shown in Figure 5 (a spectrum from a Cary-14 spectrometer provided peak wavelengths). Absorbance response linearity of RSSI was measured using Cu(SO₄) solutions prepared according to a standard procedure. RSSI was found to be linear (measured at 750 nm) to $1.6 \pm 1\%$ A. A Ho₂O₃ filter, a secondary wavelength standard, was used with RSSII and RSSIII for wavelength calibration. A Ho₂O₃ spectrum acquired from RSSIII is shown in Figure 6. The peak absorbances shown in Figure 6 are in agreement with those obtained from a Cary-15 except for the very sharp peak at 360 nm (Cary-15 A = .9) and the 453 nm peak (Cary-15 A = 2) of the triplet.

The 360 nm peak height was resolution limited. The 453 nm peak height was limited primarily by resolution and secondarily by stray light. The reason behind the stray light limitation will be discussed later. The response linearity of RSSIII was measured at 520 nm using Co(NO₃)₂ solutions and was found to be linear to $2.0 \pm 1\%$ A at 520 nm.

To examine the UV capabilities of RSSIII a spectrum of benzene vapor (obtained by placing a few drops of benzene in a 1 cm cuvette and allowing equilibrium to be obtained) was taken (shown in Figure 7). The expected fine structure was eliminated due to resolution limitations but the gross structure was apparent.

The last three figures illustrate the wavelength capabilities of a galvanometer RSS. Factors which affect the RSS output and
Figure 5

Spectrum of a didymium filter taken on RSSI. The scan rate was 50 sec/scan.
Figure 6

Spectrum of a Ho$_2$O$_3$ filter taken on RSSIII. The scan rate was 50 sec/scan.
Figure 7

Spectrum of benzene vapor in a 1 cm quartz cuvette. No reference cell. Scan rate was 50 sec/scan.
must be discussed in order to gain performance insight, including resolution, sweep wavelength linearity, light source and source power supply, sweep rate capabilities, electronic frequency response, grating, PMT response, scanning width, baseline flatness, noise, signal to noise ratio, and dynamic range.

1. Resolution

In this work resolution was defined by the Rayleigh criterion which stated that two diffraction limited lines which have zeros on either side of their maxima were resolved if the zero of one was superimposed on the maxima of the other. This was approximated by the width of a diffraction limited peak at 80% of its height.

The resolution of RSSIII was measured at 633 nm by employing a HeNe laser which replaced the light source. The width of the laser line was measured to be 0.54 nm. Figure 8 shows the laser emission trace superimposed on absorbance trace of a Ho2O3 filter showing the 0.54 nm resolution. A 10 nm wide scan of the HeNe peak is shown in an insert in Figure 8 and was used to calculate the resolution. Another definition of resolution was peak width at half height. RSSIII had a resolution of 1.2 nm using this definition.

The resolution was limited by the grating and the aberrations caused by off-axis spherical optics. Theoretically the image of the galvanometer mirror on the grating surface defined the usable width of the grating which dictated the resolving power (R) of the
Figure 8

HeNe laser peak shown in relation to a Ho\textsubscript{2}O\textsubscript{3} spectrum. Insert is a copy of a 10 nm spectrum of a HeNe peak photographed from an oscilloscope.
HeNe Laser

WAVELENGTH (nm)

INTENSITY

.54 nm

628  638
ESS. R is expressed as

\[ R = m \cdot N \]  \hspace{1cm} (2)

where \( m \) is the grating order and \( N \) is the total number of grating lines used. In RSSIII \( m = 1 \) (first order) and \( N = 5 \) mm (the diameter of the galvanometer mirror). Employing the 300 l/mm grating \( R = 1500 \). The resolution \((\Delta \lambda)\) can be calculated from

\[ R = \frac{\lambda}{\Delta \lambda} \]  \hspace{1cm} (3)

At 633 nm \( \Delta \lambda \) is theoretically .42 nm which compares reasonably well with the experimental value of .54 nm. The differences in values were probably due to optical aberrations.

2. Optical Aberrations

The optical aberrations in this type of monochromator have been discussed. The convergent illumination to the plane grating plus the aberrations caused by the two spherical focusing mirrors produced coma. Coma was produced when an image was unevenly magnified by the monochromator optics producing a "comet like" image at the exit slit. Spherical aberration (lack of focus) was a secondary effect. It has also been noted that the monochromator "f" number should be large to obtain good resolution. The f number of the RSS was calculated by dividing the focal length of the monochromator by the diameter of the limiting aperture stop which was
the galvanometer (diameter = 5 mm). The focal length of the
spherical mirror (M3, Figure 2) was 15 cm. The f number was therefore \( f/30 \) which was large compared to monochromators typically used in medium resolution spectroscopy \( (f/4.4 \) with similar resolution\(^6\)).

3. Sweep Wavelength Linearity

The wavelength linearity of RSSIII was measured by recording a \( \text{H}_{2} \text{O}_{2} \) filter spectrum (350-650 nm) on an X-Y recorder. The linearity was measured by computing wavelengths scanned per inch quantity. The constancy of this quantity indicated that the wavelength linearity was within measurement error (approximately 1 nm). At 100 scans/sec, 300 nm wide, the RSSIII wavelength response appeared to meet specifications (±1 nm) as viewed on an oscilloscope and at the maximum scan rate of 340 usec/scan (100 nm) the last 30 nm was linear to ± 1 nm as per the manufacturer's specifications.

Wightman, et al.\(^{26}\) have commented on the effect of galvanometer warm-up on sweep linearity. Their RSS employed a Bell and Howell galvanometer which has its coils fluid damped and the effect seen by them was probably due to fluid heating. The General Scanning galvanometer did not employ fluid damping and no apparent warm-up effects were seen.

4. Light Sources and Light Source Power Supplies

The utility of a light source depended on the brightness of the source, wavelength distribution of light emitted, and the light
source stability. These factors affected the usable wavelength region and the sensitivity of the RSS (in addition to grating and PMT effects).

The 75W Xenon arc obtained from Illumination Industries was rated at a brightness of 80,000 candela/cm² and operated at approximately 6000 °K. The 75W tungsten lamp had an approximate brightness of 12,000 candela/cm² and operated at about 3300 °K. The brightness rather than power of the source must be considered in evaluating the suitability of the source since the brightness affected the light throughput.

The Xenon arc emitted an estimated 50% of its total output in the UV-visible (to 800 nm) region whereas the tungsten bulb emitted only a few percent. The tungsten lamp was a filament approximately 1 x 10 mm while the Xenon arc was a point source approximately 5 mm in diameter. Employing a 75W tungsten lamp, versus a 75W Xenon arc caused approximately 1/100 of the light throughput (estimated as 1/10 of usable wavelength x 1/2 of usable height x 1/5 as bright = 1/100).

The tungsten lamp emitted substantial NIR light and did not have the strong emission lines that the Xenon arc had. The tungsten lamp was used in RSSI because of the NIR emission. The Xenon atomic emission lines at about 450 nm affected the sensitivity of the RSS because the PMT gain must be decreased in order to prevent saturation of the following circuitry.
Stability of the light output from the Xenon arc was a function of power supply noise and noise originating from the arc itself. The regulation of the PEK power supply was measured to be .02% of the applied voltage and arc positional changes and plasma instabilities appeared to be 1-2% of the emitted output. Most of the arc noise was cancelled since the RSS was a dual beam instrument but due to optical and electronic differences between the sample and reference sides of the RSS instrument some source noise remained. A more complete discussion of source noises has appeared elsewhere. Some success has been achieved in stabilizing a Xenon arc by both optical and electronic techniques. No additional stabilization of sources was attempted in this work although an evaluation of the possible benefits from stabilization would be a worthwhile effort.

The Xenon arc had an inhomogeneous wavelength emission across the width and height of the arc. This inhomogeneity at the exit slit resulted in an uneven splitting of the light beam by the beam splitter which resulted in a non-flat (non-constant) absorbance baseline. The effect of the inhomogeneity was partially eliminated by the Xenon arc housing described earlier.

5. Scan Rate Capabilities

The scan rate of RSSIII was the time required to scan a spectrum. Repetition rate was the maximum number of scans per second. Scan frequency was the reciprocal of the scan rate. An examination of
the scan rate capabilities of RSSIII was undertaken in order to define the instrument's capability as a rapid scan spectrometer. RSSIII employed a sawtooth drive wave which was compensated to ensure linear galvanometer response by computing, digitally, and overdrive voltage via a feed forward digital technique. This compensation provided a linear scan at any scan rate within scan width limits. The repetition rate was limited by the time required by the galvanometer to return to its starting position and settle down. This limited the repetition rate to 218 scans/sec. The time required for the 100 nm scan at the fastest scan rate was 340 usec. Figure 9 shows three oscilloscope trace photographs of the 450 nm Ho\textsubscript{2}O\textsubscript{3} triplet taken at various scan rates. It should be noted that neither the peak positions change or peak distortion occurs. At the fastest scan rate, the intensity of the strongest Ho\textsubscript{2}O\textsubscript{3} peak decreased by approximately 10% due to frequency response limitations of the RSSIII electronics.

6. Frequency Response

It was important to be sure that the RSS electronics had sufficient frequency response so that the absorbance signal at fast scan rates was not distorted. Particular concern was directed to the log-amp since these devices generally have poor frequency response at low input currents (i.e., high absorbances).
Figure 9

Traces of 100 nm wide Ho$^{0.2}$ spectra taken at three scan rates.

A. 40 msec/scan
B. 4 msec/scan
C. 340 usec/scan
The frequency response of the instrument was measured in the following ways: (1) The output of the RSSIII PMT and the following current-to-voltage convertor (i-E) were checked using a light emitting diode (LED). (2) The Xenon arc was turned on and the frequency response using the LED was checked at low absorbance. And (3) Step (2) was repeated at high absorbance. This set of experiments gave an indication of what the frequency limitations were in RSSIII and the sources of the limitations.

The LED was used to provide a light source which could be rapidly pulsed on and off and superimposed on the Xenon arc or studied independent of the Xenon arc. LED's were known to have sub usec on-off times and the response time of the LED did not limit the measurement. The LED was suspended in the sample compartment of RSSIII so that the light struck the PMT photocathode but did not interfere with the Xenon arc light.

The oscilloscope trace shown in Figure 10 was acquired by recording the voltage output from the PMT current-to-voltage convertor while pulsing the LED. The rise time (to 90% of max) was about 5 usec which was equivalent to a bandpass of 200 KHz. Figure 11 shows a trace acquired by superimposing the pulsed LED light on the Xenon arc light and monitoring the log-amp absorbance output. This trace reflected the frequency response of the log-amp and the rise time was 5 usec, indicating that the log-amp did not measurably reduce the bandpass of RSSIII at an absorbance of zero. Attempts to study the rise time of the LED superimposed on a high absorbance
Figure 10

Photograph of an oscilloscope trace showing output of PMT and current-to-voltage convertor in RSSIII while pulsing a LED.
Figure 11

Photograph of an oscilloscope trace showing rise time of RSSIII in an absorbance mode. Light source is a LED superimposed on the Xenon arc source light.
(\(A = 1.5\)) were difficult since at high absorbances the absorbance change caused by the LED was buried in noise. It was estimated to be 10 usec. The total absorbance change caused by the LED was 0.05 A. The causes of increased noise at high absorbances are discussed later.

7. Grating

The properties of the grating controlled resolution and wavelength efficiency. The former was dictated by the number of grating grooves per millimeter and the latter by blaze angle.\(^{63}\) The mechanical imperfections of the grating caused stray light.\(^{64}\)

The grating blaze efficiency governs the amount of monochromatic light diffracted in the order being measured (first order in this case) relative to the light reflected by a flat mirror. The physical control over the properties which affected the grating efficiency were termed "blazing" and were related to the geometry of the groove shape. Hence the "blaze angle" or "blaze wavelength" were employed to give an indication of maximum wavelength efficiency. For a grating blazed for 400 nm, the maximum wavelength intensity of diffracted light will be about 400 nm and the intensity will drop to about 50-70\% of the maximum intensity at 200 nm and 800 nm.\(^{63}\) These intensity figures assumed a constant energy distribution of incident radiation and operation of the grating in a Littrow configuration.
(Littrow configuration occurred when the angle of light incidence relative to the grating perpendicular was equal to the angle of reflectance. The RSS III optics approximate this configuration.

Stray light (or ghosts) produced by the grating was a result of periodic variations in grating groove spacing and non-perfect flatness of the reflecting surfaces. The effect of stray light (both grating caused and the stray light caused by imperfect optics (e.g., non-flat mirrors, scratches, etc.)) was to limit the maximum absorbance value which could be measured. The stray light limited the RSS to an absorbance value which was the log ratio of the reference PMT current to the stray light current from the sample PMT.

8. Wavelength Range

The wavelength range or wavelength width a RSS could scan was dependent on the type of grating (number of grooves/mm), grating diffraction order, and width of the focusing mirror (M3 in Figure 2).

The wavelength range could be calculated from a knowledge of the usable width of M3, the radius of M3, the number of grating grooves used, and the grating diffraction order used. From simple trigonometric considerations, the usable width (5 cm), and radius (30 cm) of M3, the total angle change of light incident on the grating was calculated. Employing the grating equation

$$m\lambda = a (\sin \alpha \pm \sin \beta)$$  \hspace{1cm} (4)
where \( \lambda \) is the defracted wavelength at a fixed angle, \( a \) is the grating groove spacing, \( m \) is order, \( \alpha \) is the angle of defraction relative to the grating normal, and \( B \) is the angle of incident light on the grating relative to the grating normal. A total change in \( B \) of approximately \( 10^\circ \) gave a total wavelength range of nearly 500 nm.

9. PMT Response

The two PMT's used in an RSS transduced the electromagnetic radiation to an electronically monitorable signal. PMT's were particularly suited as detectors in spectrometers due to their high internal gain. Other characteristics required for optimum performance were high gain, high quantum and collection efficiency, low thermionic and Ohmic dark current, broad wavelength response, and fast frequency response.

The gain of the PMT was controlled by the number of stages and magnitude of the high voltage. All PMT's had the anode at ground potential and the photocathode at negative high voltage, and typical gain values were \( 10^5 \) to \( 10^7 \).

The primary type of dark current was caused by non-photon induced current pulses (thermionic). Thermionic dark current was dependent on the high voltage value and temperature in a non-linear fashion increasing rapidly with high voltage. Ohmic dark current through the dynode resistors provided Johnson noise at the dynodes, which caused dynode potentials to change and pulse amplitude variation adding to the shot noise.
The construction material of the photocathode determined the wavelength response characteristics of the PMT. The spectral response curves for the three types of PMT's used in this study are shown in Figure 12. These curves show the relative photocathode radiant sensitivity (to a constant amount of light at each wavelength) versus wavelength. The quantum efficiency (probability that a photon of given energy would cause an emission of an electron from the photocathode) is also indicated. The EMI 9529B (S-11) tube was used for visible and UV work in the visible region because of its high quantum efficiency and high gain. The Hammatsu 7102 (S-1) tubes were chosen because of their NIR response, but, unfortunately, had a low quantum efficiency. The RCA GaAs tubes had an excellent wavelength response and quantum efficiency between 200 nm and 900 nm.

Frequency response was not generally an important consideration since PMT's in general have 10 to 50 MHz bandwidths but was of importance in photon counting applications.

Since two PMT's were used, it was important that both tubes had the same spectral response characteristics (i.e., for equal light intensity at a given wavelength, each PMT produced equal currents). PMT's were purchased as matched sets from the manufacturers. An example of emission response curves from RSSII employing the EMI 9529B tubes are shown in Figure 13. These traces are nearly identical although some mismatch occurs at both ends of the spectrum. The mismatch may have been due to PMT response differences or optical imperfections which caused the ratio of light reaching each PMT vary.
Figure 12

Spectral response curves for the three types of PMT's used.

Data reproduced from reference 76.
Transmission response curves of RSSII EMI 9529B PMT's. Current from each PMT is measured as voltage drop across of 100 KΩ resistor. Spectra recorded at 50 sec/scan.
10. Baseline Flatness

The baseline in a RSS was the signal obtained from the log-amp as a function of wavelength, and the flatness should be zero (or constant) at all wavelengths. The baseline generally varied from zero and was a function of wavelength but could be maintained to within $\pm 0.025$ Å over 350 to 600 nm. The non-flatness usually exceeded the stated limits outside of the 350 to 600 nm region although adjustments could be made to "flatten" the baseline in the UV or NIR as required. A subsequent loss of flatness in other wavelength regions was to be expected. The baseline was affected primarily by Xenon arc source alignment, PMT spectral response differences, and minor optical misalignment as described earlier.

The consequences of a non-flat baseline were that during the measurement of small absorbances the absorbance of the baseline must be subtracted from the sample absorbance. This technique was readily done but was inconvenient and undesirable. Baseline correction by hand calculation could be eliminated by computer acquisition of baseline and sample spectrum and subtracting the baseline spectrum from the sample spectrum and at rapid scan rates the baseline limited the sensitivity of the RSS because the baseline limited the amount of external electronic amplifier gain which could be employed without saturating (limiting) the amplifiers. The last effect is discussed in more detail in Chapter V.
11. Noise

Noise in an ESS limited the ultimate sensitivity of an absorbance measurement. Noise was mainly random and possible sources were PMT shot noise, 1/f noise from the light source, both thermal (white) noise and 1/f noise from the electronic components and noise (particularly "baseline noise") which arose at rapid scanning rates.

The primary source of noise from the PMT was shot noise which arose from the superimposition of photon generated electronic pulses and was known to be random in nature. The resultant signal from the PMT was an average DC signal on which the shot noise was superimposed. Secondary types of PMT noise (PMT flicker noise, background radiation flicker noise, secondary emission noise) were described elsewhere. The shot noise became important and predominant at msec scan rates since the shot noise could not be filtered out without distorting the signal.

Light source noise was predominately 1/f type noise and was attributed to, in the Xenon arc case, arc wander. The magnitude of the noise at low frequencies (i.e., those recorded by a Houston 2000 X-Y recorder) was approximately $5 \times 10^{-3}$ A peak-peak at 450 nm. This was measured by recording absorbance spectra over several hours. The low frequency noise was $3 \times 10^{-3}$ A at 300 nm and $8 \times 10^{-3}$ A at 600 nm. It has been noted that Xenon arcs can have noise components with frequencies to 3 KHz. RSSI low frequency noise was $10 \times 10^{-3}$ A peak-peak at 700 nm. Wavelength dependence was not examined.
The low frequency noise figure also reflected contributions from the electronics (i.e., log-amp) which followed the PMT. Low frequency noise due to the PMT current-to-voltage convertor and log-amp was estimated without light striking either PMT. The resulting output was the log ratio of the PMT and electronic dark current and reflected any time dependent variations in these quantities. A value of .1 mv p-p at frequencies below 60 Hz was obtained.

Noise at frequencies to 100 KHz were $4 \times 10^{-2}$ A at an absorbance of zero and $1.2 \times 10^{-2}$ A at an absorbance of 1.5. These figures reflected the PMT shot noise and the electronic amplifier broad band white noise.

The shot noise increase at high absorbances was due to the taking a log of a high PMT current/to low PMT current. The log-amp output noise increased because the shot noise on the low light (high absorbance) PMT increased as the number of photons reaching the photocathode decreased. Also, the log-amp had increased noise at higher absorbances because the gain of the log-amp increased, causing the signal noise to be amplified to a greater extent. The high frequency noise values were a factor of 3.5 larger than those measured by Wightman, et al. and were due to the higher frequency bandpass of RSSIII.

The high frequency noise could be eliminated by a RC filter with a 10 msec time constant. The high frequency noise thus became an important limitation to the sensitivity of RSSIII where filtering
cannot be used because it limited the amount of external gain which could be used without limiting the amplifier output. Signal averaging via a computer reduced the high frequency noise problem and is discussed in Chapter V.

12. Dynamic Range and Signal-to-noise Ratio

The dynamic range was defined as the maximum signal at which response was linear \((2.0 \text{ A in RSSIII})\) divided by the noise and was equivalent to the signal-to-noise ratio \((S/N)\). In RSSIII the dynamic range is illustrated by three values. The optimum value at 450 nm would be at X-Y recorder speed where the noise was \(5 \times 10^{-3} \text{ A}\) giving a dynamic range of 400. At fast scan rates the noise was \(4 \times 10^{-2} \text{ A}\) and the dynamic range would be 40 and finally employing the noise value of \(12 \times 10^{-2} \text{ A}\) at high absorbances the dynamic range was 17. All dynamic ranges were quoted at maximum resolution and could be improved by increasing slit widths which decreased the shot noise due to increased photon flux.

E. Discussion

The earlier sections of this chapter were intended to give an insight into the parameters affecting RSS performance. Of course the final RSS signal is a convolution of all these parameters and all must be considered together. In particular, the usable wavelength range of RSSIII was a convolution of Xenon arc source
spectral distribution, grating blaze and efficiency, and PMT spectral response.

A summary of RSS capabilities is given in Table 1. Certain points, relative to the table, will be brought out in particular. RSS instruments are capable of operating from 220 nm to 900 nm by optimizing of the output in a given region (grating, PMT). Wavelength scan ranges of 0 nm to 500 nm are possible. RSS scan frequency capabilities are DC to 250 spectra/sec with good wavelength linearity (± 1 nm) at all scan frequencies. Of course the maximum permissible scan rate is a function of the wavelength range scanned decreasing to 100 nm at the fastest scan rate. The repetition rate is limited to 218 spectra/sec due to the electromechanical limitations of the galvanometer. The repetition rate will not increase substantially unless the mechanical galvanometer is eliminated from the monochromator. The RSS sensitivity varies from 5 x 10^-3 A at slow scan rates to .12 A at fast scan rates and high absorbances.

The present limitations of the RSS appear to be of the following three sources: (1) the Xenon light source noise which might be eliminated by employing optical feedback or better power regulation; (2) the PMT shot noise which is fundamental and cannot be eliminated; and (3) the baseline flatness which limits sensitivity and might be eliminated by employing a chopped, single PMT RSS.
TABLE 1

GENERAL CAPABILITIES OF THE RSS

1. Output is direct absorbance
2. Wide variation in scan width - 0 nm to 500 nm
3. Scan rates of DC to 340 usec/scan
4. Maximum repetition rate of 218 scans/sec
5. Spectral range of 220 nm to 900 nm
6. Absorbance sensitivity of .005 A at 0 A
7. Reproducibility of scan is $\pm$ 0.5 nm
8. Resolution is .54 nm at 630 nm
9. Readily adaptable to a wide variety of applications (stopped-flow, HPLC, flame emission, derivative spectroscopy, spectroelectrochemistry, and normal absorbance spectroscopy)
1. RSSIII

The Harrick RSSIII has several features in the electronics module which are worth mentioning. The wavelength controls are set via digital readout potentiometers and are experimentally verified to be accurate and reproducible within $\pm 1$ nm. Scan rate controls are digital as well as the function controls (start, stop, single sweep, etc.). RSSIII contained an internal Ho$_2$O$_3$ filter whose position is digitally controllable. A decade (1 Hz to 100 KHz) RC filter is also available and digitally controllable. Optimization of the galvanometer drive wave for maximum frequency response is possible through two front panel controls. RSSIII can be placed under computer control and the required interface hardware and software exists for this purpose.

2. A Comparison

It is important to consider the capabilities of RSSIII as compared to the Vidicon and LDA. In one sense, comparing a galvanometer RSS with the Vidicon and LDA is akin to comparing apples to oranges, because Vidicon and LDA RSS's are multielement detectors and are light integrating detectors and the RSSIII measures instantaneous light. These RSS's are being applied to the same types of measurements and a comparison will be made. Table 2 lists various capabilities of the galvanometer, Vidicon, and LDA RSS's. There are several other types of RSS but the three mentioned appear to be the predominant forms currently in use.
### TABLE 2

CAPABILITIES OF THE GALVANOMETER, VIDICON, AND LDA RSS's

<table>
<thead>
<tr>
<th>Capability</th>
<th>RSSIII</th>
<th>Vidicon</th>
<th>LDA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. scan</td>
<td>.34 msec-DC</td>
<td>4 msec-1 sec</td>
<td>13 usec-4 sec</td>
</tr>
<tr>
<td>2. frequency scan</td>
<td>4.25 KHz-0 Hz</td>
<td>250 Hz-10 Hz</td>
<td>77 KHz-.25 Hz</td>
</tr>
<tr>
<td>3. repetition rate</td>
<td>218 Hz</td>
<td>250 Hz</td>
<td>77 KHz(9.7 KHz)</td>
</tr>
<tr>
<td>4. frequency response</td>
<td>200 KHz</td>
<td>250 Hz</td>
<td>77 KHz(917 KHz)</td>
</tr>
<tr>
<td>(1 RC time const)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5. dynamic range</td>
<td>400</td>
<td>10^4</td>
<td>10^3</td>
</tr>
<tr>
<td>6. scan width</td>
<td>0-500 nm</td>
<td>400 nm</td>
<td>12.8 nm-200 nm</td>
</tr>
<tr>
<td>7. resolution</td>
<td>.54 nm</td>
<td>1 nm</td>
<td>.1 nm-.6 nm</td>
</tr>
<tr>
<td>8. resolution elements</td>
<td>infinite</td>
<td>10^3x10^3</td>
<td>128-1024</td>
</tr>
<tr>
<td>9. maximum sensitivity</td>
<td>5x10^-3 A</td>
<td>2x10^-3 A</td>
<td>6x10^-3 A</td>
</tr>
<tr>
<td>10. output mode</td>
<td>absorbance</td>
<td>transmittance</td>
<td>transmittance</td>
</tr>
<tr>
<td>11. integrating</td>
<td>no</td>
<td>yes</td>
<td>yes</td>
</tr>
</tbody>
</table>
The scan rate of the LDA is predicted to be the fastest (13 usec) for a 128 photodiode LDA. This value is an extrapolated value based on a maximum clock rate (10 MHz) of the LDA. The maximum scan rate of a LDA depends on the number of photodiodes. 128 to 1024 element photodiode LDA's are available and have been used. The slowest scan rate of the LDA RSS is limited by integration of thermal dark current. The Vidicon RSS has demonstrated scan rates of 4 msec/scan to .1 sec/scan. The fastest scan rate reflects the Vidicon detector beam discharge lag which is due to the finite time required to recharge the Vidicon surface. This effect does not occur in a LDA. RSSIII scan rates are .34 msec/scan to DC. RSSIII is limited by the electromechanical nature of the galvanometer.

The scan frequency values are misleading and should be considered in combination with the repetition rate and scan rate. The LDA has a definite advantage in the fastest possible scan rate and repetition rate at 77 KHz for a 128 element LDA. The Vidicon (250 scans/sec) and RSSIII (218 scans/sec) are comparable in repetition rate although RSSIII operates at a scan frequency of 4.25 KHz for a 100 nm scan wavelength width. Neither the Vidicon nor LDA have any wavelength width dependence on scan rate as RSSIII does.

As pointed out by Wightman, et al., the frequency response of the integrating array detectors is very limiting. The Vidicon and LDA have a frequency response which is directly proportional to the time light is integrated on their surface. The Vidicon has a maximum repetition rate of 250 Hz. Therefore, for some changing spectra, the maximum frequency components of the signal must be 1/2
of the repetition rate. This is the Nyquist criterion. Therefore the maximum frequency component in a changing spectra must not exceed 125 Hz. The LDA RSS, in principle, does not have such severe frequency limitations. A frequency component of 38.5 KHz could be accurately sampled by a 128 element photodiode array operating at the maximum scan rate. RSSIII has a similar sampling rate to a Vidicon RSS when scanning (218 scans/sec) but the RSS is capable of DC (fixed wavelength) absorbance-time measurements. In a DC mode RSSIII has a bandpass of 200 KHz and could sample a frequency component of 100 KHz (assuming no external sampling limitations).

This past argument is based solely on Nyquist frequency considerations. Another point to be considered about integrating detectors is the effect of integration time on signal amplitude. This is related to apertature time of a sample and hold circuit which causes an error voltage on the output. Again the magnitude of the error will depend on the frequency components of the signal. For example, a sine wave of amplitude Vm will have an error voltage (ΔV) expressed by:

\[ ΔV = Vm \sin (2πft_a) \]

where \( f \) is the sine wave frequency and \( t_a \) is the apertature time.

The Vidicon and LDA RSS's aperture times (integration time) are the same as the sampling time (scan rate) and voltages output by the integrating device could be in error as much as 100% for frequency components on the order of 1/2 of the scan rate. The error on
frequencies lower than the scan rate will be less. The galvanometer RSS has the same problem except seriously affected frequency components are on the order of 100 KHz.

The data presented by Pardue, et al. and Horlick, et al. appear accurate and do not show the aperture time effect but the changing signals measured may not have high frequency components. The effect should certainly be present and must be considered when examining rapidly changing signals.

The dynamic range figures indicate over what range a RSS will have linear response. RSSIII dynamic range measurements are made using various absorbance solutions as recommended in a NBS circular. The RSSIII dynamic range figure includes monochromator stray light and is an accurate reflection of the instrument's performance. Measurements of dynamic range on Vidicon and LDA are made with a mechanical optical attenuator employing accurately calibrated polaroid filters to attenuate the light. This procedure is certainly applicable to measurements of a detector's dynamic range but the use of a mechanical optical attenuator to measure a spectrometer's dynamic range is questionable because the monochromator stray light is attenuated as well as the dispersed light and effectively eliminating stray light effects. Neutral density filters have also been used but their absorbance is broad and the same effect may occur.

The scan width of RSSIII is dependent on the grating groove width and optical limits. The Vidicon and LDA RSS's are dependent on their physical width and the wavelength width of light focused on
their active surface which is in turn a function of the physical characteristics of the monochromator. Since the array detectors consist of discreet photosensitive elements, the resolution of the monochromator should be five times the wavelength width sampled by a photosensitive array element. If this is not the case the array detector will limit the resolution of the RSS. The resolution required in RSS's is dependent on the application involved. Atomic spectroscopy generally requires high (1 or 2 Å) resolution while in solution absorbance measurements 1 or 2 nm is sufficient. It can be stated that as resolution requirements increase the possible wavelength width must decrease.

Number of resolution elements has been discussed in the preceding paragraph with accent on RSS resolution. Galvanometer RSS's have an effectively infinite number of resolution elements, assuming a continuous analog drive wave. RSSIII has a digitally stepped analog drive giving it a 10 bit (1/1024 of the scan width) resolution.

RSSIII sensitivity is a function of scan speed with a maximum sensitivity of 5 x 10⁻³ Å at DC and a minimum of .12 Å at maximum scan speed. Wightman, et al. show better sensitivity figures but RSSIII has a substantially higher bandpass and more shot noise is passed by the electronics. Vidicon sensitivity has been reported as 2 x 10⁻³ Å at 250 scans/sec. LDA absorbance sensitivity is reported as 6 x 10⁻³ Å (in atomic absorption analysis). Recent data
on a Vidicon RSS indicates that this instrument suffers from a rather severe sensitivity problem at high absorbances. Data given indicates a standard deviation of 0.007 %T at 0.01 %T. It is assumed by this author that the standard deviation given is ±0.007% (stated to be 2S) versus ±0.0035%. At 0.01 %T the absorbance is 2.0 and, including the given error, absorbance ranges of 1.77 to 2.52 are possible with the ± standard deviation at a 95% confidence limit (if ±0.0035% error is assumed, the absorbance values are 1.87 and 2.2).

The output mode (transmittance or absorbance) is important since absorbance is directly proportional to concentration. RSSIII produces absorbance directly; the Vidicon and LDA produce transmittance. The Vidicon and LDA are integrating but RSSIII is not.

Which is the better RSS? This question probably has no answer. There are advantages and disadvantages to all three types. It seems that for rapid scan spectral studies RSSIII has a distinct advantage. The statement is primarily based on versatility considerations. RSSIII has a wide variation in both scan speed and wavelength range. The possible use of RSSIII as a fixed wavelength spectrometer for fast kinetic studies is a definite advantage. The RSSIII can also be used as a slow sweep spectrometer. The fact that RSSIII produces a direct absorbance output eliminates further signal processing. Array detectors are generally used with a computer to convert transmittance to absorbance. RSSIII does not have the frequency limits (integration time) that integrating array detectors have.
Array detectors have an advantage in size and ruggedness (particularly the LDA) and can be readily adapted to most standard monochromators. The integrating capabilities may be a particular advantage in high noise (flame) studies for multielement analysis although present sensitivities are less than an equivalent PMT instrument. The "ruggedness" of RSSIII is lacking requiring a certain amount of knowledge and intuition for optimum performance. Research is in progress to improve the two-dimensional array detectors and the state-of-the-art devices have been reviewed by Talmi.

F. Conclusion

Important improvements could possibly be made in the galvanometer RSS. A higher repetition rate would allow faster kinetic changes to be examined. This may be difficult without a sacrifice in wavelength linearity due to the electromechanical nature of the galvanometer. A light throughput improvement could be obtained by increasing the size of the galvanometer (as well as resolution increase) with a sacrifice in scan speed.

It should be possible to improve the sensitivity of the galvanometer RSS by elimination of the low frequency noise components. Since these appear to be due to Xenon arc fluctuations, an appropriate stabilization circuit may be helpful. The high frequency noise components are fundamental to the PMT and cannot be eliminated except by signal averaging or reducing the scan speed and employing a filter.
Baseline flatness could be improved by using a single detector and in some way chopping the light beams. The use of a single PMT eliminates both PMT mismatch and wavelength inhomogeneities. Various modulation techniques are under investigation in this laboratory with some encouraging results.\textsuperscript{25}

It should be obvious the RSS are currently enjoying a great success as analytical instruments for a variety of applications. As the research in improving all forms of RSS continues, a continued advancement in RSS capabilities and applications must be expected. Eventual use of RSS as a routine analysis instrument is certainly possible.
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CHAPTER II

COMPUTER SYSTEM

The digital computer has definitely become common equipment in the chemistry laboratory. Applications range from data calculations to instrument automation. Many analytical instruments are now supplied with some form of integral computer. A recent book by Perone and Jones\textsuperscript{1} describes many types of computer applications prior to 1973. Examples of recent computer applications to many techniques may be found in most chapters of the Fundamental Reviews of Analytical Chemistry.\textsuperscript{2} The availability of inexpensive microprocessors has presented new possibilities for application of computers to chemical problems. There have been recent articles on microprocessor hardware\textsuperscript{3} and possible microprocessor applications.\textsuperscript{4}

A computer system has been developed for use in this laboratory with the following goals in mind: (1) automate the experimental system involving indirect coulometric titrations of bio-components; (2) control the Harrick\textsuperscript{5} Rapid Scanning Spectrometer (RSSIII); (3) perform experiments in electrochemistry (chronoamperometry, cyclic voltametry, chronocoulometry) and spectroscopy (rapid scanning spectroscopy, fixed wavelength spectroscopy) commonly used in this laboratory; and (4) run two programs concurrently.
in the computer. The last goal allows two users to share the computer system resources and is possible through the use of the manufacturer's operating system (Real Time Disk Operating System (RDOS)). The first three goals are provided through the design and construction of interface hardware and writing software. We have also maintained that the computer system should have a high degree of "user" versatility, that is, it should be easy to use by personnel unfamiliar with the computer system and should only be used when the benefits would justify the effort (i.e., time and possibly money).

In general, the computer may be useful when one or more of the following circumstances exists: (1) An experiment is run repetitively a large number of times under relatively similar conditions. In this case a single program is written to acquire and to mathematically and/or statistically analyze the data, (2) Experiments are long term and require little operator supervision. In this case, the computer can monitor time, control relays, and acquire and analyze data. The automated indirect coulometric titrations described in Chapter VI are a prime example, (3) Experiments where a computer can acquire data more accurately than would otherwise be possible. The use of an oscilloscope versus computer data acquisition in stopped-flow kinetics is an example of this case, and (4) Experiments which cannot be done any other way. The determination of the spectra of short lived intermediates via RSS described in Chapter V is an excellent example.
Three other conditions were imposed on computer use. Firstly, the experimental system should not be dependent on the computer to operate if at all possible. This allows valuable equipment to be employed in other uses if the computer is not available. Secondly, the computer is not permitted to make intuitive decisions so that it does not control the chemistry. The experimenter is required to observe the data and make decisions. And thirdly, valuable data (i.e., enzyme titration data) are recorded redundantly in some way. This redundancy permits a reference set of data to be maintained in case a question arises as to the validity of the computer acquired data or a computer hardware failure occurs and the computer acquired data are lost.

There were several trade-offs in our methods of computer interfacing. We use RDOS which requires a substantial portion of computer memory (i.e., 8 to 10 K out of 32 K computer core memory) but RDOS supports all standard peripherals (i.e., disk, paper tape reader, etc.). User control of these peripherals is accomplished by simple English keyboard commands (i.e., DELETE, RUN, LOAD, etc.). RDOS requires a minimum of 20 K of computer memory to compile a Fortran program. The extra 12 K of computer memory allows both the execution of foreground/background programs or large Fortran programs.

The alternative to RDOS is assembly language programming which may provide programs with shorter execution times. The offsetting factor is the time required to write a program. The computer is used with a variety of experiments which would dictate that a great
number of complicated assembly language programs be written and that experimenters would need to learn assembly language in detail even though their sole desire would be to acquire data employing the computer.

This computer system has a relatively large amount of core memory since most of the programs employed in this laboratory would execute in 8 K of core memory if the programs were optimized in assembly language. Considering the relatively low cost of computer memory we feel the advantages of RDOS and the benefits of a large amount of computer memory outweigh any advantages (particularly cost) of a small core memory computer.

A. Computer Hardware

The computer system was a Nova 800 minicomputer with 32,768 16 bit words of memory. The Nova 800 was a 4 accumulator computer with a central processor unit (CPU) cycle time of 800 nsec. The CPU had hardware multiply/divide logic. Communication, by the CPU, to computer peripherals occurred in two days. Programmed ("software") transfer of data is used with slow peripherals such as a teletype. In a programmed transfer, a desired device is selected and then data, contained in an accumulator, is transferred to that device. Data could be transferred to a device at a maximum rate of 50 KHz using programmed transfers.

DMA is used with fast peripherals such as a disk or fast analog-to-digital convertor. DMA allowed data transfer to a series of
preset, hardware determined, memory locations automatically. DMA operated on a cycle stealing technique where the DMA logic interrupted ("steals") the CPU for one machine cycle. A data point was transferred to a memory location during the interrupted cycle. DMA hardware transferred data to a series of memory locations, kept track of the number of data transfers, and stopped the transfers at a predetermined number. In the Nova 800 any number of devices (up to 61) may use DMA since DMA logic was contained within the interface of the device. The use of DMA was advantageous because it eliminated software overhead required to acquire one point at a time and allows high speed (to 1 MHz) data transfer rates.

A block diagram of the computer configuration is shown in Figure 14. Mass storage was provided by a 1.2 million word moving head disk (Diablo model 31). The disk was double sided and was capable of processing data at the rate of one word every 11 usec. The average time to seek a disk track was ca. 60 msec. The average time for the desired sector to pass under the read/write heads was 20 msec. Auxiliary equipment included: high speed paper tape reader (300 characters per second), real time clock programmable at frequencies of 10, 60, 100, 1000 Hz, cathode ray tube (CRT) terminal (Hazeltine model 1000) operating at 480 characters per second, and 10 character per second teletype (TTY) (Teletype model ASR33).

The computer system also contained three separate experiment interfaces. These are (as depicted in Figure 14) as follows:

(1) Interface 1 contained an analog-to-digital convertor (ADCI)
Figure 14

Block diagram of the Nova 800 computer system.
and two digital-to-analog convertors (DAC), (2) Interface 2 contained a second analog-to-digital convertor (ADCII), and (3) Interface 3 contained digital input/output (I/O) and two more DAC's. These three interfaces will be discussed later.

It should be noted that the two ADC interfaces and the disk transferred data to computer memory via the direct memory access (DMA) lines. The digital I/O interface and the other standard devices employed standard programmed data transfers.

Figure 14 indicates three sets of I/O lines and two sets of DMA lines. In reality the I/O bus is one set of 16 bidirectional synchronous lines, 9 control lines, 6 device select lines, and several interrupt sense lines. The DMA used the same set of 16 bidirectional synchronous lines, associated control lines, and several additional DMA control lines. The I/O and DMA structures are discussed in a Data General publication.

B. Interface Hardware

The interface hardware consisted of three separate computer devices. Each interface was constructed on a 15" x 15" printed circuit card purchased from Data General (basic model 4040) and contained I/O bus drivers/receivers, interrupt and control logic, and DMA logic if required (the digital I/O interface did not employ DMA). Approximately one-half of the board was available for integrated circuit (IC) sockets. Two of these interfaces were constructed in this laboratory and the third (ADCI) was constructed elsewhere.
The first interface contained an ADC (ADCII), eight channel analog multiplexor (MUX), dual sample and hold amplifiers (SH), system programmer, and 12 bit programmable clock.

The configuration of the ADCII interface is shown in Figure 15. ADCII is a 12 bit, two's complement, successive approximation convertor (Datel, Inc. ADCM-12B). ADCII has a specified conversion time of 13 usec although it has a measured conversion time of about 11 usec. ADCII's input range was ± 5 volts. The analog multiplexor has 8 channels and a channel settling time of 1 usec (Datel, Inc., MM-8). The SH amplifiers (Datel, Inc., SHM-4) have an acquisition time of 7 usec and an aperture delay of 40 nsec. The system programmer is a logic circuit designed to control and synchronize the rest of the interface components. A twelve bit programmable clock is fed by a decade frequency which is derived by dividing a 10 MHz clock (Vectron Inc.) by $10^N$ where $N=1$ to 7. The decade frequency and a preset value for the programmable clock are selected under program control.

The ADCII interface had two separate modes of operation. First data could be converted by ADCII on any analog channel (random mode). The programmable clock controlled the rate of conversions. The second mode was a sequential mode where a series of conversions could be made on a sequential series of analog channels. Hardware was provided which automatically caused the analog multiplexor channel value to increment after a conversion and then caused ADCII to convert data from the new analog channel. This sequence continued
Figure 15

Block diagram of the ADCII interface.
until all desired channels had been converted. Conversions in the sequential series were caused by the hardware to proceed at the fastest rate permitted by the ADCII conversion time and analog MUX settling time. The programmable clock controlled the rate of each sequential conversion series in the sequential mode.

The ADCII transferred data to computer memory by DMA. The interface hardware required an initial memory location for storing data and a value equal to the negative number of words to be converted. Two hardware registers were provided by Data General for these functions. The values in the hardware registers were incremented after each conversion was completed and after a required number of conversions the interface stopped the operation and an interrupt was generated. The interrupt signalled a program in memory that the conversions were completed.

The second interface to be described contained four 12 bit digital output storage registers, two 12 bit DAC's, 12 bit presettable event counter, and two 16 bit digital input registers. A schematic representation of this interface is shown in Figure 16.

The data in four 12 bit storage registers and two 12 bit DAC's were controlled by a digital multiplexor. The digital MUX was capable of guiding an input pulse to one-of-sixteen outputs based on the value of the 4 bit address lines at the digital MUX address inputs. A load pulse from the computer I/O bus was used to load one of the 12 bit registers or one of the DAC's in this manner. Four free outputs from the digital MUX were used as triggers for experiments. The DAC's were 12 bit ± 10 V, two's complement convertors (Datel,
Figure 16

Block diagram of digital input/output and DAC interface.
I/O INTERFACE

COMPUTER I/O BUS

12 BIT DATA LINES

12 BIT I/O

DIGITAL MUX.

4 BIT ADDRESS LINES

12 BIT 1/O

READ COUNTER

12 BIT DAC

READ REGISTER

12 BIT PRESETTABLE EVENT COUNTER

READ REGISTER

12 BIT I/O

16 BIT INPUT REGISTER

16 BIT I/O

12 BIT 1/O

6 BIT INPUT REGISTER

12 BIT DAC
Inc., DAC-VK-12B). The 12 bit presettable event counter was designed to count digital pulses from an external source (i.e., an experiment). The incoming pulse frequency could be divided by 1, 10, 1000, 10,000 as required under program control. A 10 KHz free running clock was available and could be manually switched into the event counter circuit. Two 16 bit digital input registers were also included in this interface.

The third interface contained ADCI, SH, 12 bit programmable clock, and two 12 bit DAC's. The ADCI was a 12 bit, two's complement, successive approximation convertor (Datel, Inc., ADCN-12B). ADCI had a conversion time of 4 usec and an analog input range of ± 5 V. The SH had a 100 nsec acquisition time and a 10 nsec aperture delay (Datel, Inc., SH-2). The 12 bit programmable clock, although of different design, performed the same function as the programmable clock in the ADCI interface. The two DAC's were identical to those on the digital I/O interface.

The circuitry for the first two interfaces is given in Appendix A with a description of their electrical and computer operation.

C. Computer Software

The programs used in driving the interfaces were written as assembly language subroutines and were called from a main Fortran program. This procedure was employed so that only a knowledge of the subroutine format and the function performed by the subroutine
were required. Generally the subroutines were written in a modular fashion. Each subroutine performed a minimum function which allowed an understanding of how the interface operated without examining a complicated subroutine call format.

Subroutines were written to drive ADCI, the digital I/O registers, and the digital counter. Subroutines to drive the DAC's were written as X-Y plotter (or X-Y oscilloscope) drivers although they could be used as voltage sources. The plotting capability was also available in the Basic language but the other interface capabilities were not. Listings of the assembly language interface drivers written for the ADCII and digital I/O interfaces are given in Appendix B.

The computer memory was allocated to a Fortran program by BDOS which, upon request, loaded the program into memory and caused execution to begin.

RDOS contained programs for standard peripheral I/O, disk file management and disk allocation, and device interrupt handlers. RDOS also supported foreground/background programs, multitask programs, disk overlays, and program chains and swaps. Foreground/background programs are two different programs competing for the computer system resources concurrently. Multitask programs contained subsections ("tasks") which performed differing functions and competed for the computer system resources concurrently or on a predetermined priority basis. Disk overlays were program subsections which resided on disk and were loaded into computer memory and executed when
required. A program chain is the loading of a second program by the first while destroying the first and a program swap is the loading of a second program while the first program is written onto disk for possible later use.

User communication with RDOS using the TTY was through a Command Line Interpreter (CLI). The CLI accepted English word commands from the TTY and translated the command into a form usable by RDOS. RDOS generally executed the command although the CLI executed some simple commands. RDOS and CLI were discussed in a Data General reference manual.⁹

The foreground/background programming was accomplished by partitioning the available computer memory into two sections as illustrated in Figure 17.

It should be noted that the Nova architecture provided 256 words of "page 0" memory which could be directly addressed from any other location in memory. Page 0 was used by RDOS and user programs for subroutine addresses. RDOS resided in the uppermost addresses of memory. The foreground program had assigned starting addresses (page 0 address and memory address) which forced the program to run in approximately the top half of the available page 0 and memory. The actual addresses for a foreground program depended on its size. Once a foreground program was loaded and running, a background program could be loaded and executed in the remaining memory if space was available.
Figure 17

Computer memory allocation under RDOS in a foreground/background environment.
MEMORY ALLOCATION IN
FOREGROUND - BACKGROUND
ENVIRONMENT

32K

RDOS
OPERATING SYSTEM

~ 25K

FOREGROUND
MEMORY

BACKGROUND
MEMORY

FOREGROUND PAGE 0

BACKGROUND PAGE 0

RDOS PAGE 0
D. Results and Discussion

This computer system has been used to completely control RSSIII, to automate the indirect coulometric titrations of bio-components, to determine spectra of short-lived intermediates, and to acquire data for a variety of experimental methods (linear diode array spectrophotometry, cyclic voltammetry, chronocoulometry, chronoamperometry, and stopped-flow kinetics).

It is interesting to note that computer controlled RSS has been applied to HPLC, flame emission spectroscopy, and stopped-flow.

The Harrick RSSIII has been interfaced to the computer and could be completely controlled by the computer. Such items as scan speed, fixed wavelength position, filter bandwidth, and start/stop functions could be controlled. The position of the galvanometer could also be monitored. The RSSIII hardware required three of the digital output registers and one of the digital input registers from the digital I/O interface. A special assembly language driver for RSSIII was written to simplify control.

An extremely useful and successful example of a use of the two interfaces was the automation of the indirect coulometric titration of bio-components. The experimental procedure, developed in this laboratory, required extensive intervention by the experimenter to control charge, monitor time, and record spectra. The actual techniques employed in the automation procedure are described in Chapter VI. It is considered important to note that the purpose of
automation was to relieve an experimenter of a variety of menial
tasks which then allowed him to be occupied with other more important
tasks.

The ADCII interface was also applied to cyclic voltammetric
data acquisition. The inclusion of two sample and holds (SH) allowed
acquisition of current and voltage data. The ADCII control hardware
causd both SH's to hold data at the same point in time and then the
ADCII sequentially converted two analog channels. This procedure
eliminated the need to interpolate the data points so that current
at some potential could be calculated. The simultaneous acquisition
of both potential and current allowed plotting, by the two DAC's on
a X-Y recorder, directly as potential versus time. In addition, the
rate of potential change could be calculated accurately from the
voltage data by knowing the frequency of data acquisition which was
defined by the user program. An alternative procedure was to
acquire current as a function of time. Potential could be computed
if the rate of potential change was predetermined.

The ADCII and digital I/O interfaces have also been used in
chronocoulometry and chronoamperometry.

The fast ADCI interface (data conversion rate of 200 KHz) has
been applied to the determination of the spectra of short-lived
intermediates generated under chronoamperometric conditions at an
optically transparent electrode (see Chapter V) and to the acquisition
of spectra from a linear diode array spectrometer$^{14}$ (Chapter IV).
The capability to have foreground/background provided for more effective computer usage. Two data acquisition programs could be concurrently executed or an acquisition program along with data plotting or text editing program. In general the foreground/background worked reasonably well except for two limits. The first limitation was the size of the two programs. It was generally best to keep the Fortran arrays to a minimum size and eliminate extraneous statements. It was also best to keep the amount of calculation to a minimum so that greater amounts of computer time were not lost performing calculations. A program requiring excessive calculation time prevented the other program from the computer. The second limitation was that the foreground program must be loaded before the background and this fact required a certain amount of cooperation and planning between the two experimenters desiring access to the computer.

The computer ESS combination presents a unique possibility for continuous optimization of measurement. In applications such as HPLC\textsuperscript{10} or flame emission spectroscopy\textsuperscript{11} the computer could control scan speed and filter bandwidth. The number of scans per peak could be optimized according to the peak shape or the filter bandwidth could be optimized for maximum signal-to-noise ratio during peak scans.

E. Conclusion

The computer system continues to be applied successfully to a number of experiments in the laboratory. The interface hardware
and software have broad capabilities and will be appropriate for most future needs. An important feature of our computer system is that it is relatively easy to use even by those inexperienced in computers.
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CHAPTER III

DERIVATIVE SPECTROSCOPY

In our RSS, the dispersed spectrum from the grating was swept across the exit slit by an oscillating mirror in a galvanometer mount (see Chapter I). By driving the galvanometer with a triangular or sawtooth waveform, the wavelength of the spectra, in principle, was linear with time. This method of scanning can be easily modified to provide a simple and convenient way of modulating the wavelength for derivative spectroscopy. It is only necessary to superimpose a small sinusoidal waveform on the main waveform drive. In this chapter, wavelength modulation using the RSS will be discussed.

The usual mode of modulation is amplitude modulation where the intensity of the light beam is mechanically "chopped" or the source itself is modulated through the power supply. Amplitude and wavelength modulation have been used in atomic absorption spectrometry. Bonfiglioli and co-workers have described a self-modulating derivative spectrometer. Bonfiglioli and Trench have recently discussed signal recovering in wavelength modulated spectrometers. The analysis of trace levels of $SO_2$ by wavelength modulation has been discussed by Hager, and in his instrument (available commercially from Lear Siegler) the entrance slit was oscillated to provide modulation.
In this laboratory the analysis of trace amounts of SO₂ was done employing a wavelength modulated galvanometer RSS of modified optical design. Hart, et al. have described a wavelength modulated spectrometer for reflectance studies of semiconductors. Generally, most modulation instruments employed modulation frequencies less than 100 Hz except in the Hart instrument which used 378 Hz.

The principles and advantages of derivative spectroscopy have been described elsewhere. The important advantages are accentuation of overlapping spectral bands and improvement of signal-to-noise ratio by elimination of the low frequency flicker noise (1/f type noise) and the periodic noise (i.e., 60 Hz noise from the power lines). The accentuation of overlapping spectral bands was particularly evident in a study of native proteins. The 1/f noises present in the spectrometer system associated with the light source and electronic components can be effectively reduced by coupling higher frequency modulation with band-pass filtration. A lock-in amplifier band-pass filters the incoming signal at the modulation frequency eliminating the noise which did not appear at this frequency. Of course any noise whose frequency components were the same as the modulation frequency would also be passed. Hieftje has discussed improvements in signal-to-noise ratio due to modulation techniques.

The signal resulting from a wavelength modulation derivative spectrophotometer should be directly proportional to concentration to be most useful as an analytical tool. Differentiation of the
absorbance form of the Beer-Lambert law with respect to wavelength yields

\[
\frac{\text{d}A}{\text{d}\lambda} = C \cdot B \frac{\text{d}\varepsilon}{\text{d}\lambda}
\]  

(1)

where \(C\) is molar concentration, \(B\) is path length in cm, and \(\varepsilon\) is the molar absorptivity thus \(\frac{\text{d}A}{\text{d}\lambda}\) is directly proportional to concentration.

A. Experimental

The bulk of the work on wavelength modulation was done employing RSSII although RSSIII was also successfully used as a wavelength modulated derivative spectrometer.

A schematic drawing (Figure 18) of the wavelength modulated spectrometer illustrates how frequency modulation was accomplished. The lock-in amplifier served as the demodulator and reference sine wave generator. The sine wave was added to the triangle scanning waveform through a conventional operational amplifier adder circuit and was fed to the galvanometer through the driving amplifiers. The galvanometer mirror caused the wavelength to scan linearly since the mirror's angular displacement was proportional to the current passing through it, and for a linear waveform, the scan was linear in time. The superimposed sine wave caused a corresponding small wavelength modulation whose magnitude and frequency could be adjusted by the sine wave.
FIGURE 18

Block diagram of wavelength modulated spectrometer system.
The lock-in amplifier was a Princeton Applied Research Model 126 with a Model 116 Preamplifier. A Hewlett-Packard 3300 A Function Generator provided the main waveform drive and the spectra were recorded on a Houston Model 2000 X-Y recorder.

Calibrations of the derivative output versus concentration were done using $K^2$Fe(CN)$_6^-$ solutions (concentration range 0.1-1.0 M) at 420 nm. The $K^2$Fe(CN)$_6^-$ was a Baker Analyzed Reagent.

Modulation parameters were optimized for maximum signal intensity. These parameters included frequency and amplitude of the sine and triangle waves and the phase of the lock-in amplifier. The modulation frequency was 500 Hz at 10 mv p-p. A mechanical phase lag in the galvanometer caused a 90° lag in the signal. There was no detectable phase lag in the log amp at this frequency. The frequency of the triangle was limited to less than 1 Hz in order to maintain the overlap of sine waves needed to obtain the derivative spectra.

B. Results and Discussion

Figure 19 illustrates the absorbance and derivative spectra of a holmium oxide optical filter. The frequency, $f_1^\prime$, of the scan was 0.01 Hz. To obtain the derivative spectrum, the frequency of modulation, $f_2^\prime$, was set at 500 Hz. The $f_2^\prime$ signal during scan was demodulated and rectified by the lock-in. Besides the obvious enhanced sensitivity and selectivity to small absorbance bands or bands appearing as shoulders on larger bands, the derivative mode
FIGURE 19

Absorbance and derivative spectra of Ho$_2$O$_3$ filter.

Top: Absorbance spectrum taken on X-Y recorder at 50 sec/scan.

Bottom: Derivative spectrum with modulation frequency, $f_2$, at 500 Hz. Phase of Lock-In amplifier was adjusted to give maximum derivative amplitude.
is extremely useful for rejection of spurious, periodic noise (i.e., 60 cycle). An example of this noise rejection was illustrated by comparing absorbance and derivative spectra of the holmium oxide filter while a magnetic stirrer was operating in the cell compartment near the photomultiplier tubes. The oscillating magnetic field of the stirrer affects the photomultiplier such that a low frequency, large amplitude oscillatory noise of approximately 0.1 A was superimposed on the absorbance spectrum. The derivative spectrum was, however, free from the noise and was identical to that shown in Figure 19.

The derivative spectrum was dependent on the values of \( f_1 \) and \( f_2 \) and the amplitude of \( f_2 \) for a given slit setting, i.e., optical resolution. A 10-mV peak-to-peak sine wave into the galvanometer driver amplifier corresponded to a 1.5 nm wavelength modulation.

In Figure 20 a plot of amplitude of the derivative signal for absorbance band at 536 nm is shown as a function of modulation frequency (\( f_2 \)) and phase adjustment of the lock-in. The phase was adjusted to give a maximum derivative amplitude for each setting of \( f_2 \) and maximized at approximately the resonance frequency of the galvanometer. Thus, the phase lag exhibited the largest change near the resonance frequency. At large values of \( f_2 \), the amplitude of the galvanometer oscillations decreased. The driving voltage to the galvanometer could be increased but the galvanometer oscillatory motions become distorted and complex at higher frequencies. The
FIGURE 20

Spectral signal versus frequency and phase.

-0-0-0- Peak amplitude of $\text{Ho}_2\text{O}_3$ absorbance band at 536 nm as a function of modulation frequency at optimized phase.

--- Peak amplitude measured as a function of phase and modulation frequency.
characteristics of each individual galvanometer must be studied for operational optimization.

The response linearity measurements yielded the same values as the direct absorbance measurements for RSSII (0.00 to $1.6 \pm 1\% \text{ A}$).

Unfortunately, the $1/f$ noise did not substantially decrease due to modulation apparently because the lock-in has internal $1/f$ drift or the signal at $f_2$ has substantial noise components which are amplified and appear as noise. Therefore, the $S/N$ did not increase. Wavelength modulation may have distinct usefulness in applications where noise is not fundamental (i.e., 60 cycle) and has a well defined noise spectrum. Wavelength modulation allows transfer of the signal to a frequency where no interference occurs. The wavelength modulation derivative spectra technique does appear to have an advantage in increased detectability of overlapping or small bands. There may be an advantage to using wavelength modulation in the spectral examination of turbid solutions to eliminate the DC absorbance offset while maintaining the spectral information (at the modulation frequency).

This approach to derivative spectroscopy shows promise but requires a complete examination and optimization of the technique in applications where normal absorbance spectroscopy is limited by some non-fundamental noise.
LIST OF REFERENCES


CHAPTER IV

EVALUATION OF A SELF-CONTAINED LINEAR DIODE ARRAY
FOR RAPID SCANNING SPECTROMETRY

The basic types of designs and performance characteristics of rapid scanning spectrometers (RSS) were reviewed in Chapter I. This chapter discusses use of a linear diode array (LDA) for an alternative design of a RSS.

The use of a LDA as a detector for RSS was possible when a complete ready-to-operate 1024 element LDA became commercially available. This LDA will be evaluated in terms of its performance characteristics and spectral properties for rapid scanning of visible absorption spectra. In a LDA spectrometer the dispersed spectrum is kept stationary and a single row of elements can then be responsive to the light. The response from each element corresponds to a wavelength width dependent on the linear dispersion of the spectrum and the physical size of the element. In the LDA the elements are interrogated in sequential mode and the readout is indicative of the spectral intensity distribution.

The LDA has seen limited usage. Horlick and Codding employed a 256 element LDA for determining emission spectra. Dessey has computerized a 128 element LDA for RSS applications.
Horlick et al. described a computer automated 512 element LDA for direct current arc time studies. Horlick and Codding have also described a LDA spectrometer for simultaneous multielement and multiline atomic absorption analysis and for dye laser measurements.

The LDA had several characteristics which made a performance evaluation desirable. These characteristics were a large number of elements (1024); spectral sensitivity from the near UV to the near IR; associated electronics were together as a self-contained unit; and fast interrogation times (up to 10MHz).

A. Description of Hardware and Operating Principles

The Reticon LDA unit contained 1024 photodiode elements, the signal processing, and the timing logic circuits. The unit was made up of three printed circuit (PC) boards. One PC board, the driver amplifier board, contained the LDA chip and the current-to-voltage convertor. The second, the RC400 board, contained the clock circuits. The third, the CASH-1B board, had circuitry to generate a boxcar output from the voltage pulses which was then displayed as required.

The LDA driver amplifier board contained a 22 pin socket for the LDA chip, a CAIOA op amp to convert the current pulses from the photodiode elements to voltage pulses, and a TTL to MOS level shifter to provide the correct voltage levels for driving the LDA detector's internal MOS circuit. The LDA chip consisted of 1024 photodiode elements and a corresponding charge storage capacitors \( C_d \). The photodiode elements were divided into even and odd elements.
The even numbered elements were connected to an "even" video line through a MOS transistor switch which was turned on by an even shift register. Correspondingly, the odd numbered elements were connected to an "odd" video line. Two shift registers, internal to the LDA chip, turned the MOS switches on and off in the correct sequence. Thus charging (i.e., sampling) of the $C_d$ capacitors occurred in numerical sequence.

The mechanism of the LDA chip operation was that each $C_d$ capacitor was charged to a common charge level (equivalent to a charge of $4 \times 10^{-12}$ coul on the capacitor) during each scan. This charging occurred when the MOS switch was turned on by one of the shift registers. During charging, if charge was removed from any of the capacitors, due to dark current or light induced current, a charging current would appear as a current pulse or as a train of $1024$ current pulses since the capacitors were sequentially charged. The magnitude of charge depletion was proportional to the intensity of light incident on the photodiode element, the quantum efficiency at the incident wavelength(s), and the rate of dark current leakage. The length of time between each sampling of the LDA chip (integration time) provided the final magnitude of signal.

The second PC board, the RC400, contained the digital logic required to start a scan, clock the LDA chip shift registers, and determined the scan time and time between consecutive scans (cycle time). The scan time (time required to sample $1024$ photodiode elements) was determined by either an internal clock or an external clock (selectable via jumper on RC400 PC board). Clock rates of up
to 10 MHz were possible. The RC400 used in this study had a preset internal clock rate of 100 KHz. The scan time was therefore 1.024 x 10^{-2} sec. The cycle time was determined by a 12 bit presettable binary counter mounted on the RC400 PC board. Binary switches on the RC400 PC board determined the initial counter setting. The minimum cycle time was 1028 clock pulses and the maximum cycle time was 4096 clock pulses. Therefore, repetition rates between 97 scans/second and 24 scans/second were possible using the 100 KHz internal clock. Figure 21 represents this sequence. The scan rate (\Delta T_1) is fixed for a given clock rate but the cycle time (\Delta T_2) is variable through the use of the 12 bit presettable counter.

The RC400 PC board provided a start pulse to initiate the scan on the LDA chip. A four phase clock was generated with phase 1 (\phi_1) and phase 2 (\phi_2) sequencing the even shift register; the phase 3 (\phi_3) and phase 4 (\phi_4) clocks sequenced the odd shift register. Four clock pulses were required by the LDA chip before the first diode element is sampled. This was the reason for a minimum cycle time of 1028 clock pulses. When the last two diode elements were sampled, each shift register generated an end of scan (EOS) pulse. The EOS pulse reset the LDA clocking circuitry so a new scan was initiated when the 12 bit binary counter was full.

The third PC board, the CASH-1B, contained three amplifiers, a filter, and the associated timing circuitry. Two of the three amplifiers were sample and hold modules (SH 1 and SH 2). A third amplifier had its input multiplexed between SH 1 and SH 2. A
Figure 21

General representation of the relation between \( \Delta T_1 \) and cycle time \( \Delta T_2 \).
retrace blank generator held the LDA unit output at zero volts when the LDA detector was not being sampled. The timing logic commanded one SH to hold while the other was sampling. At the correct moment the multiplex amplifier selected and amplified the output of the SH in the hold mode. SH 1 and SH 2 integrated the voltage pulses producing a convenient boxcar output. This procedure also reduced the fixed pattern clock noise which integrated to zero. The final output was a DC signal (as opposed to an AC pulse train) which can be displayed on an oscilloscope or fed to an analog-to-digital converter for computer processing. A trigger and clock outputs were available for synchronizing a computer or oscilloscope.

A detailed description of the operation of these components is provided in the manufacturer's literature and in a recent paper. The clock frequency, generated by the RC400, determines the frequency of photodiode sampling (one clock pulse per photodiode). Clock rates of up to 10 MHz were possible. The RC400 used in this study had a preset internal clock rate of 100 KHz. The scan time was therefore $1.024 \times 10^{-2}$ sec.

The overall unit had physical dimensions of 6.5 in x 4.5 in x 2 in. The LDA detector active surface geometry can be pictured as a $1.024 \times 0.017$ in aperture defined by an opaque mask. Each photodiode element was $0.001 \times 0.017$ in and the photodiode elements extended across the entire aperture. The entire aperture was photosensitive. The photodiode elements were not discrete. An area $5 \times 10^{-4}$ in $\times 0.017$ in about the center was considered 100% sensitive for the photodiode element in question.
The $5 \times 10^{-4}$ in area which bordered the most sensitive area in the middle decreased linearly in sensitivity as one moves toward the edge of the photodiode element. Therefore, there was a region where two adjacent photodiode elements responded to light at the same time.

B. Optical System

The optics for the LDA RSS were constructed using RSSII and the LDA optical configuration is shown in Figure 22.

As shown in Figure 22 the basic optical configuration for a galvanometer RSS was retained. The PMT's were removed and the LDA sampled a portion of the dispersed light. A cylindrical lens may be used to focus the vertical axis of the dispersed light on the LDA, but was not required here since the light intensity was sufficient. Note that the LDA was placed at the exit slit plane and could be considered as individual slits.

Optical configuration was retained since it had several advantages which are useful to retain in a LDA optical system. The galvanometer allowed simple variation of the sampled wavelength region and gave adequate resolution for most work (theoretical = .48 nm using 300 l/mm grating at 633 nm). The galvanometer also allowed a convenient setting of the spectral region which was to be sampled. This configuration gave an adequate wavelength sampling region which can be adjusted to user requirements. A final reason for the choice was the availability of this optical configuration in the laboratory.
Figure 22

Optical layout of the spectrometer

Components:

- **source:** Tungsten lamp or Xenon lamp.
- **S:** Slit (Harrick Scientific Corp.).
- **M₁:** Concave mirror, focal length 12.7 cm.
- **Gₐ:** Galvanometer (Bell and Howell Corp.).
- **M₂:** Concave mirror, focal length 15 cm.
- **G:** Diffraction grating (Bausch and Lomb, 300 l/mm blazed 400 nm).
- **M₃:** Plane mirror.
- **LDA:** Linear diode array detector.
- **Δλ:** Wavelength width sampled by LDA.
C. Experimental

The Reticon LDA unit (received May, 1974) contained 1024 photodiode elements, the signal processing, and the timing logic circuits.

The diode array (RL1024C-17), clock circuitry (RC400), and signal processing circuitry (CASH-1B) were purchased from Reticon Corporation.¹

Mounting the CASH-1B circuit board was a problem since it contains 13 trim pots which must be accessible for adjustment to optimize the LDA performance. In our spectrometer, the dispersing optics were mounted on a flat plate about six inches above the spectrometer base. The LDA unit was mounted in one half of a Bud box on a three axis translation stage. The unit was set to the proper optical level by a stem which fits into an optical mount fastened to a triangular optical rail. The unit can also be conveniently removed and replaced if this should be required. While it is possible to detach the circuit board containing the array from the other two boards (electrical connection made with a 14 wire, flat ribbon cable) undesirable noise was introduced by this change. Optimization of the unit was performed about once a week to maintain the desired performance level.

External power supplies (+15 V and +5 V) were purchased from Elasco Power Supply Corporation. The supplies were mounted separately from the LDA unit and external to the spectrometer. Cables were used to transfer the power.
Co(NO₃)₂ solutions were used to determine the response linearity. These solutions were prepared using Baker Analyzed Reagent Chemicals in 1% H₂SO₄. The absorbances of these solutions were determined using a Cary 15 spectrometer (absorbance measured at 520 nm). The response linearity spectra were acquired via computer and signal averaged 100 times.

The light source for the spectrometer was a 100 W Tungsten Iodine quartz lamp or a 75 W Xenon arc lamp powered by a highly regulated power supply.

Alignment of optical components and positioning of the LDA were performed using a TRW Argon Ion Laser No. 71B or a Spectra Physics Model 125 HeNe laser as a light source. Resolution and dispersion were also determined using these lasers. Dispersion was determined by measuring the number of photodiodes between two laser peaks. Resolution was measured by determining the wavelength width at one half-peak height of a single laser line. Dispersion and resolution using a laser were recorded via oscilloscope with Polaroid camera attachment.

Computer acquired spectra were taken by a Data General Nova 800 computer system described in Chapter II of this work. The ADCI interface was used. ADCI had 200 KHz data conversion rate.

The trigger pulse from the LDA was used to initiate the computer hardware, and the LDA clock was used to synchronize the ADCI conversion. 1024 data points were taken at 10 usec intervals.
D. Results and Discussion

To illustrate performance, typical computer acquired transmittance spectra (using a Tungsten source) of a baseline, Holmium oxide filter spectrum and background with the light source off are shown in Figure 23. These spectra are a result of 100 averaged scans. The shape of these transmittance spectra is the result of the convolution of the light source wavelength distribution, diffraction grating wavelength efficiency, and LDA photodiode wavelength response. A high pressure Xenon arc lamp has also been used with the LDA unit. In general this lamp provides more light throughput, since it is a better point source, particularly at shorter wavelengths as compared to the Tungsten lamp. Narrower slit widths or shorter integration times can then be used with the Xenon arc lamp. The main problem with the Xenon arc lamp was the atomic emission lines which appear in the visible region centered about 480 nm. The Xenon arc emission lines were a problem because they appear as sharp peaks superimposed on the Xenon source continuum. The emission lines had two important effects: 1. The LDA detector signal-to-noise ratio was limited by the emission lines (i.e., the slit widths had to be decreased so that the LDA detector was not saturated in the emission line region). 2. In the transmission mode, sample absorption peaks whose wavelength distribution overlap the emission line wavelengths had their spectral bandshape distorted (i.e., the sample absorption peak had the emission lines superimposed on the absorption peak which may cause difficulty in visually
Figure 23

Typical LDA spectra

A. Tungsten source baseline.
B. Holmium Oxide filter.
C. Zero line (light source off).

All spectra were signal averaged 100 times. Cycle time was $4.096 \times 10^{-2}$ sec.
interpreting the sample spectrum). The seriousness of the last effect was dependent on the sample spectral bandshape, molar absorptivity, sample concentration, and degree of wavelength overlap between the emission lines and the sample peak and could effectively be eliminated by computer subtraction of a baseline spectrum from a sample transmission spectrum.

Figure 24 shows an absorbance spectrum of the same filter taken with a RSS unit using photomultiplier tubes.

To determine the maximum resolution, a HeNe laser was used as the light source. Figure 25 shows the spectrum of this laser. The wavelength axis was expanded using the oscilloscope controls to display approximately 11 nm. The resolution (peak width at 80\% height) was measured to be .6 nm. This resolution was determined by measuring the number of diodes at one-half peak height (approximately 3 photodiodes) and multiplying the number of photodiodes by 0.19 nm/diode (one photodiode corresponds to a width of 0.19 nm). This resolution was comparable to a RSS using photomultiplier tube detectors.

In this spectrometer, the wavelength width (\(\Delta\lambda\)) sampled by the LDA detector was a direct function of the optical gain. The optical gain was determined by a ratio of the distance between the entrance slit image (as formed by \(M_1\) in Figure 25) and \(M_2\) to the distance between \(M_2\) and the exit slit image. In this spectrometer the optical gain was determined to be 3 and resulted in the entrance slit being magnified by a factor of 3 at the exit slit plane. The width and height of the dispersed beam was also
Figure 24

Absorbance spectra acquired from a conventional RSS unit used in this laboratory with PMT detectors.
Figure 25

Oscilloscope trace of HeNe laser peak used in calculating resolution. Each diode covers 0.19 nm. ($\lambda_{\text{max}} = 633$ nm).
magnified (although resolution remained constant). The $\Delta \lambda$ can be adjusted readily anywhere between 190 to 250 nm. With an optical gain of 3 and a $\Delta \lambda$ of 190 nm a maximum signal (Tungsten source baseline) was obtained at an integration time of 40.96 msec. No additional optical focusing element (i.e., a cylindrical lens) was used to focus the vertical exit slit plane on the photodiode because image distortion would have occurred.

The width could have been increased by using a Xenon source and increasing the optical gain by adjusting the image distances. Additional light throughput could also be obtained by replacing the galvanometer with a larger flat mirror (the galvanometer was the limiting aperture stop in this spectrometer).

A non-linearity in wavelength also existed in the optical system due to the curved exit slit focal plane. To calculate the magnitude of this error simple trigonometric functions were used. The error for a 190 nm $\Delta \lambda$ was 0.03% at each end of the LDA detector (center of detector was assumed to have zero error). This error was considered insignificant with the present resolution and $\Delta \lambda$.

We will discuss several important parameters which determine the usefulness of a detector. Among these are signal-to-noise ratio (S/N), spectral response, response linearity, and convenience of use.

The maximum S/N was the ratio between the maximum signal output and the noise at the LDA unit output. The maximum signal was equivalent to 5.00 volts (saturation voltage). The minimum signal was 0.00 ± 0.02 volts. This 5 volt change corresponded to a
The noise in the LDA unit arose from four principle sources. First, the fixed pattern noise from the clock circuits was capacitively coupled onto the video lines. The CASH-1B sample and hold circuits partly eliminated this noise since the clock noise integrated to zero. The LDA unit output showed clock frequency noise caused by the switching between the two SH amplifiers on the CASH-1B. As can be seen in Figure 26, which shows a trace of several photodiode elements, spikes appear on the output due to this switching. The second source of noise was dark current. This dark current was charge which leaked from the photodiode charge storage capacitor and was not due to light striking the photodiode element. The dark current was specified by Reticon to be 0.01 pCoul or 0.25% of the maximum output signal at 22°C with an integration time of 10.28 msec. Experimental verification of this figure was done by varying the integration time with no light striking the LDA detector. The third source of noise was the fundamental thermodynamic noise. This was specified by Reticon to be 0.1% of the maximum output. The fourth source of noise was the electronic noise induced on the output by the additional LDA unit processing components (switching transients, drift, etc.) and appeared to be the major noise source in the LDA unit.

The noise was experimentally measured by the computer acquisition of 4096 repetitive spectra. The computer saved the digital value for a known photodiode element during each scan. The 4096 saved points were then plotted. In this manner the peak to
Oscilloscope trace of a non-linear response area in the LDA detector. Vert = 0.5 V/div.
peak and the RMS noise dependence on light intensity, wavelength, and time was examined as well as obtaining the actual noise figure. The noise did not appear to be wavelength or light intensity dependent. The RMS noise value was measured to be 50 mV. The experimental maximum (no signal averaging) signal-to-noise value was calculated to be 100:1 (1% transmittance).

The response linearity was evaluated using known standard solutions of Cobalt Nitrate. The absorbance was found to be linear from 0.00 to 1.5 + 1% absorbance units.

The spectral response of the LDA detector was similar to that of a typical photodiode detector with a maximum response at ca. 900 nm. It decreased to about 10% of the maximum response at 1100 nm and 250 nm.

As the data indicate, the LDA unit has rather limited applications due to its lack of sensitivity. Horlick's data suggested that the LDA detector was a suitable RSS detector but his signal processing circuitry was different. In comparison to his results, we conclude that the principle noise source must be in the signal processing electronics. We have not yet attempted to modify the circuits to see if the noise could be reduced. Since the Reticon LDA unit requires no additional circuitry for operation (except a + 15 V and + 5 V power supply), it was operational as received.

The LDA gave a non-uniform output which is seen in Figure 26. The non-linear portion of the LDA encompasses a width of about fourteen photodiodes and appeared to be independent of wavelength.
and light intensity. This non-linearity was believed to be due to imperfections (i.e., impurities) in the LDA active surface.

An important point to consider is what possible maximum scan rate could be achieved with this device. If the upper specified clock rate of 10 MHz is used, the maximum would be 9.7 KHz. The scan rate is higher than any other rapid scanning spectrometers for comparable wavelength scan width and repetition rate. The present limitation of using such high clock rates is to obtain sufficient light throughput and to obtain a means of acquiring data. Computer data acquisition at 10 MHz (if all 1024 diodes are sampled) is difficult. Special techniques such as transient recorders with large internal memories would be required. The problem of data acquisition and storage is not trivial if analysis of all spectra taken at high scan rates is desired.

There are several major drawbacks associated with the LDA detector which we feel should be mentioned. The manner of the LDA detector operation requires that the entire LDA detector must be sampled. Since in many types of work the entire sampled spectrum may not be of interest, time is wasted sampling unwanted data. An addressable readout mode would eliminate this problem but this type of device is not available, although the technology certainly exists to produce it. The device would generally require a computer to take maximum advantage of the device.

An example of an application has been the analysis of SO₂ in the wavelength range of 280-315 nm. Sensitivity of 100-2000 ppm of SO₂ in a cell of 50 cm length was reported.⁹
It should be mentioned that Reticon indicates that the LDA unit is intended for prototype applications. As such, this unit was probably designed and intended for a broad variety of applications and performance may be improved by designing circuitry for specific applications.

A widespread usage of the solid state array detectors is predicted because of the attractiveness of being able to electronically (versus mechanically) control all aspects of the spectral acquisition.

The work described in this chapter has been published.10
LIST OF REFERENCES

1. Reticon Corporation, 450 E. Middlefield Road, Mountain View, California, 94043.


CHAPTER V

DETERMINATION OF THE SPECTRA OF SEVERAL ELECTROCHEMICALLY
GENERATED REACTIVE INTERMEDIATES BY RAPID
SCANNING SPECTROMETRY

Spectroscopy combined with electrolysis at optically transparent
electrodes (OTE) has proven to be a useful technique for studying
reaction mechanisms, determining kinetic parameters, and characterizing
generated species, particularly reactive intermediates. This
technique, commonly termed spectroelectrochemistry (SEC), has the
unique advantage of providing quantitative, molecular information on
the nature and behavior of generated species at an electrode inter­
face only a few hundred angstroms thick.¹

SEC has been used extensively in this laboratory for studying
first order catalytic processes,² second order catalytic processes,³
homogeneous electron-transfer reactions using internal reflection
SEC,⁴ rates of bipyridylium radical cation reaction with cytochrome
C⁵,⁶ and cytochrome c oxidase,⁷ and kinetics of several aromatic
hydrocarbons.⁸ Kuwana⁹ has reviewed the theory and practical
applications of SEC.

In applying SEC to the determination of kinetic parameters it
is important to know the spectra of any intermediates so that their
absorbance-time behavior can be studied at an appropriate wavelength.
The development of the galvanometer RSS by Gruver, et al.¹⁰ allowed
the spectrum of intermediates to be determined in a single experiment. Examples of this use of RSS are by Gruver and Kuwana\textsuperscript{8} for reduced aromatic hydrocarbons and by Wells\textsuperscript{11} for nitrobenzene radical anion. Gruver and Kuwana\textsuperscript{8} attained RSS sensitivity of $10^{-3} \text{ A}$, via signal averaging, during several hundred msec SEC experiments.

In this work we have determined spectra using RSS III, during controlled potential oxidation at platinum OTE, of several substituted electron-rich olefins, which were of interest in preparative organic chemistry.\textsuperscript{12-16} Oxidized olefins generally dimerize to form unique products. Spectra of the generated intermediates were recorded for the following: (1) 1,1'-dianisylethylene (DAE), (2) 4,4'-dimethoxy-stilbene (DMS), (3) 3,4-dimethoxypropenyl benzene (DMPB), and (4) tetraphenylethylene (TPE). Sensitivity was improved and shorter times utilized. Spectra were acquired in 5 msec during 10 msec potential steps, and RSS sensitivity was demonstrated to be $10^{-4} \text{ A}$ via signal averaging by computer.

A. Experimental

The reactions were carried out in acetonitrile using tetra-butylammonium perchlorate as supporting electrolyte. Thin platinum films, vapor deposited on Pyrex glass, were used as the OTE. The basic design of the electrochemical cell has been described elsewhere.\textsuperscript{17} The body of the cell was made from Teflon rather than Lucite for use with nonaqueous solvent. To minimize the solution resistance between reference and working electrode, a pseudo reference platinum
wire was inserted into the solution next to the working electrode. This technique did not permit accurate redox potential measurements but minimized uncompensated resistance effects. The potentiostat was a standard three electrode type with iR compensation and is shown in Figure 27. The modified electrochemical cell, solutions, and substituted olefins were prepared and supplied by E. Steckhan.\textsuperscript{18}

The RSS-SEC instrumentation is shown schematically in Figure 28. A function generator (Hewlett-Packard Model 3300-3302A) was used to supply a variable amplitude/frequency square wave to the potentiostat. The internal bias of the potentiostat was used to adjust the square wave to the correct potential for oxidation, which was initially determined by cyclic voltammetry. IR compensation was accomplished by using an oscilloscope to monitor the current behavior during the potential step and increasing the degree of compensation until a slight ringing of the current occurred. A variable gain amplifier with bias was used to increase the magnitude of the absorbance signal before conversion of the data by the ADC and an oscilloscope was used to continually monitor the absorbance signal.

The values of the RSS parameters (i.e., scan frequency, filter, wavelength scan width, wavelength center, and external gain) were set manually based on the speed (duration of the potential step) of the experiment and the spectral characteristics of the absorbing intermediate. The computer program was initialized and the data acquisition began.
Figure 27

Conventional three electrode potentiostat with iR compensation. Operational amplifiers (OA) are Teledyne Philbrick 1026 or 1009. A Hewlett-Packard Model 467 A was used as a booster amplifier (B).
Figure 28

Block diagram of the SEC-RSS experiment. Function generator is Hewlett-Packard Model 3300A with 3302A Trigger/Phase Lock Module. Oscilloscope is a Tektronix Model 7613. External gain operational amplifiers were Teledyne-Philbrick 1026.
A representative timing diagram for the data acquisition is shown in Figure 29. After the computer program initialization, the first data acquired was a baseline spectrum (scan without potential step) which was added to a baseline averaging array. After the function generator triggered the potential step, the computer waited a predetermined amount of time (At) before triggering the RSS to acquire a spectrum. This At allowed the intermediate concentration to increase to a maximum (or steady state value) and the value was estimated from the approximate rate of the follow-up chemical reaction(s). The spectrum was added to the spectra averaging array.

After acquiring the spectrum, the computer program waited at least 100 times the potential step time to allow initial solution conditions to be re-established at the electrode-solution interface. The program repeated the above procedure, signal averaging the data, until either a predetermined number of cycles were completed or a TTY keyboard interrupt occurred. The plotting segment of the program followed termination of that cycle.

The computer plotting program included plotting of the averaged baseline spectrum and the averaged spectrum (the averaged spectrum was computed by dividing the corresponding summed array by the number of cycles completed). The difference between the data and baseline spectra corresponded to the intermediate absorbance spectrum multiplied by any external amplifier gain. The computer program also allowed data scaling to increase the peak amplitude, as well as the noise, for display purposes. If more signal averaging was required,
Figure 29

Timing diagram for RSS-SEC experiment.
POTENTIAL

SCAN

TRIGGER A

TRIGGER B

ACQUIRE BASELINE SPECTRA

ACQUIRE SAMPLE SPECTRA

TIME
then the averaging process could be continued. The program used (SECAV) is given in Appendix C.

The computer acquired 1000 data points during a 5 msec scan (using ADCI). The filter of RSSIII was set for a bandpass of 10 KHz. A Holmium Oxide filter was used to calibrate the wavelength region for each run. The resolution of RSSIII was degraded to 3-5 nm to increase the signal-to-noise ratio.

B. Results and Discussion

The spectra for the intermediates are shown in Figures 30-33. Figure 30 and 31 are spectra for the oxidized DMPB intermediate, showing a doublet absorbance band centered at 515 nm and a singlet absorbance band is centered at 415 nm. The spectrum in Figure 32 is the DAE intermediate and a single absorbance maximum is at 508 nm. Figure 33 is the spectrum of the DMS intermediate (absorbance bands at 417 nm, 526 nm, and 604 nm). The figure captions provide experimental conditions (i.e., concentration, gain, times) used in each experiment. The indicated gain reflects the external amplifier gain multiplied by the software scaling value. The absorbance indicated on each figure was calculated by dividing the voltage of signal output on the computer recorder (approximate figure scale: 0.7 in = 1 volt) by the gain.

There were several problems which must be discussed in order to understand the inherent advantages and limitations of rapid scan SEC.
Figure 30

Spectrum of DMPB intermediate, 882 averages of a 5 msec spectrum acquired 4.5 msec after initiation of a 10 msec potential step. Total gain on displayed spectrum is 825. The initial concentration of DMPB was approximately 5 mM. Spectrum is 150 nm wide.
Figure 31

Spectrum of DMPB intermediate. Spectrum is a 100 nm scan centered at 415 nm. 811 averages. Total gain is 400. Other conditions as in Figure 30.
Figure 32

Spectrum of DAE intermediate, 200 averages of a 5 msec spectrum taken 9 msec after initiation of a 20 msec potential step. Total gain on displayed spectrum is 100. Initial concentration of DAE was 5 mM. Spectrum is 150 nm wide.
Figure 33

Spectrum is for the DMS intermediate and is 600 averages of a 5 msec spectrum taken 9 msec after initiation of a 20 msec potential step. Total gain of displayed spectrum is 480. Initial concentration of DMS is 5 mM. Spectrum is 200 nm wide.
Since the RSS produced a spectrum by log ratioing the currents from the two PMTs, mismatch (error) of these currents generally occurred. This mismatch produced a baseline which was not flat (i.e., the baseline absorbance was not constant (zero absorbance) as a function of wavelength). The degree of mismatch depended on several factors as discussed in Chapter I. The baseline flatness could be maintained to within $\pm 0.025 \text{ A}$ over 250 nm (350-600 nm). The optimum flatness over 100 nm was about $\pm 0.01 \text{ A}$. The baseline mismatch limited the amount of external amplifier gain which could be employed on the RSS signal. If the baseline flatness was within $\pm 0.025 \text{ A.U.}$ then a maximum gain of about 200 was theoretically possible. This figure excluded the high frequency PMT shot noise which limited the gain to approximately 100. The final signal (just before entering the ADC) must be maintained (biased) within $\pm 5 \text{ V}$ to match the range of the ADC.

An important problem was the generation of a product which absorbed in the spectral region of interest. Of course the product concentration increased as the experiment proceeded. The absorbance of a product could readily cause the observed signal to move out of the voltage range of the ADC ($\pm 5 \text{ V}$) (if the product absorbance band was broad the baseline appeared as DC shift) or distort the baseline so that it was over amplified and out of range of the ADC (this could be rectified by decreasing the gain). If a product absorbance or DC drift caused the signal to move out of range of the ADC, the operator could adjust the bias of the signal between potential
steps. The spectral signal was displayed on an oscilloscope, in parallel to the computer, for this purpose.

Wells\textsuperscript{9} has employed a somewhat different approach to obtain spectra from a RSS-SEC experiment (his RSS was of the original Kuwana\textsuperscript{8} design). His procedure was to acquire a baseline spectrum, mathematically smooth the signal to remove the noise, and then store this data in computer memory. Concurrently with acquiring the spectrum, the computer DAC output the prerecorded baseline to a difference amplifier which subtracted the baseline spectrum from the spectrum. This procedure had the advantage of eliminating the product spectrum and baseline mismatch but the spectrum acquisition must be relatively slow. The synchronization of the DAC output and acquisition of the data by the ADC under software control would require an estimated 15 usec to 65 usec per point. These estimated times depended on the computer and ADC conversion time which was not stated in the publication. A second possible disadvantage would be caused by the resolution of the DAC. This error excludes other noise caused by the DAC. Wells' procedure was to acquire and smooth a new baseline spectrum each RSS-SEC cycle (eliminating the spectrum of a continually increasing product concentration). If a 12 bit \pm 10 V DAC were used (5 mV resolution) the difference between the spectrum and the DAC supplied baseline spectrum could be different by \pm 5 mV (due solely to quantization error of the DAC) which when amplified by 100 gave an error of \pm 500 mV. Our procedure
has the advantage of speed and simplicity but has the disadvantage of inability to eliminate unfavorable background.

   It should be noted that Wells did not give exact data acquisition times. Wells' work is presented to illustrate the advantages and disadvantages of his approach.

   During an experimental run prior knowledge of the spectral region where the intermediate absorbed was helpful. For olefins this region was approximately 400 nm to 600 nm. The width, which was employed in an experiment, was limited by following four factors: (1) the baseline flatness which dictated the maximum electronic gain; (2) the gain which was required to enable the intermediate spectrum to be seen; (3) the physical constants of the chemical reaction and intermediate species involved in the reaction; and (4) the RSS scan rate limitations.

   These four factors were intimately related. Probably the most important factor was the physical constants. "Physical constants" were the molar absorptivity of the intermediate, the rate constant of any homogeneous follow-up step(s), and the initial reactant concentration. These constants controlled the absorbance intensity of the intermediate species and when related to instrumental sensitivity, determined the amount of electronic gain which was required in order to detect the intermediate. The amount of electronic gain was related to the baseline flatness which was a function of scan width which could be increased or decreased. The RSSIII scan rate
limitations (ca. 200 nm for a 5 msec scan) must not be exceeded or the scan will not be linear.

There were several other instrumental characteristics which affected the sensitivity of the RSS-SEC experiment.

It is important that the potentiostat be able to charge the electrochemical double layer to the correct potential in a short time relative to the duration of the potential step. A fast potentiostat response time was particularly important in fixed wavelength SEC studies where kinetic information was desired. A fast charging time allowed a precise t=0 to be established which was required for accurate kinetic measurements. No extraordinary steps (except for modification of the reference electrode as described earlier) were taken to optimize the potentiostat response.

The resolution of the ADC has a resolution of 2.5 mV (1/4096 of 10 V). Therefore the signal change must exceed 2.5 mV to be detected (ideally the signal magnitude should change several times the ADC resolution). This was an inherent limitation to all digital conversions which were not present in analog averagers such as waveform eductors. But, a waveform eductor has a serious problem of long term drift. The resolution of the ADC dictated that a high gain be used in order that measurements could be made.

The RSS unit has several important effects on the RSS-SEC experiment. This instrument must have a finite, ultimate sensitivity. This sensitivity limit was defined as the point at which the RSS cannot distinguish between two different absorbers and no amount of signal processing could recover the signal. This point is difficult
to quantitate. The maximum signal-to-noise ratio we obtained was ca. twenty at 0.002 Å for a 5 msec scan at 417 nm by signal averaging 600 times (see Figure 33). The RSS sensitivity in this case was $10^{-4}$ Å. In a fixed wavelength spectrometer an absorbance sensitivity of $10^{-5}$ Å has been reported.\(^7\)

Signal averaging 900 times appeared to be a practical limit. Nine hundred averages gave a S/N enhancement of 30 and did not exhaust the reactant. To increase the S/N by 300 through signal averaging 90,000 averages would be required (exhaustion of the reactant would occur and long times would be required; i.e., 90,000 sec at 1 cycle/SEC). We consider it doubtful that signal averaging alone could substantially improve the sensitivity unless the RSS baseline can be "flattened" to allow for greater external gains. The lower sensitivity in the fixed wavelength work reflects the higher electronic gain.\(^7\)

Another type of application for the SEC-RSS combination was to rapid scan an electrochemically generated intermediate as a function of time. This was done with tetraphenylethylene (TPE) oxidized to the radical cation in a $\text{CH}_2\text{Cl}_2/10\%\text{CF}_3\text{COOH}$ solvent system. Spectra acquired once every two seconds during this electrolysis are shown on the three dimensional plot, Figure 34. This application has also been demonstrated by Gruver and Kuwana\(^8\) and by Wells.\(^11\)

C. Conclusion

As can be seen from the foregoing discussion the SEC-RSS experiment is a viable means for rapidly determining the spectrum
Figure 34

Time dependence of the appearance of TPE intermediate. Spectra displayed are acquired at 2 sec intervals with a gain of 5. The solvent was 10% trifluoroacetic acid in methylene chloride. Initial concentration of TPE was approximately 5 mM.
of an absorbing intermediate generated during a double potential step experiment.

The spectral observation provides strong evidence for the existence of an intermediate. The actual identity of the intermediate must be made by either reference to spectra of stable analogs of the intermediate or by stabilizing the intermediate. If the detected and stabilized intermediates have identical spectral characteristics then they can reasonably be assumed to be identical. The possibility of other intermediates existing in a reaction sequence cannot be eliminated because they may not be at a detectable concentration. Also the appearance of a spectroscopically detectable product during the time the intermediate spectrum is acquired may complicate spectral interpretation. Ideally the intermediate spectrum would have different spectral characteristics from those of any product.

This study has demonstrated the applicability of the RSS-SEC technique to the determination of the intermediates generated in the oxidation of several substituted electron rich olefins. These spectra provide information for fixed wavelength SEC studies to obtain kinetic parameters and mechanistic information.

Certainly research will continue to determine what the ultimate capability of the technique is and what can be done to extend this capability.
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18. Work conducted in collaboration with Dr. Eberhard Steckhan, Organisch-Chemisches Institut der Universität Münster, D-44 Münster, W. Germany, during the summer of 1975.
Two heme proteins, cytochrome $c$ and cytochrome $c$ oxidase, have been extensively studied in this laboratory\textsuperscript{1-5} to accurately determine the stoichiometry and energetics of their reactions and hence, to gain insight into their electron transfer mechanism.

The indirect coulometric titration was developed\textsuperscript{2,6} to quantitatively transfer charge to or from these enzymes via a mediator titrant using an OTE and then recording the absorbance spectrum.

A plot of change in absorbance versus charge ($\Delta A - \Delta Q$) was then made and, in simple systems, the number of electrons transferred ($n$ value) can be determined\textsuperscript{7} from

$$n = \frac{\Delta \varepsilon \cdot b}{m \cdot V}$$

(1)

where $\Delta \varepsilon$ is the difference in molar absorptivity at some wavelength between oxidized and reduced species, $b$ is the cell path length, $V$ is the volume of the cell, and $m$ is the slope of the $\Delta A - \Delta Q$ plot. It is also possible to evaluate the redox potentials of the components if the redox potential of one component is accurately known.\textsuperscript{2}
The advantages of indirect coulometric titration are that the enzyme(s) can be repetitively oxidized or reduced without changing the solution, large or small amounts of charge can be injected as desired, and the simplicity of experimental procedure.

Operator tasks, after filling and setting up the electrochemical cell in the E3S, were recording injected charge, switching a magnetic stirrer, recording a spectrum after homogeneous solution conditions are attained, recording time between spectra, and determining when to reverse the redox direction. The elapsed time for a single "run" (a series of redox titrations) was generally several hours.

Indirect coulometric titrations seemed an ideal system with which to test the computer system (Chapter II) and provide useful information. Computer software and hardware were used to control the menial operations but the computer was not allowed to make intuitive judgements (endpoint, applied potentials, or "quality of data"). The primary goal was to relieve the experimenter from the tedious control tasks allowing him to prepare for the next run, interpret data, etc.

Computer programs were written to plot spectral data as difference spectra, ΔA vs ΔQ plots, dual wavelength plots, and data in several other formats for visual interpretation.

Dual wavelength difference spectroscopy was developed by Chance, et al. 8-11 to examine small spectral changes in turbid solutions with large background absorbance and uses a wavelength, to
which all other measurements were referenced. The dual wavelength
difference spectra program allowed selection of any wavelength to be
used as the reference wavelength, which was an advantage over the
instrumental dual wavelength technique which required a decision
before the experiment as to what wavelength to use as a reference.

A. Experimental

The experimental procedure for solution preparation, degassing,
and cell construction were described elsewhere. The equipment for
an indirect coulometric titration is represented by the block
diagram in Figure 35.

The potentiostat was a standard three electrode type. A relay
disconnected the auxiliary electrode and imposed a unity gain on
the control operational amplifier which would otherwise limit.
The difference in potential between the reference electrode and the
working electrode (potentiometric potential) was measured by a
digital volt meter (DVM) and was required to correct the \( \Delta A - \Delta Q \)
plots for background charge. The potential of the working electrode
was manually adjusted to the desired value. Current was measured
by converting the voltage drop across a 500 ohm load resistor to
digital pulses, whose frequency was directly proportional to voltage,
with a voltage-to-frequency convertor (V/F : Vidar Model 240). This
V/F outputs \( 10^6 \) counts/(V·sec). These counts were fed into a
digital counter (Beckman Preset Eput Model 5311DR) which functioned
as an integrator. The amount of charge injected into the solution
Figure 35

Block diagram of the typical indirect coulometric titration.
was calculated from the number of counts. A magnetic stirrer was used to stir the solution during charge transfer.

A block diagram of the computer control of this experiment is given in Figure 36. Note that the computer was added in "parallel" to the normal experimental equipment (i.e., the X-Y recorder, counter, and DVM were retained for monitoring the experiment). The computer auxiliary electronics (excluding the computer interfaces) for automation were as follows: (1) A relay was added so the computer could control the auxiliary electrode; (2) A digital inverter was used to drive the pulses from the V/F to the computer's counter; (3) The absorbance signal was amplified five times and filtered using a RC filter (RC = 10 msec) before transmission to the computer; and (4) A difference amplifier was used to drive the electrode potential voltage to the computer.

All signals were transmitted over coaxial cable (RG-58/u, 75 ft. long because the experiment instrumentation was away from the computer). Signal bounce in the V/F cable was eliminated by placing a 250 ohm resistor in series with the cable at the computer end producing a RC filter using the coaxial cable capacitance.

The magnetic stirrer was turned on or off via a digitally controlled triac which was mounted inside the computer interface panel. An AC line carried power to a Variac which, in turn, regulated the speed of the stirrer. A HP function generator was triggered by the computer to drive the wavelength axis of the RSS.
Figure 36

Block diagram of the computer controlled indirect coulometric titration experiment.
Two main Fortran programs were written. TITCONTROL acquired data and controlled the experiment, and TITREAD displayed the data. These programs are listed and described in Appendix C.

A typical operator/computer dialogue for TITCONTROL is shown in Figure 37. The program requests the following information:

1. experiment times;
2. number of counts from the V/F;
3. data is to be stored (on disk);
4. spectra are to be plotted; and
5. type potential information. The program then waits for the experimenter to strike a teletype key before continuing.

TITREAD was independent of TITCONTROL and could be run at any time after the titration experiment was completed and allowed display of data acquired by the TITCONTROL in a variety of formats. TITREAD consisted of a main program which allowed the experimenter to execute a desired display subprogram which was stored on the disk in an overlay file. The overlay procedure was used because it conserved computer memory.

B. Results and Discussion

The operation of the computer controlled experiment was verified by several techniques.

The accuracy of the acquired absorbance data was established by acquiring H$_2$O$_3$ spectra. Care must be taken to insure that the RC filter, used to remove the high frequency noise, did not distort the absorbance spectrum. A RC time constant of 10 msec was adequate to remove the high frequency noise but not distort the sharp 450 nm
Figure 37

TTTCONTROL initialization output.
TITCONTROL

ALL TIMES ARE IN SECONDS

STIRRER START UP TIME 5
NUMBER OF COUNTS 2000
DELAY AFTER COUNTING 50
STIRRER STOP TIME 10
PLOTTING TIME 50
DO YOU WANT TO SAVE DATA? YES=1
PLOT DATA AS TAKEN? YES=1
TYPE POTENTIAL INFO AS TAKEN? YES=1
FILENAME?
DATAFILE1
PAUSE READY TO START
triplet at a scan time of 50 sec and a 150 nm scan width. The Ho$_2$O$_3^-$ spectrum was recorded simultaneously by a recorder and by the computer and then compared. A decrease in absorbance of the sharp 450 nm triplet indicated that the RC time constant was too large.

The verification of the rest of the experimental set-up was accomplished by titrating a solution of cytochrome c. Reductive titrations employed electrochemically generated MV''+ (parent: 1,1'-dimethyl-4,4'-bipyridylium dichloride; common name: methyl viologen (MV)) and oxidative titrations employed electrochemically generated HMF+ (parent: 1,1'-bis(hydroxy-methyl) ferrocene (HMF)). An absorbance versus charge plot yielded a straight line (n value equal to 1.00 ± 0.05) for both oxidation and reduction indicating that the titration and electronics were behaving correctly. The computer acquired and the recorded data agreed to better than 0.2%.

A titration of cytochrome c oxidase was carried out using electrochemically generated BV' (parent: 1,1'-dibenzyl-4,4'-bipyridilium dichloride; common name: benzyl viologen (BV)) for reductions and HMF+ for oxidations and will serve to illustrate the advantages and limitations of the automation. The TITREAD program allowed selection of a spectrum from those acquired by the computer for use as a reference baseline. The reference baseline could be subtracted from a series of acquired spectra (i.e., spectra from an oxidative or reductive titration) to give difference spectra. Also, any wavelength(s) may be selected for dual wavelength data.
A series of spectra for an oxidative titration of oxidase are shown in Figure 38. The difference (oxidized minus reduced) spectra (Figure 38B) are computer calculated.

The spectra can also be plotted in several other formats. For example, difference spectra plotted as reduced minus oxidized spectra are shown in Figure 39A. An example of difference spectra using a reference baseline which is approximately one-half reduced is illustrated in Figure 39B.

The data presented in Figures 38 and 39 illustrates the data display formats now available. We have found the selective removal of background absorbance allows examination of the spectrally resolvable components of oxidase (the high potential heme and low potential heme). In addition, the examination of selected portions of the titration spectra has yielded information on oxidase-oxidant (both HMF+ and the physiological oxidant, O₂) equilibrium effects.

Data can also be displayed in several additional ways: (1) three dimensional projections; (2) absorbance at a wavelength versus charge increment; (3) OTE potential versus charge increment; and (4) tables of potentials, absorbances, times data were taken, and experimental information (times, counts, etc., input by the operator to TITCONTROL).

The computer program allowed comparison of the absorbance versus charge behavior for a set of reductive or oxidative titrations and could be used to search for trends in data behavior.
Cytochrome c oxidase titration

Spectra A: computer acquired absorbance spectra

Spectra B: oxidized minus reduced difference spectra

Conditions: cytochrome c oxidase concentration was 14 μM, BV was 1 mM, HMF was 1 mM, all in pH 7 Titrisol phosphate buffer.
Figure 39

Cytochrome c oxidase titration spectra

Spectra A: reduced minus oxidized difference
Spectra B: reduced minus 1/2 oxidized difference

Conditions: as in Figure 38.
As can be seen from the foregoing discussion, the automated indirect coulometric titration experiment is restricted to experimental parameters which can be controlled or monitored better by a computer. Intuitive parameters such as the applied potential and, more importantly, determination of the end points are not controlled by the computer. The former could be added to the automation program by using one of the computer's DAC's to supply the potential. This was not done because the value of the applied potential depended on the experimental conditions which could vary during the titration.

The endpoint is more difficult to automate. The endpoint is determined (in cytochrome c oxidase oxidative titrations) by a change in absorbance at 560 nm which is an isosbestic point for the enzyme. In a reductive titration using one of the viologens as the mediator titrant, it would be possible to do this since MV\(^{2+}\) cation has a large molar absorptivity \((E_{550} = 8,200, E_{605} = 12,500)^{13}\) and would cause the isobestic to change rapidly at the endpoint when excess radical is generated. Oxidative titrations would require the computer to sense when the 605 nm oxidase band stops changing (if HMF\(^{+}\) is the mediator titrant since it has an \(E_{605}\) of approximately 385 and would not cause a significant spectral change until a large excess concentration of HMF\(^{+}\) is generated).

An alternative to an endpoint detection would be to select a predetermined number of charge increments during the reductive and oxidative titration. This requires the concentration of the enzyme
to be accurately known. The enzyme must also behave in a reproducible and predictable way from sample to sample which is not always the case.

C. Conclusion

There are several additional points that should be brought out in the procedure employed in this automation. Firstly, the experiment can be operated independently of the computer. Therefore, a computer hardware failure does not force shut-down of the experiment. Secondly, the critical data (the spectra) are recorded redundantly preventing data loss in case a catastrophic computer hardware failure occurred. In addition, the recorded data serves as a reference in case a question arises about the integrity of the computer acquired data.

It has been demonstrated that indirect coulometric titrations were amendable to restricted computer automation. The computer hardware and software were allowed to control all functions of the experiment except those which require experimenter intuition or decision. The computer control eliminated the menial tasks previously done by the experimenter freeing him to prepare new solutions, interpret data, etc. An extensive plotting capability allowed display of raw data in a variety of formats for analysis.

There are several extensions which may be added to the computer automation, if desired. The addition of applied potential and endpoint detection would allow unsupervised computer control of the
experiment. Mathematical analysis of raw data has not been added to the program. This hopefully will be done in the future to allow large amounts of data to be analyzed and statistically compared. This would require a program to mathematically fit the raw data to a theoretical function by some iteration procedure instead of the current procedure\textsuperscript{13} of estimating parameters (i.e., redox potentials) by the experimenter and then comparing a theoretical curve to the experimental curve.
LIST OF REFERENCES


12. Cytochrome c oxidase experiment carried out in conjunction with R. Szentirmay, Ohio State University, Columbus, Ohio 43210.

13. L. Mackey, Ph.D. Dissertation, Ohio State University, Columbus, Ohio (1975).
CHAPTER VII

THE 1,1'-BIS-HYDROXYMETHYL FERROCINIUM OXIDATION OF REDUCED CYTOCHROME c

Since 1970 a major effort in this laboratory has been directed towards elucidation of the electron transfer mechanisms involving cytochrome c and cytochrome c oxidase. Investigations have employed indirect coulometric titrations for thermodynamic measurements. Cytochrome c transfers an electron to cytochrome c oxidase which reduces molecular oxygen to water in a four electron process.

Cytochrome c has been extensively studied since the work of Keilen in 1925. Dickerson and co-workers have determined crystal structures of both the oxidized and reduced forms. The knowledge of the crystal structures should provide the information required to establish cytochrome c reaction mechanisms but as is evidenced from the recent literature this is not the case. Kinetic studies employing small molecules as oxidants or reductants suggest two possible electron transfer mechanisms. Some evidence indicates the electron transfer occurs at the exposed edge of the heme porphrin ring via an outer sphere mechanism and the second proposed mechanism indicates an inner sphere attack which requires opening of the heme crevice and electron transfer occurring at the iron center.
Recent literature on kinetic rates is summarized in Table 3 (reductions) and Table 4 (oxidation). Except for dithionite and Cr(II) (aq) reductions proceed via the outer sphere mechanism. Dithionite and Cr(II)(aq) exhibited a mixed inner and outer sphere mechanism. The number of oxidative studies has been restricted by a limited number of suitable oxidants. Of those studied all were attributed to the outer sphere mechanism except for the inner sphere reactant, bromomalononitrile. The differentiation of inner/outer sphere cytochrome c reactions has involved by varying pH, ionic strength, anion variations or reactant/product structure differences. As suggested by Sutin, et al.,\textsuperscript{18} there is a definite need to undertake further oxidative studies in order to correctly assign a pathway to cytochrome c electron transfer.

Our familiarity with the probable outer-sphere oxidant HMF+ prompted its use to further study oxidative cytochrome c kinetics. Earlier work\textsuperscript{2} via SEC yielded a rate constant of $1 \times 10^7$ M\textsuperscript{-1} SEC\textsuperscript{-1}.

Concentration gradients in the diffusion layer during SEC kinetic measurements generally prevent resolution of multiple path reactions, and, hence, require a reaction order to be measured (second, in this case). In order to verify this assumption and the rate constant we decided to use the stopped-flow technique for kinetic measurements on the enzymes mentioned. Therefore, we adapted a stopped-flow unit to RSSIII and used it to study the oxidation of electrochemically reduced cytochrome c via
TABLE 3

CYTOCHROME c REDUCTION

<table>
<thead>
<tr>
<th>Oxidant</th>
<th>$k_2 (M^{-1} \text{SEC}^{-1})$</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(CN)$_6^{4-}$</td>
<td>$2.6 \times 10^4$</td>
<td>6,7</td>
</tr>
<tr>
<td>Fe(EDTA)$_2^2$</td>
<td>$2.6 \times 10^4$</td>
<td>8</td>
</tr>
<tr>
<td>Ru(NH$_3$)$_6^{2+}$</td>
<td>$3.8 \times 10^4$</td>
<td>9</td>
</tr>
<tr>
<td>Ce$^{4+}$</td>
<td>$1.9 \times 10^4$</td>
<td>8,10-13</td>
</tr>
<tr>
<td>S$_2$O$_4^{2-}$</td>
<td>$1.2 \times 10^4$ (outer)</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>$6 \times 10^4$ (inner)</td>
<td></td>
</tr>
<tr>
<td>Ferrocyanochrome $c$</td>
<td>$1 \times 10^3$ (self-exchange)</td>
<td>15</td>
</tr>
<tr>
<td>Ru(NH$_3$)$_5$(Imidazole)$^{2+}$</td>
<td>$5.8 \times 10^4$</td>
<td>16</td>
</tr>
<tr>
<td>MV$^{+*}$</td>
<td>$&gt;10^8$</td>
<td>2</td>
</tr>
<tr>
<td>Reductant</td>
<td>$k_2^s (M^{-1} \text{sec}^{-1})$</td>
<td>Ref.</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>---------------------------------</td>
<td>-------</td>
</tr>
<tr>
<td>Fe(CN)$_6^{3-}$</td>
<td>$1.2 \times 10^7$</td>
<td>7,17</td>
</tr>
<tr>
<td>tris-(1,10-phenanthroline)Co$^{3+}$</td>
<td>$1.5 \times 10^3$</td>
<td>18</td>
</tr>
<tr>
<td>Bromomalononitrile</td>
<td>5</td>
<td>19</td>
</tr>
</tbody>
</table>
electrochemically produced HMF+. The instrument is described and possible mechanistic implications of the results are discussed.

A. Instrumentation

An Aminco-Morrow stopped-flow module (American Instrument Co.) was adapted to RSSIII via the amount shown in Figure 40 and was designed to rigidly hold the stopped-flow between the sample compartment and PMT. The observation cell (length = 1 cm) coincided with the light beam and a lens (f1 = 12 cm), mounted on a three axes stage was used to focus the light beam on the observation cell. Teflon gaskets were used at stopped-flow/mount interfaces to make the unit air tight. Quartz plates were epoxied on the mount where light enters the observation cell. The stopped-flow could be purged with nitrogen.

The ADOI interface was used and acquired 1000 points. The stopped-flow stopping syringe provided a signal to the computer, after mixing was complete, to start data acquisition. An external series of amplifiers were used to increase the magnitude of signal change. The data were immediately stored on the disk and then analyzed. To improve signal-to-noise ratio and smooth the data, a three point weighted average, digital smoothing routine was used. The stopped-flow mixing time was 4 msec. The instrument was tested, per American Instrument Co. instructions, by employing the redox reaction between ascobate and 2,6-dichlorephenolindophenol. The measured rate agreed within 4% of the expected value.
Figure 40

Diagram of stopped-flow mount.
B. Experimental

The horse heart cytochrome c was obtained from Sigma (Type VI) and purified by C.R. Hartzell\textsuperscript{24} according to Margoliash.\textsuperscript{23} Phosphate buffer (Titrisol, m=0.15), pH 7.00 was obtained from E. Merck Co. The 1,1'-dimethyl-4,4'-bipyridylium dichloride (benzyl viologen, BV) was from K and K Laboratories and the HMF was from Strem Chemical Co. Prepurified (2 ppm O\textsubscript{2}) nitrogen gas was from J.T. Baker. Water was doubly distilled from an all glass still.

Stock solutions of cytochrome c were prepared by diluting the purified cytochrome c 1:25 in the phosphate buffer. All stock solutions were stored at 0°C.

Cytochrome c was reduced with electrochemically generated MV\textsuperscript{2+} in an external electrolysis cell. The reduced cytochrome c concentration was determined using a Cary-15 spectrometer at 550 nm assuming a molar absorptivity of 18,500 M\textsuperscript{-1}cm\textsuperscript{-1}. Enzyme solutions were initially degassed using nitrogen and diluted to the desired concentration level after reduction. HMF\textsuperscript{+} was oxidized at a platinum gauze electrode also in an external electrolysis cell. The concentration of HMF\textsuperscript{+} was determined spectrophotometrically at 625 nm and then diluted to the desired concentration. The stopped-flow runs were performed with a minimum time lag between preparation and reaction to minimize decomposition of HMF\textsuperscript{+}. Reactants were transferred to the stopped-flow apparatus using glass syringes.
C. Results and Discussion

A second order kinetic plot

\[
\frac{2.3}{\text{Cytc}^0 - \text{HMF}^0} \log \left( \frac{\text{HMF}^+ - \text{Cytc}^0 + \text{Cytc}_t}{\text{Cytc}_t} \right) \text{ vs time}
\]

was linear for the analysis time and the average rate constant was \(1 \pm 0.4 \times 10^7 \text{ M}^{-1}\text{s}^{-1}\). Cytochrome c concentrations were typically 5-20 M (before mixing) and HMF+ concentrations were 5-40 M. All runs were duplicated. A representative sampling of data are shown in the following table:

<table>
<thead>
<tr>
<th>HMF+ x 10^6 M</th>
<th>Cytc x 10^6 M</th>
<th>rate constant (M^{-1}s^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>5</td>
<td>9.6 x 10^6</td>
</tr>
<tr>
<td>5</td>
<td>2.5</td>
<td>9.5 x 10^6</td>
</tr>
<tr>
<td>20</td>
<td>10</td>
<td>1.4 x 10^7</td>
</tr>
</tbody>
</table>

To simplify the calculations the concentration of HMF+ was always in excess and the reverse reaction was neglected (this is reasonable since the equilibrium constant, calculated from standard reduction potentials, is 4000). Solutions were deoxygenated with nitrogen although effects of oxygen would be expected to be minimal. Because of the fast rate of reaction and the 4 msec mixing time of
the stopped-flow, only about 20% of the reaction time was observed. A typical computer acquired trace of the reaction is shown in Figure 41.

Mackey and Kuwana used SEC to study this reaction and have reported a second order rate constant of $10^7 \text{M}^{-1}\text{s}^{-1}$ to $10^8 \text{M}^{-1}\text{s}^{-1}$ in excellent agreement with the rate determined by the stopped-flow technique. A Marcus theory calculation of the reaction yielded a value of $2.9 \times 10^7 \text{M}^{-1}\text{s}^{-1}$ indicating that the reaction proceeds via the same mechanism as the electron self exchange reactions (i.e., via the exposed heme edge of cytochrome $c$) and that there are no kinetic limitations (i.e., opening of the heme crevice) which control the rate although some extremely fast rearrangement cannot be eliminated. Agreement with experimentally determined rates has been shown with other oxidants and reductants.

D. Conclusion

Relating the information obtained via reactions with non-physiological probes to the mechanism of electron transfer in mammalian respiratory system remains a challenging problem. The work of Margoliash, et al., involving cytochrome $c$ amino acid residue modifications, indicates that the oxidative electron transfer occurs in a separate area of the molecule from that of reductive electron transfer. Sutin, et al., believes there is no reason to assume a more complex mechanism than is required. Availability of the structures of cytochrome $c$ oxidase and reductase would certainly help clarify the problem.
Figure 41

Trace resulting from oxidation of cytochrome c by HMF+.
Absorbance of cytochrome c at 560 nm was followed. HMF+ concen-
tration was 10 um and cytochrome c was 5 um after mixing. The
smoother curve results from repeated use of the three point smoothing
routine.
LIST OF REFERENCES


2. L. Mackey, Ph.D. Dissertation, Ohio State University, 1975.


16. C. Creutz and N. Sutin, private communication.


APPENDIX A.

This appendix describes the interface hardware designed and constructed.

The interface hardware will be described in a modular fashion when possible. A complete description of the input/output (I/O) structure of this computer is available in the Data General Manual. The I/O structure will be described when it is needed for understanding the operation of the interfaces.

All circuits have the chip type written on the drawing. Standard symbols (INVERT, OR, NOR, AND, and NAND) were used and these components were not labeled as to type unless they were of an unusual nature. Graphic symbols were used as indicated: 1. arrow heads ( dõi ) indicate data to or from the Nova computer; 2. small squares ( □ ) indicate signals transferred to or from the interface to the interface panel; and 3. arrows ( → ) indicate intra interface signals. Symbols were also numbered to indicate where connections were made. Flip-flops were labeled as to type of input or output function (i.e., clear (Cl), preset (P), clock (C), data (D), Q, and Q̅). The flip-flops (FF), one-shots (OS), and logic gates (G), are numbered to ease understanding.

The initial discussion will involve the interface containing the digital I/O, the 12 bit counter, and the two DAC's.
A total of 48 digital output bits were required on the interface. Of these 36 bits were designed for controlling the Harrick RSS control module and 12 bits were available for general use.

The circuit employed for generating the 48 output bits is shown in Figure 42. Four 12 bit registers are used consisting of two 74174's each. A 74174 is a register capable of storing six bits of information. The strobe (load) inputs of two of the 74174's are connected together. The correct strobe for each of the 12 bit registers is obtained from the 74154. A 74154 was a 1 line-to-16 line decoder. Four bits of data are required to select one of the 74154's 16 outputs. The strobe pulse to the 4 registers is the Nova DATA-OUT-C (DOC) pulse. The DOC pulse is guided through the 74154 to one of the 4 output registers. The software driver is required to place a 4 bit select code in the four most significant bits (DATA0, DATA1, DATA2, and DATA3) of the data word to be output. The 12 least significant bits of the same word contain the data to be output and loaded to one of the output registers. This procedure is necessitated because the Nova I/O structure operates by first selecting an I/O device and then placing the 16 data bits on the I/O bus and finally issuing the DOC. Hence the data for the 74154 and the register are at their inputs when the DOC is issued. The 74154 guides the DOC to the appropriate output register.

To illustrate, a "7" (0111₂) appearing at the inputs of the 74154 causes the first register (REG0) to be loaded with whatever data appears at REG0's input. A 1₀, 1₁, 1₂ (subscript "n" indicates
Figure 42

Schematic of four twelve bit digital output registers.
an octal number) corresponds to the second (REG1), third (REG2), and fourth (REG3) registers respectively. REG1, REG2, REG3 are dedicated to the Harrick RSS (RSSIII); REGO was available for general use.

All 48 output bits were connected to two Cinch Jones 44 pin connectors mounted on the side of the interface board. The signals were transferred to an interface panel via two 44 wire cables. These two cables were constructed by using double sided printed circuit boards which mated with the Cinch Jones connectors on the interface board and the interface panel. Single strand wire (28 gauge ca. 6 ft. long) was used. The digital input registers, counter input and triggers were also carried by these two cables.

The four triggers generated by the interface are also shown in Figure 42. These triggers are derived by using 4 free outputs on the 74154. The software driver loads the correct 4 bit code to the 74154 to obtain the desired trigger. In this case, $13_8$, $14_8$, $15_8$, and $16_8$, correspond to TRIGA, TRIGB, TRIGC, and TRIGD, respectively.

REG1, 2, 3 were wired directly to a 50 pin Cinch "D" type connector on the interface panel. A cable connected the Harrick RSS and the interface panel. REGO was connected to a 12 pin Cinch "D" type connector. In addition the three most significant bits of REGO were connected to one of three AC(115 v) line driving circuits. The AC line drivers will be discussed later. The 6 least significant bits of REGO were connected to one of six BNC connectors mounted on the rear of the interface panel.
The circuitry used for driving the two DAC's is shown schematically in Figure 43. These DAC's are 12 bit ± 10 v output with an internal digital storage register. The digital data is loaded into the DAC's by employing the same procedure used in the loading the digital output registers. The 74154 is used to guide the DOC pulse to one of the DAC's according to the 4 bit code at the 74154 data inputs. A 4 bit code of 0 loads DAC 1, a 4 bit code of 1 loads DAC 2.

The DAC analog signals were transferred to the interface panel via RG 174 coaxial cable through connectors on the interface board and interface panel. Power (+15 v) was supplied to the DAC's through the same connector. The +15 v supply was contained in a separate power supply module.

Two 16 bit digital input registers were also available on the interface. The circuit for these two input registers is shown in Figure 44. Each register consists of four 74125's. The 74125 is a hex, tri-state inverter. Tri-state logic consisted of the normal high (+5 v) and low (0 v) with the addition of a high impedance "off" state. The use of Tri-state logic allowed several registers to use a single input bus (labeled IDATA0-IDATA15) asynchronously. Three interface subsystems (the two input registers and the digital counter) employ this technique. The two 16 bit input registers are distinguished by the input pulse which is used to load them. The DATA-IN-B pulse (DIB) was used to strobe input register 0 (IREGO) and the DATA-IN-C pulse (DIC) was used to strobe input register 1.
Figure 43

Schematic of driving the two digital to analog converters.
Schematic of the two 16 bit digital input registers.
(IREG1). When DIC goes to 0 volts the 74125's outputs are turned on and their outputs reflect the inverted input. High current nand gates (7438) are used to provide sufficient current to strobe a complete register. The input bits for IREGO (I100-15) and IREG1 (IR10-15) are transferred from the interface panel to the interface board using the two 44 wire cables described earlier.

IREG1 was dedicated to the Harrick RSS through the "D" type 50 pin connector. The six least significant bits of IREGO were wired to BNC connectors mounted on the rear of the interface panel.

A 12 bit digital counter was also incorporated into this interface. This counter was designed for use as either a digital counter (specifically for counting pulses from a voltage-to-frequency convertor) or a digital timer. The digital timer incorporated a 10 KHz clock which will be discussed later.

The counter circuit used is shown in Figure 45. The counter input is fed through a series of 7490's which divided the initial counter pulse input by $10^0$, $10^1$, $10^2$, $10^3$, or $10^4$. A 7490 is a decade divider. The desired division is selected by a 74151A. A 74151A is a 8 line-to-1 line multiplexor.

Three address bits are decoded by the 74151A to select one of 8 lines. The resulting pulse train was fed to a series of three 74193's. A 74193 is a four bit binary presettable counter. The value for the three bit address for the 74151A (DATA0, 1,2) and the 12 bit preset value for the 12 bit counter (DATA15-DATA4) is loaded into the counter by issuing a DATA-OUT-A pulse (DOA).

Issuing a start (START) sets the flip-flop (FF1) and enabled the
Figure 45

Schematic of the digital counter circuit.
counter to count. The 74193's are wired as down counters. When
the counter reaches zero the DONE line is set causing the interface
to generate an interrupt. Issuing a clear (CLEAR) resets FF1 which
in turn resets the decade dividers and binary counters. Issuing a
clear also resets the interface interrupt logic. The current
count value of the 74193's could be read at any time by issuing a
DATA-IN-A (DIA). The DIA causes the value of the four 74125's to
be read. These 74125's were tri-state invertors identical to
those described earlier. This feature allows measurement of
counts per unit time in addition to a preset number of counts. The
three bit address which are input to the 74151A require a $4$ (in
bits DATAO, 1, 2) to perform a divide by $10^0$ and $0$ to perform a
divide by $10^4$ function. The maximum preset value to the counter
was $4096_{10}$ which gave 4097 counts before interrupt. A preset value
of zero gave one count. The software driver calculated the correct
output word from the designated decade divider constant and
number of counts.

The interface contained several miscellaneous circuits which
are shown in Figure 46. All digital input and output bits have a
light emitting diode (LED) to indicate in what state (high or low)
the digital bit is in. The LED's and drivers are mounted in the
interface panel. The LED circuit is shown in the Figure labeled
"A". An open collector invertor (7406) is used to turn the LED on
or off depending on the 7406 input value. An unregulated 9 volt
(+V) power supply is used to power the LED's. A 120 ohm resistor
limits the current through the LED. The AC power controls (Figure 46
Figure 46

Miscellaneous circuits

Circuit A: LED driver circuit
Circuit B: 115 V. AC power control circuit
Circuit C: Signetics 555 wired as a 10 KHz clock
"B") are turned on or off using a triac which is controlled by a relay. The relay coil is driven by a 7406. The 10 KHz clock signal is provided by a Signetics 555 chip. (Wiring diagram is shown in Figure 47 and labeled "C".)

The second interface to be described contained the analog-to-digital convertor (ADC), 8 channel multiplexor (MM8), two sample-and-hold modules (S+H), and programmable clock. This interface transferred digitized data automatically to a predetermined memory location via use of the direct memory access channel (DMA) of the computer. The interface also monitored the number of words digitized, stopping after a predetermined number of conversions. The DMA logic was provided by Data General on the interface board.

This interface also provided the capability of sequentially acquiring data from two or more analog channels. Dual S+H amplifiers were simultaneously "held", holding data on analog channels zero and one enabling the simultaneous conversion of data. Data points could be digitized from these two channels.

The programmable clock used in the ADC interface is only slightly different than the design used for the digital counter. The circuit for the ADC clock is shown in Figure 48. The clock section of the ADC interface consists of a 16 bit data register made up of two 74174's and one 74175. A 74174 is a six bit register and a 74175 is a four bit register. This 16 bit register contains a three bit address code for the 8 line-to-1 line decoder (74151) and 12 bits of information for the preset value to a 12 bit
Figure 47

Schematic of the ADC interface clock and register select circuit.
presettable binary down counter (74193). A 10 MHz clock is used as the main interface clock. The 10 MHz frequency is then divided by a software-designated power of 10. Divider values possible are $10^n$ where $n = 1$ to 6. (It should be noted that in this circuit a value of 7 in bits DATA1, 2, 3 corresponds to a 10 MHz divided by 10 frequency (100 KHz) being sent to the 12 bit counter. A value of 1 in bits DATA1, 2, 3 corresponds to a 10 MHz divided by 10 MHz (1 Hz) frequency being sent to the counter.) The desired decade frequency is selected by the $7^{151}$. The decade frequency selected is fed into the 12 bit preset down counter. The 16 bit data register is loaded by issuing a DATA-OUT-A (DOA). The DOA also resets all the decade dividers to zero. Resetting the decade counters to zero allowed a precise divide by $10^n$ to be obtained. (If this were not done each decade counter could be in a state between a division of 0 to 9. The decade counters count to ten internally and then pass one count to the next counter.) The decade frequency dividers and 12 bit counters are, in principle, accurate to ± 50 nsec (% 10 MHz cycle). The 12 bit counter was initially preset by issuing the computer "start" (START) command. The START causes the counters to load the preset value at their inputs. As can be seen from Figure the START pulse is passed through an AND gate and then through two invertors to the ADC section of the interface and, also, to the DMA and interrupt section of the interface. It should be noted that in order to accurately start this clock in operation the software must issue a "DOAS" mnemonic in the assembly language driver. When a "DOAS" was issued the START pulse arrives
approximately 150 nsec after the DOA pulse. The interface, in fact, will not work unless the "DOAS" is issued. The reason for this will be seen later.

Upon counting to zero the 12 bit counter passes the next clock pulse to the 74123 one-shot which produces a 100 nsec pulse. The 100 nsec pulse is passed through the AND gate and causes the counters to reload their preset value and the pulse is passed to the ADC section to initiate the next conversion. The one-shot must produce a pulse whose duration is less than one cycle of the fastest clock input to the counters or one count will be lost (i.e., one clock pulse would occur before the counters are completely preset).

The principle advantage of this clock was the time resolution which could be obtained. Data points could be taken at intervals of 1 μsec (excluding ADC conversion time limitations) to 4097 μsec or 1 sec to 4097 sec. Any value between these was possible.

There is also a small subcircuit drawn of Figure 47. This circuit consists of a 74155. A 74155 is a one line-to-eight line multiplexor. This circuit was required since three registers (the DMA word count register, the DMA address count register and analog multiplexor word) must be loaded and only two output pulses are available (DOB and DOC). This procedure is accomplished by loading the Data General provided output data (OD) register via the DOB. The three most significant bits in this register provide a three bit address for the 74155 which allow it to select the LOAD WC DATA or LOAD CA DATA output. When the DOC is issued it is
passed either to LOAD WC DATA or LOAD CA DATA, loading one of these registers with the appropriate data. The next step involves releading the OD register with the new three significant bits. The DOC is reissued loading the opposite register to the first DOC. The last step is to reload the OD register but placing information for the ADC multiplexor in bits OD5-7 and OD13-15. The significance of these bits will be discussed later.

It should be noted that the ADC clock ran continuously and the clock frequency and preset value are changed by reseting the clock.

The operation of the ADC section of this interface will now be described. This ADC interface was capable of digitizing data on one of eight single channels (random mode) or on a consecutive sequence of channels (sequential mode). The sequential mode allowed a first channel to be defined (channel 0 to channel 6) and a last channel to be defined (channel 1 to channel 7). The first channel must be less than the last channel.

The ADC circuit is shown in Figure 48. The OD register contains the channel information for the ADC circuit as well as a bit to indicate whether the conversion is random or sequential. OD13, OD14, and OD15 contain the first channel to be converted. OD12 (R/S) indicates whether the conversion is random (R/S=0) or sequential (R/S=1). In the random mode the triple input AND gate (G1) has its output in the high state, independent of the other two inputs. This blocks the sequential scanning circuitry. The receipt of a clock pulse from the ADC clock causes two things to occur. First the 74193 (binary presettable counter) is preset to
Figure 48

Schematic of the ADC timing and control circuitry.
the analog channel address value in OD13, OD14, OD15. This channel is immediately available to the three bit analog multiplexor address inputs. The multiplexor allows the appropriate analog input to be fed through the multiplexor to the ADC. Second, the clock is OR'ed (G2) with a sequential signal from G1 (not relevant in the random mode) and then is fed to a series of two one-shots. The first one-shot (OS1) produced a 1 μsec pulse. This procedure delayed the clock 1 μsec to allow sufficient time for the analog multiplexor to settle on the desired channel. The OS2 100 μsec pulse is input to the convert input of the ADC. This positive pulse causes the ADC to reset (if it is converting) and initiate a new conversion when the pulse returns to zero volts.

Coincident with the initiation of a new conversion the EOC line goes high and is fed through the dual input OR gate (G3) to the two S+H amplifiers causing them to switch from the sample to hold mode. When the ADC finishes the conversion the EOC goes low releasing the S+H's allowing them to again sample the incoming signal. The discussion of the S+H's is relevant only if analog channels 0 or 1 are used. The trailing edge of the EOC is also used to load an input data register and request transfer of the digitized data to the computer memory. This will be discussed later. The random conversion sequence is now complete. A new clock pulse will cause a new conversion sequence to be started.

Certain timing considerations must be mentioned with respect to a series of conversions in the random mode. The fastest data
conversion rate that can be converted was limited by the ADC conversion time, the S+H acquisition time (time required to go from a hold mode to sample mode and correctly sample a signal which has changed from -5 V to +5 V) and the 1 μsec settling time for the analog multiplexor. The ADC conversion time was specified to be 13 μsec (although the measured time was about 11 μsec) and the S+H acquisition time was 7 μsec (to 99.95% accuracy). Therefore, a data conversion rate of 13 μsec + 7 μsec + 1 μsec (approximately 50 KHz) was possible. Faster rates were possible if less accuracy was permissible when digitizing a rapidly changing signal or the signal changes relatively slowly (i.e., the time required to sample a signal which has changed 1 volt was less than that for a 10 volt change). Rapidly changing signals should not be digitized on non S+H channels since the digitization would be inaccurate (the signal would change while the ADC was converting). The degree of error depended on the ADC conversion speed and the rate of change of the analog signal).

The sequential conversion of more than one analog channel will be illustrated with an example of a three channel conversion. Initially, the OD register has loaded in it 0 (start at analog channel 0) in OD13, OD14, OD15 and 2 (analog channel 2 is the last channel) in OD5, OD6, OD7. The R/S (OD12) will be one indicating a sequential scan.

Upon receipt of the clock pulse the initial procedure described for the random scan occurs with data on channel 0 being converted initially. Upon completion of the first conversion
several events occur. The EOC is inverted \( G \) and fed to the 74193 counter and the EOC trailing edge (rising edge when inverted) causes the 74193 contents to increment. The trailing (falling) edge of the EOC causes a one shot (OS3) to generate a 100 nsec pulse. The 100 nsec pulse travels through the three input AND gate (G1) and through the OR gate (G2) causing a conversion on channel one to be started. It must be remembered that the EOC increments the 74193. When the first channel is converted, the CONVERT CLOCK is fed to a flip-flop (FF2) which resets. The Q output (high state) of this flip-flop (FF2) is ANDed (G5) with R/S and fed to the OR gate (G3) driving the S+H's. This causes both S+H's to remain in the hold mode until after the last channel conversion is complete.

After conversion of the second channel the 74193 is incremented to two and the next conversion (analog channel 2) is started. When the output of the 74193 is two the output equals the last channel to be converted. The outputs of the 74193 and the last channel bits (OD5, OD6, OD7) are fed to the inputs of a 4 bit address comparator (7485). The 7485 compares the value of the last channel bits with the outputs of the 74193, which now contain the value two. Since both sets of inputs are equal the A=B output of the 7485 goes to a high state. The A=B transition causes the D input of the flip-flop (FF2) to go high and the positive A=B transition causes the AND gate (G6) to fire a one-shot (OS4) to generate a negative going 6 \( \mu \)sec pulse (the OS4 functions as a delay. The A=B transition is delayed until after the last conversion starts). This 6 \( \mu \)sec
pulse serves as the C input to the flip-flop (FF2) which causes the A=B 0 volt level to be transferred to the Q output of the flip-flop (FF2) changing the output of the following AND gate (G2) to zero volts. This change allows the S+H's to release from the hold mode when the third conversion is complete (note that the S+H's will not release until conversion is complete). The 6 μsec pulse also sets the Q output of the flip-flop (FF3) to 0 volts blocking the passage of a new EOC caused clock pulse through G1. Thus the sequential cycle is complete. A new sequence will start when a new ADC clock pulse is received.

The rate of data conversion in the sequential mode was dependent on the number of sequential channels converted. Seven μsec must be allowed for the S+H acquisition time; thirteen μsec for each channel converted (ADC conversion time). One μsec must be allowed for the multiplexor settling time. The conversion time for a sequential conversion is expressed in the following equation:

$$\text{Sequential conversion time} = 7 \mu\text{sec} + N \cdot 13 \mu\text{sec} + N \cdot 1 \mu\text{sec}$$

(1)

where N is the number of channels converted.

The last part of the ADC circuit to be described is the interrupt and DMA synchronization logic. The circuit required for this is shown in Figure . (This section of the interface was designed, in part, elsewhere.)
Figure 49

Schematic of the interrupt and DMA synchronization logic.
The initial required "DOAS" which starts the clock also sets the DMA and DONE logic for operation. The DOA pulse is extended by the one-shot (OS5). The one-shot pulse extends so that it overlaps with the START pulse. The START and one-shot pulses are NANDed together (G7). The output of the NAND (G7) gate causes the flip-flop (FF4) Q output to go high enabling the NAND gate (G8) driving the DATA CHAN, SYNC SET TERM and the LOAD DATA REG lines. The Q output of FF3 disables the NAND gate (G9). This prevents the done line from being set until all conversions are complete. The EOC from the ADC fires a one-shot (OS6) on the rising (trailing) edge (i.e., when conversion is complete). If the DOA and START have been issued the one-shot pulse sets the DATA CHAN SYNC SET line requesting a DMA transfer. The one-shot (OS6) pulse also causes the ADC digital data bits to be loaded into the Data General supplied input register (IDAT4 to IDAT15) on Figure 48. The DOA and START cause (via the one-shot (OS5)) to set the Q output (via NAND gate, G10) of the flip-flop (FF5) to a high level. This enables the CLOCK to pass through the NAND gate (G11) to a second NAND gate (G9) which NANDs the Q (low state) of the flip-flop (FF4) with the CLOCK. This allows done to be set when Q (FF5) and CLOCK are high (after the last conversion). The last conversion causes the most significant bit of the word count register (WCO) to go high. This causes the flip-flop (FF4) to switch states (Q → 0, → 5V) blocking further data transfers. The flip-flop (FF4) Q transition also enables done to be set on the next clock. The CLEAR or IORST
cause the interface to reset for the next conversion sequence. CLEAR sets Q(FF5) to zero volts preventing DONE from setting and resets the DMA and interrupt logic. CLEAR is specific to a given interface. IORST resets the entire computer.

Several miscellaneous connections were made on the ADC interface. These connections are shown in Figure 50. These connections allow correct operation of the DGC DMA circuitry.

Logic components were produced by Signetics or Texas Instruments. The interface logic used was standard TTL type and complete descriptions of the various logic components can be found in the manufacturer's literature.
Figure 50

Schematic of miscellaneous circuit connections required for interface operation.
load output register input #2

DCH SELECT, DCH IN

ADDRESS REGISTER CLOCK

OUT DATA BUS #2

WORD COUNT REGISTER CLOCK

ADDRESS ENABLE

ENABLE ADDRESS REGISTER
APPENDIX B.

This appendix describes the assembly language subroutines used to drive the two interfaces described in Appendix A. It is assumed that the reader has an understanding of DGC assembly language. The programs will be described in the same sequence as the interface hardware. All the programs were Fortran callable subroutines using standard Fortran subroutine format. All parameters passed to and received from Fortran were integers. All assembly language routines employed two Fortran Library routines. The first was Fortran Library Routine .CPY1 which saved the main Fortran program state (state refers to information which was required for the main program to operate after returning from the assembly language subroutine). .CPY1 also provided an address, through which data can be passed back and forth from Fortran. A second routine, .FRET, provided an orderly return to Fortran and restarted the main Fortran program.

The first program to be discussed is the program which controls the four twelve bit output registers. This program, titled REGS, is shown in Program 1. Although the title is REGS there are eight internal subprograms which are the names appearing in an actual Fortran subroutine call. Four of these subprograms simply reset the entire register in question. These programs,
Program 1

Program, REGS, used for controlling the four digital output registers.
• TTTL REGS
• ENT CREG0 CREG1 CREG2 CREG3
• ENT SREG0 SREG1 SREG2 SREG3
• EXTD • CPYL • FRET
• NREL
0 ;NO PARAMS

CREG0: JSR @ • CPYL ;SAVE STATE
LDA 0,SIX ;LOAD MUX ADDRESS
DOC 0,65 ;CLEAR REGISTER
JSR @ • FRET ;RETURN
SIX: 60300
• NREL
0 ;NO PARAMS

CREG1: JSR @ • CPYL ;SAVE STATE
LDA 0,SXX ;LOAD MUX ADDRESS
DOC 0,65 ;CLEAR REGISTER
JSR @ • FRET ;RETURN
SXX: 70000
• NREL
0 ;NO PARAMS

CREG2: JSR @ • CPYL ;SAVE STATE
LDA 0,SX ;LOAD MUX ADDRESS
DOC 0,65 ;CLEAR REGISTER
JSR @ • FRET ;RETURN
SX: 100000
• NREL
0 ;NO PARAMS

CREG3: JSR @ • CPYL ;SAVE STATE
LDA 0, SZ ;LOAD MUX ADDRESS
DOC 0,65 ;CLEAR REGISTER
JSR @ • FRET ;RETURN
SZ: 110000
• NREL
1 ;ONE ARGUMENT PASSED

SREG0: JSR @ • CPYL ;SAVE STATUS
LDA 1,-167,03 ;GET DIGITAL WORD
LDA 0,128M ;GET 12 BIT MASK
AND 0,1 ;AND VALUE IN
LDA 0, SXX
ADD 0,1
DOC 1, DIOR ;OUTPUT WORD TO REG0
JSR @ • FRET ;RETURN
• 128M: 7777

SXX: 60300
• DJSR DIOR=65 ;DEVICE CODE 65
• NREL
1 ;ONE ARGUMENT PASSED

SREG1: JSR @ • CPYL ;SAVE STATUS
LDA 1,-167,03 ;GET DIGITAL WORD
REGS

LDA 0, 1BM          ;GET 12 BIT MASK
AND 0, 1            ;AND VALUE IN
LDA 0, SIEZ         
ADD 0, 1
DOC 1, DIOR         ;OUTPUT WORD TO REG0
JSR 0, FRET         ;RETURN

1BM: 7777
SIEZ: 070000
• DUSR DIOR=65      ;DEVICE CODE 65
• NREL
1               ;ONE ARGUMENT PASSED
SREG2: JSR 0, CPYL  ;SAVE STATUS
LDA 1, -167, 03    ;GET DIGITAL WORD
LDA 0, 2BM         ;GET 12 BIT MASK
AND 0, 1            ;AND VALUE IN
LDA 0, SIEK
ADD 0, 1
DOC 1, DIOR         ;OUTPUT WORD TO REG2
JSR 0, FRET         ;RETURN

2BM: 7777
SIKE: 100000
• DUSR DIOR=65      ;DEVICE CODE 65
• NREL
1               ;ONE ARGUMENT PASSED
SREG3: JSR 0, CPYL  ;SAVE STATUS
LDA 1, -167, 03    ;GET DIGITAL WORD
LDA 0, 2BM         ;GET 12 BIT MASK
AND 0, 1            ;AND VALUE IN
LDA 0, SIEK
ADD 0, 1
DOC 1, DIOR         ;OUTPUT WORD TO REG3
JSR 0, FRET         ;RETURN

• BM: 7777
SIKE: 110000
• DUSR DIOR=65      ;DEVICE CODE 65
• END
clear register (CREG), reset the appropriate register to all zeros. The names CREGO, CREG1, CREG2, and CREG3 are used to indicate which register is to be cleared. The operation of each CREG program is the same. Examining the entry point (starting point) of the assembly language code for CREGO, the .CPY1 routine is called first. Secondly, a word is loaded into accumulator 0 (ACO). The word in ACO contains the 4 bit multiplexor (mux) select code for REGO but the twelve data bits contain zero. The ACO word is then output to the interface (device code 65 or menonically DIOR) by issuing a DOC 0, 65 (DIOR). The .FRET subroutine is called next and return to Fortran is executed. The procedure for CREG1, CREG2, CREG3 is identical except for the different mux address.

The subprograms (SREGO, SREG1, SREG2, SREG3) used to load data into the output registers require that an integer, with the desired bits set, be passed from Fortran to the assembly language routine. Examining the entry point for SREGO the first step (.CPY1 will not be counted as a step) is to load the integer from Fortran. This integer must have the desired bits set or cleared contained in the twelve least significant bits. The next step is to load a twelve bit mask (bit 4 through bit 15 set to one) and then AND this twelve bit mask with the integer. The ANDing process makes the sixteen bit integer into a twelve bit integer. The first four bits became zero through this process. The next step is to load ACO with the word containing the four bit mux address. The twelve bit integer and the four bit mux address are ADDed together producing
a 16 bit word. This 16 bit word is then output to the interface via a DOC 0, DIOR. The SREG1, SREG2, and SREG3 are identical except for the four bit mux address.

The various triggers are generated using a program with the general title TRIGS. TRIGS contains four subprograms which generate the separate triggers. The subprograms are titled TRIGA, TRIGB, TRIGC, TRIGD. The TRIGS program is shown in Program #2. Examining the entry point of TRIGA it can be seen that the first step is to load ACO with the four bit mux address word for trigger A. This word is then output to the interface via a DOC 0, DIOR. The TRIGB, TRIGC, and TRIGD programs are identical except for the four bit mux address word.

The assembly language driver for the two DAC's is given as Program #3. This program is titled PLOT1. PLOT1 obtains the two values (X and Y) to be output to the DAC's from Fortran. PLOT1 makes X a 12 bit word and adds in the four bit mux address for DAC#1. This value is then output to DAC#1. The above procedure is repeated for Y and DAC#2. PLOT1 also obtains a software delay from the Fortran program. This delay value was counted down using a loop within a loop (a delay of seven caused seven to be counted to 0 seven times). The delay was a mechanism used when plotting on an x-y recorder to slow the plotting speed down.

The value of the input registers was read using a program titled RREG. This program is shown in Program #4. RREG contains two subprograms titled RREG0 and RREG1... These subprograms are simple, requiring a DIB 0, DIOR (to read input REGO) or a DIC 0,
Program 2

Program, TRIGS, used for controlling the four output triggers.
TRIGS

• TITL TRIGS
• ENF TRIGA TRIGB TRIGC TRIGD
• EXTD • CPYL • FRET
• NREL

0 ; ZERO PARAMS

TRIGA: JSR @ • CPYL ; SAVE STATE
LDA 0 • TWELVE ; LOAD MUX CONSTANT FOR TRIGA
DOC 0 • 65 ; OUTPUT TRIG
JSR 0 • FRET ; RETURN

TRIGB: JSR 0 • CPYL ; SAVE STATE
LDA 0 • THIRTEEN ; LOAD MUX CONSTANT FOR TRIGB
DOC 0 • 65 ; OUTPUT TRIG
JSR 0 • FRET ; RETURN

TRIGC: JSR 0 • CPYL ; SAVE STATE
LDA 0 • FOURTEEN ; LOAD MUX CONSTANT FOR TRIGC
DOC 0 • 65 ; OUTPUT TRIG
JSR 0 • FRET ; RETURN

TRIGD: JSR 0 • CPYL ; SAVE STATE
LDA 0 • FIFTEEN ; LOAD MUX CONSTANT FOR TRIGD
DOC 0 • 65 ; OUTPUT TRIG
JSR 0 • FRET ; RETURN

TWELVE: 120000
THIRTEEN: 130000
FOURTEEN: 140000
FIFTEEN: 150000

• END
Program 3

Program, PLOT1, used for driving the two DAC's.
PROGRAM TO DRIVE THE SECOND PLOTTER

CALL PLOT1(NX, NY, NDELAY)

WHERE NX=0 TO 4095

NY=0 TO 4095

NDELAY=1 TO 32768

ALL VALUES MUST BE INTEGERS

THREE PARAMETERS ARE PASSED FROM FORTRAN

1. CXL
2. C Y L
3. CS

PARAMETERS ARE

LOAD -2048 FOR -10 VOLTS

ADD TO X AND Y VALUES

LOAD 7777 FOR 12 BIT MASK

MAKE X AND Y 12 BIT WORDS

LOAD 4 BIT CONTROL WORD FOR X DAC

ADD TO X VALUE

LOAD Y DAC CONTROL

ADD TO Y VALUE

 biased

LOAD DELAY VALUE

STORE IN LOOP COUNTERS

DSZ PDEL2

IDS AND CHECK FOR 0 IN OUTER LOOP

JMP +2

NOT 0; GO TO INNER LOOP

JSR @. FRET

THEN RETURN

DSZ PDEL2

IDS AND CHECK FOR 0 IN INNER LOOP

JMP -1

NOI 0; JUMP BACK

JMP DELA

0 GO TO OUTER LOOP

PDELA:

0

PDEL1:

0

PDEL2:

0

12BM:

7777

NBIALS:

174000

XAXIS:

0

YAXIS:

010000

DUSR PLTR1=65

DEVICE CODE FOR PLOTTER 1

END
Program 4

Program, RREG, used for reading the value of the two 16 bit digital input registers.
RREG

.TITL RREG
.ENT RREG1,RREG0
.EXTD .CPYL *FRET
.NREL
0

RREG0:
JSR 0 *CPYL  *SAVE STATE
DIB 0 *DIOR  *READ INPUT REG 0
STA 0,-167,03 *SEND VALUE TO FORTRAN
JSR 0 *FRET  *RETURN
0

RREG1:
JSR 9 *CPYL  *SAVE STATE
DIC 0 *DIOR  *READ INPUT REG1
STA 0,-167,03 *SEND VALUE TO FORTRAN
JSR 0 *FRET  *RETURN
*DJSR DIOR=65 *DEVICE CODE OF INTERFACE
*END
DIOR (to read input REG1). The value read from the interface is then sent to Fortran.

The program used for driving the digital counter was more complex than the programs described previously. The counter program is titled COUNTER and contains four subprograms. These subprograms perform the following functions: 1. set initial count and decade divider (SETCT), 2. read current value in counter (REDCT), 3. check if clock has counted to zero and interrupted (CKDNE), and 4. clear clock interface (CKCIR). The program COUNTER is shown as Program #5. The first entry point in COUNTER is SETCT. SETCT initially identifies the clock, as a device which can generate an interrupt to RDOS via the RDOS system command .IDCF. SETCT then obtains the decade divider value and the number of counts from Fortran. The number of counts is deincremented by one (this is done because the counter counts down to zero not one). The rest of the assembly language code constructs the correct sixteen bit word for output to the clock. The sixteen bit word is then output to the counter and the clock started via the DOAS O, CONTR statement. CONTR is defined as equivalent to the device code 65.

The next subprogram, REDCT, simply reads the current value of the counter via a DIA1, CONTR statement. The value is then sent to Fortran.

The third subprogram, CKCIR, clears the counter interface and removes the counter from the RDOS list of interrupting devices via the RDOS system command .IRMV.
Program 5

Program, COUNTER, used to control the digital counter.
SETCT: JSR @CPYL

;SETCT SETS COUNTER DIVIDER AND PRESETS 12 BIT COUNTER
;FORMAT CALL SETCT(NDIVIDE,NCOUNT)
;WHERE NDIVIDE=4,3,2,1,0
;
SUB 0,0
STA 0,CDONE ;0 IN DONE WORD
STA 3,SAVE ;SAVE AC3
LDA 0,CKDVD ;LOAD COUNTER DEVICE CODE
LDA 1,CKDC ;LOAD ADDRESS OF COUNTERS
DCT
;SYSTM
;IDEF
HALT ;ERROR RETURN
LDA 3,SAVE ;RELOAD AC3 AND CONTINUE
LDA 1,-167,03 ;LOAD DIVIDER VALUE
LDA 0,-167,1,03 ;LOAD COUNT
SUBEL 2,2 ;GENERATE A ONE,
SUB 2,0 ;SUBTRACT FROM COUNT
LDA 2,128M ;LDA MASK FOR 12 BIT WORD
AND 2,0
MOVS 1,1
MOVE 1,1
MOVE 1,1
MOVE 1,1
MOVE 1,1
ADD 1,0
DOAS 0,CONTR ;OUTPUT WORD AND START COUNTER
JSR 0,FRET
1
REDCT: JSR @CPYL
;REDCT READS CURRENT COUNTER VALUE
;VALUE RETURNED IS BETWEEN 0-4096
COUNTER

; DIA 1, CONTR ; READ COUNTER
STA 1, -167, 03 ; SEND TO FORTRAN
JSR 0, FRET
0

CKCLR: JSR 0, CPYL
; CKCLR CLEARS COUNTER AND REMOVES ITS INTERRUPT
; FROM RDOS
LDA 0, CKDVD ; LOAD DEVICE CODE FOR COUNTER
NIOC CONTR ; CLEAR COUNTER
* SYSTM
* IRMV
JMP +1 ; NO ERROR RETURN
JSR 0, FRET
1

CKDONE: JSR 0, CPYL
; CKDONE RETURNS A ONE IF THE COUNTER
; HAS INTERRUPTED A 0 IF NOT
LDA 0, CDONE ; LOAD DONE FLAG
STA 0, -167, 03 ; SEND VALUE TO FORTRAN
JSR 0, FRET

* CKDC: CKDCT

CKDCT: * SAVA ; BWORD SAVE AREA
37777
CKINT

* SAVA: ; BLK 10

CKINT:
NIOC 65
SUBZL 0, 0 ; GEN A 1
STA 0, CDONE ; STORE IN DONE WORD
NIOC CONTR ; CLEAR COUNTER
* UIEX

* DUSR CONTR=65
* 12BM: 7777

CKDVD: 65
CDONE: 0
* SAVE: 0
* END
The fourth subprogram is CKDNE. This program loads a flag word (CDONE) and returns the value of CDONE to Fortran. If CDONE equals zero the counter has not interrupted. If CDONE equals one the counter has interrupted (counted to zero). The value of CDONE is set by the counter interrupt handling program, CKINT. When the counter generates an interrupt RDOS gives program control to CKINT. This program places a one in CDONE. Return from CKINT to Fortran is made via the RDOS .UIEX program. A further discussion of RDOS interrupts and interrupt handling is in a DGC publication.  

ADI is a program which operated the ADC interface. This program consists of six subprograms. ADCIR clears the ADC interface. ADCHAN constructs the analog multiplexor word for a random or sequential mode. ADSET constructs the ADC clock word for the desired rate of conversion and obtains the number of points to be acquired. ADRUN starts the ADC conversion. ADRED sends the converted data to Fortran. ADDNE checks to see if the ADC has completed the conversion sequence and has caused an interrupt. ADI is shown as Program #6. The first entry point in ADI is ADCIR. ADCIR clears the ADC interface and then removes the ADC device code (668) from the RDOS list of devices capable of generating an interrupt.

The second subprogram entry point is ADCHAN. ADCHAN requires three parameters to be passed to it from Fortran. The first parameter is an integer constant indicating a random (value equal
Program 6

Program, AD1, used for controlling the ADC interface.
ADCLR:
JSR @ CPYL  ; SAVE MACHINE STATE
NIOC ADCl  ; CLEAR DEVICE
LDA 0, ADIDV  ; LOAD ADCl DEVICE CODE
SYSTM  ;
IRMV  ; REMOVE ADCl FROM RDOS
JMP +1  ; NO ERROR RETURN
JSR @ FRET  ; RETURN
3  ; THREE PARAMS TO BE PASSED

ADCHAN:
JSR @ CPYL  ; SAVE STATE
LDA 1,-167,03  ; LOAD RND/SEQ CONSTANT
LDA 0,-167+1,03  ; LOAD FIRST CHANNEL
SUB 2,2  ; 0 REG 2
MOV 1,1,SZR  ; TEST FOR ONE OR ZERO
LDA 2, .4BMK  ; MAKE BIT A ONE
ADD 2,0  ; ADD R/S TO FIRST CHANNEL
MOV 1,1,SNR  ; TEST FOR 1
JMP SChan  ; IF 0 GO SAVE CHANNEL
LDA 1,-157+2,03  ; LOAD FINAL CHANNEL
MOVS 1,1  ; SWAP HALFS
ADD 1,0  ; ADD INTO CHANNEL WORD
SChan:
STA 0,CHANA  ; SAVE CHANNEL WORD
JSR 9,FRET  ; RETURN

.4BMK:
1B12

CHANA:
0  ; FORMAT: CALL ADSET(MUX,MULT,NPTS)
; MUX=CLOCK FREQ.
; MULT=NUMBER OF PULSES TO BE COUNTED

ADSET:
JSR @ CPYL  ; SAVE MACHINE STATE
SUB 0,0  ; GENERATE A 0
STA 0,CDONE  ; CLEAR DONE FLAG
LDA 0,-167,03  ; LOAD MUX ADDRESS
LDA 1,-167+1,03  ; LOAD CLOCK COUNT
SUB2L 2,2  ; GENERATE A ONE
SUB 2,1  ; SUB FROM CLK COUNT TO KEEP
; THE HARDWARE HAPPY
LDA 2,.12BM  ; LOAD 12 BIT MASK
AND 2,1  ; MAKE CLOCK COUNT 12 BIT WORD
MOVS 0,0  ; SWAP MUX TO HIGH ORDER BYTE
MOVE L 0,0  ;MOVE
MOVE L 0,0  ; TO
MOVE L 0,0  ;
ADD 1,0  ;ADD INTO CLOCK WORD
STA 0,0  ;STORE FINAL CLOCK WORD
LDA 1,-167+2,03  ;LOAD NUMBER OF AD POINTS
NEG 1,1  ;NEGATE VALUE
STA 1,NPTS  ;STORE VALUE
LDA 0,ADIDV  ;LOAD DEVICE CODE OF ADC1
LDA 1,ADDT  ;LOAD ADDRESS OF ADC1 DCT
*SYSTM
*IDEF  ;IDENTIFY ADC1 TO RDOS
HALT  ;ERROR RETURN
JSR 0,FRET  ;RETURN
*128M: 7777
CLOCK: 0
NPTS: 0

;ADRUN:
;FORMAT: CALL ADRUN(TRG)
;TRG= TRIGGER NUMBER(0 TO 3)

;ADRUN: JSR 0,CPYL  ;SAVE MACHINE STATE
LDA 0,-167,03  ;LOAD TRIG ARGUMENT
NIC 1,ADC1  ;MAKE SURE DEVICE IS CLEAR
LDA 0,SEVEN  ;LOAD MUX FOR WORD COUNT
DOB 0,ADC1  ;OUTPUT VALUE
LDA 0,NPTS  ;LOAD -NO- POINTS
DOC 0,ADC1  ;OUTPUT TO INTERFACE
LDA 0,SIX  ;LOAD MUX FOR ADDRESS
DOB 0,ADC1  ;OUTPUT VALUE
LDA 0,DATB  ;LOAD ADDRESS OF DATA BUFFER
DOC 0,ADC1  ;OUTPUT TO DMA
LDA 0,CHANA  ;LOAD MUX FOR CHANNEL
DOB 0,ADC1  ;OUTPUT MUX ADDRESS FOR AD MUX
LDA 0,CLOCK  ;LOAD CLOCK WORD
DOAS 0,ADC1  ;START ADC1
JSR 0,FRET  ;RETURN

;ADRED: JSR 0,CPYL  ;SAVE STATE
LDA 0,-167,03  ;LOAD POINT NO. TO BE READ
LDA 2,DATB  ;LOAD ADDRESS OF DATA
SUB2L 1,1  ;GENERATE A ONE
SUB 1,0  ;SUBTRACT FORM POINT NO.
;BECAUSE FORM. IS 1 TO N
ADD 0,2  ;ADD DISPLACEMENT TO DATA ADDRESS
LDA 1,0,2  ;GET VALUE
LDA 0,0,2  ;
ADD 0,1  ;
LDA 0, 7777
AND 0, 1
STA 1, -167+1, @3 ;RETURN VALUE TO FORTRAN
JSR @FRET ;RETURN
•RDX 10

DBIAS:
•RDX 8
•1B5: 1B5
•7777: 7777
BIAS: 4000
SEVEN: 160000
SIX: 140000
FIVE: 120000
•ADDT:
•ADIDT
AD1DV:
•SAV
ADIDT:
17777
;INTERUPT MASK FOR ADC1
AD1IT
;ADDRESS OF INTERRUPT HANDLER
AD1IT:
SUBRL 1, 1 ;GEN A 1
STA 1, CDONE ;STORE IN DONE INDICATER
NLCC ADC1 ;CLEAR ADC1
•UIEX ;INTERUPT RETURN
•SAV: •BLK 10
CDONE:
0
1
ADDNE:
JSR @CPYL ;SAVE STATE
LDA 1, CDONE ;LOAD DONE INDICATER
STA 1, -167, 03 ;SEND TO FORTRAN
JSR @FRET ;RETURN
•DATB:
DATAB ;ADDRESS OF DATA BUFFER
•DUSR ADC1=66 ;DEVICE CODE FOR ADC1
DATAB:
•END
BLK 2000 ;1000 WORDS OF DATA
to zero) or sequential (value not equal to zero) conversion. The second parameter is the first sequential channel or the random channel. The third parameter is only required for a sequential conversion and is the last channel to be converted. The analog multiplexor word to be output to the ADC interface is constructed. This word is then stored for use by ADRUN. ADSET is the next entry point. ADSET initially zeros the ADC interrupt done flag, CDONE. The decade divider constant and the clock multiplier are loaded from Fortran next. The clock multiplier is decremented by one (this counter also counts to zero not one) and made into a twelve bit word. The decade divider constant is placed in the correct three bits and added to the twelve bit multiplier to form the final clock value. The clock value is then stored. ADSET then obtains the number of points to be converted. The number of points is negated (the DMA word count register requires this since it counts up) and stored. The final step in ADSET is to identify the ADC interface to RDOS as a device capable of generating an interrupt.

ADRUN is the next entry point and actually starts the ADC interface. ADRUN loads the DGC output register with the correct multiplexor code to guide the next DOC to the word count register. ACO is loaded with the negative number of points and a DOC 0, ADC1 is executed. The word count register is now loaded. The multiplexor code for the memory address register is output and then the
address of the 1024 word data buffer (DATAB) is loaded into ACO and output to the memory address register. The analog channel word is now loaded and output via a final DOB 0, ADC1. The interface is now ready to operate. The final step is to load the clock word into ACO and output this word to the clock via a DOAS 0, ADC1. The interface is now running.

It should be noted that the maximum number of points which can be acquired with this program is 1024 (defined by DATAB). If a 2 channel sequential conversion is executed 512 points will be acquired from each channel.

ADRED is the next subprogram. ADRED first obtains an integer value which points to the value to be read from the AD1 1024 word data buffer. The data point is made into a twelve bit word with possible values between zero and 4095. This data point is then sent to Fortran.

The next entry point is ADDNE. ADDNE reads the value of the interrupt done flag (CDONE) and returns the value to Fortran. CDONE is zero if the ADC interrupt has not occurred and one if the interrupt has occurred. The ADC interrupt handler, AD1IT, receives program control when the ADC generates an interrupt. AD1IT sets CDONE equal to one and then executes the RDOS interrupt return (.UIEX).
This appendix describes several Fortran programs which were used in this study. The description will be on a level which requires a knowledge of DGC Fortran IV.\textsuperscript{2,3} DGC Fortran IV has several extensions to allow for file management, more effective use of the computer system, and real-time control. A knowledge of RDOS\textsuperscript{1} is also required. The Fortran programs include some comment statements and are somewhat self-explanatory. The programs which will be discussed include the titration control program (TITCONTROL), the titration plotting program (TITREAD), and the SEC signal averaging program (SECAV). The program used in rapid scan stopped flow and rapid scan SEC experiments will not be discussed since they are very similar to TITCONTROL in their procedure of operation.

TITCONTROL is the program used to control the RSS and electrochemical equipment used in the indirect coulometric titrations of bio-components. The TITCONTROL listing is presented as Program \#7. TITCONTROL requires the initial input via the teletype of all the information and the number of counts to be counted (i.e., number of millicoulombs of charge). Times required include magnetic stirrer start/stop times, delay time, and plotting time (plotting time determines the data acquisition time. Plotting time corresponds
Program 7

Program, TITCONTROL, used to control the automated indirect coulometric titrator.
DIMENSION NTIM1(3),NTIM2(3),NPOT(16)
DIMENSION NDATUM(1024),IFNAME(40)

1000 FORMAT (40S2)
IBLK=0
CALL PEN(0,500)
ND3LKS=4
NDBLK=1
I=1
NREG0=0
NC3DIV = 2
CALL SREG0(NREG0)
C
INITIALIZE TIMES AND COUNTER
TYPE"<15><15><15><15>TIMES ARE IN SECONDS.<15><15>
>1<15>"”
ACCEPT"STIRRER START UP TIME ",NSTART
ACCEPT"NUMBER OF COUNTS ",NCOUNT
ACCEPT"DELAY AFTER COUNTING ",NDAFTER
ACCEPT"STIRRER STOP TIME ",NSSTOP

C
CONVERT SECONDS TO MSEC
NSTART=NSTART*10
NDAFTER=NDAFTER*10
NSSTOP=NSSTOP*10
ACCEPT"PLOTTING TIME ",NPTIME
NPTIME=NPTIME*10

C
GET FILE SAVE AND PLOTTING INFO
ACCEPT"DO YOU WANT TO SAVE DATA? YES=1 ",NSAVE
ACCEPT"PLOT DATA AS TAKEN? YES=1 ",NPD
ACCEPT"TYPE POTENTIAL INFO AS TAKEN? YES=1 ",NPP

IF(NSAVE .NE. 1 ) GO TO 35
C
INPUT FILENAME
25
TYPE "FILENAME? 
READ (11,1900) IFNAME
CALL CFILW(IFNAME,2,IER)
IF(IER .EQ. 1 ) GO TO 30
IF(IER .NE. 12) GO TO 26
WRITE(10,1000) IFNAME
ACCEPT"FILE ALREADY EXISTS. DELETE? YES=1 ",NDEL
ETER
IF(NDELETE .NE. 1 ) CALL DELETE(IFNAME)
GO TO 25
26
TYPE"CFILE ERROR. IER = ",IER
STOP ABORT
30
CONTINUE
CALL OPEN(0,IFNAME,2,IER,512)
IF(IER .EQ. 1 ) GO TO 35
STOP ABORT AT OPEN
35
NDBLKS=4
PAUSE READY TO START
Continued

**Type**: ""

**Type**: ""

C **TURN MAG STIRRER ON**

C **SET BIT VIA FORTRAN**

CALL BSET(NREGO, 10)

CALL SREG0(NREG0)

C **WAIT FOR STIRRER TO COME TO SPEED**

CALL FDELY(NSTART)

NDONE = 0

C **TAKE POTENTIAL DATA**

CALL ADCLR

CALL ADCHAN(0, 1)

C **TAKE 16 POINTS AT 50 USEC EACH**

CALL ADSET(7, 51, 16)

CALL ADRUN(1)

C **CHECK FOR ADC DONE**

CALL ADDNEC(NDONE)

IF (NDONE .EQ. 0) GO TO 11

TPOT = 0

C **AVERAGE 16 POINTS**

DO 15 N = 1, 16

CALL ADRED(N, NPOT(N))

POT = NPOT(N) / 4096 - 5.0

TPOT = POT + TPOT

15 CONTINUE

C **CONVERT POTENTIAL TO VOLTAGE**

POT = TPOT / 16.0

POT = POT + ((POT + 118.0) / 100000.0)

NPOTENT = IFIX(POT * 1000)

C **TURN WORKING ELECTRODE ON**

CALL BSET(NREG0, 7)

CALL SREG0(NREG0)

C **READ TIME COUNTS STARTED**

CALL TIME(NTIM1, IER)

IF (IER .NE. 1) TYPE "CALL TIME ERR...IER=" , IER

CALL CKCLR

C **START COUNTER**

CALL SETCT(NCTDIV, NCOUNT)

16 CALL CKDNE(NDONE)

IF (NDONE .EQ. 0) GO TO 16

C **READ TIME COUNTS DONE**

CALL TIME(NTIM2, IER)

IF (IER .NE. 1) TYPE "CALL TIME ERR...IER=" , IER

C **WAIT FOR EQUILIBRATION THEN TURN**

C **STIRRER OFF**

CALL BCLR(NREG0, 7)

CALL SREG0(NREG0)

CALL FDELY(NDAFTER)
CALL BCLR(NREQ0, 10)
CALL SREG0(NREG0)
CALL FDELY(NSSTOP)
CALL BSET(NREG0, 5)
CALL SREG0(NREG0)
CALL SREG0(NREG0)
CALL SREG0(NREG0)
CALL BSET(NREG0, 5)

C TAKE SPECTRUM
CALL ADCLR
NDONE=0
CALL ADCHAN(0, 0)
CALL ADSET(5, NPTIME, 1000)
CALL TRIGA
CALL ADRUN(0)

17 CALL ADDNE(NDONE)
CALL FDELY(5)
IF(NDONE .EQ. 0) GO TO 17
NREQ0=0
CALL SREG0(NREG0)

C READ DATA AND PLOT IF DESIRED
20 NI = 1, 1000
CALL ADRED(NI, NDATUM(NI))
IF(NPD .NE. 1) GO TO 20
CALL PLOT(NDATUM(NI), 4*NI, NDEL)
CALL PEN(1, NDEL)
NDEL=50
CALL PEN(0, 500)

C TYPE INFO IF DESIRED
TYPE"LOOP #", I
TYPE"TIME FOR COUNTS = ", NDIF
TYPE"PO TENTIAL READING = ", POT

89 I=I+1
IF(NSAVE .NE. 1) GO TO 88

C WRITE ALL DATA TO DISK IF DESIRED
NDATUM(1001)=I-1
NDATUM(1002)=NDIF
NDATUM(1003)=-NPOTENT
NDATUM(1004)=NTIM1(1)
NDATUM(1005)=NTIM1(2)
NDATUM(1006)=NTIM1(3)
CALL WRBLK(0, NDBLK, NDATUM, NDBLKS, IER, IBLK)
IF (IER .NE. 1) TYPE "WRITE BLK ERROR IER = ", IER

IF (IBLK .NE. 0) TYPE "WRITE BLK ERROR IBLK = ", IBLK
NDBLK = NDBLKS + NDBLK
CALL RSWITCH (NSWITCH)

C CHECK FOR A -1 IN SWITCHES
C A -1 INDICATES TERMINATE RUN
88 IF (NSWITCH .EQ. -1) GO TO 99
GO TO 10
99 CONTINUE
IF (NSAVE .NE. 1) GO TO 999
NDATUM(1) = NCTDIV
NDATUM(2) = NCOUNT
NDATUM(3) = NSTART / 10
NDATUM(4) = NDAFTER / 10
NDATUM(5) = NSSTOP / 10
NDATUM(6) = NPTIME / 10
CALL DATECNTIM1*IER)
IF (IER .NE. 1) TYPE "DATE ERROR. IER = ", IER
NDATUM(7) = NTIM1(1)
NDATUM(8) = NTIM1(2)
NDATUM(9) = NTIM1(3)
NDATUM(10) = NTIM1(4)
CALL WRBLK (0, 0, NDATUM, 1, IER, IBLK)
IF (IER .NE. 1) TYPE "WRITE ERROR IER = ", IER
IF (IBLK .NE. 0) TYPE "WRITE ERROR BLK = ", IBLK
CALL CLOSE (0, IER)

C STOP. DONE
999 STOP TERMINATE
END
to the time required to plot the data on a x-y recorder (ca. 50 sec) and the data acquisition time is forced by the program to be the same). TITCONTROL then request some option information (i.e., save data, plot data, type potential). A disk file name is requested if the data is to be saved. The magnetic stirrer is started and the working electrode potential is digitized (16 potential values are acquired and averaged). The potentiostat auxiliary electrode relay is closed and the amount of charge injected into the cell solution is measured by the counter. The counter counts digital pulses produced by the V-F convertor. The frequency output of the V-F is proportional to the current passed through the cell. After the charge is injected the auxiliary electrode is disconnected and the solution is allowed to equilibrate. The magnetic stirrer is then turned off and a data spectrum is acquired. The digitized spectra is written to the disk file along with potential and time information. If at this time a value of minus one is present in the computer front panel switches the program terminates.

The final step in the program is to write the time and count information to the disk file. If the front panel switches are not -1 then the program starts a new cycle by starting the stirrer and acquiring new potential data. The amount of data which can be stored is limited by the disk space available.

The companion program to TITCONTROL is TITREAD. The TITREAD program allows the data stored on disk to be read and plotted in many different ways. TITREAD is a main supervisory program which
controls what type of plotting or data display is to be done.

TITREAD is shown as Program #8. TITREAD requests a data file name and opens this file for access to its contents. TITREAD then requests a parameter to indicate what type of data display is to be done. The actual data display is accomplished via the use of disk overlays. Overlays allow the many distinct plotting and display programs to be kept on disk and loaded when required by the main program. This procedure is more core memory efficient (uses less core) than if all the plotting programs were core resident at one time. TITCONTROL determines which overlay program is to be loaded, loads it, transfers program control to the overlay program, and then TITCONTROL waits for the overlay program to complete its operation.

The overlays used are: (1) TITA' (Program #9) plots any individual spectra in the data set. (2) TITB (Program #10) plots a series of spectra in the data set. (3) TITC (Program #11) plots any spectra in the data set and calls it a baseline spectrum. (4) TITD (Program #12) plots a series of spectra on a z axis projection (three dimensional). (5) TITE (Program #13) plots a series of baseline corrected spectra on a z axis projection. (6) TITF (Program #14) plots a series of baseline corrected spectra. (7) TITG (Program #15) plots absorbance at one wavelength versus charge increment for a series of spectra. (8) TITH (Program #16) plots measured potential versus charge increment. (9) TITI (Program #17) types a table of charge increment, potential, and time.
Program 8

Program, TITREAD, used as a main supervisory program for data display.
LINK OVERLAYS AND TASKS VIA EXTERNAL

PROVIDE DATA LINKUP VIA LABELED COMMON
EXTERNAL OVTSP, OVSPT, OVBLN, OVEAX, OVEAC, OVSPC, OVWV

EXTERNAL ISPEC, SPECT, BLINE, ZAXIS, ZAXCO, CSPEC, WAVS

EXTERNAL OVPCH, OVTCH, OVPNT, OVVAL, OVDFS, OVO, OVCPT

EXTERNAL PTCHG, TYCHG, INFO, POIN, DUWAL, DIFSP, BOXPT, CALPT

COMMON/KEYS/KEY1, KEY2, KEY3, KEY4, KEY5, KEY6, KEY7, KEY8, KEY9, KEY10,
KEY11, KEY12, KEY13, KEY14, KEY15, KEY16, KEY17, KEY18, KEY19, KEY20

COMMON/XINFO/NDBLKS, NWAVE, NDIFPNT

COMMON NDATA(1024), NBASE(1024)

COMMON IFNAME(40), NJNK(256)

FORMAT(40S2)
CALL RESET
NDBLKS = 4

OPEN OVERLAY FILE
C
CALL OVOPN(9, "TITREAD.OL", IER)
IF(IER .NE. 1) TYPE "TITREAD.OL OPEN ERROR. IER = ", IER

INPUT DATA SET FILE NAME
TYPE "DATA FILE NAME"
READ(11, 1000) IFNAME
CALL OPEN(1, IFNAME, 1, IER, 512)
IF(IER .NE. 1) GO TO 30
IF(IER .NE. 13) GO TO 40
TYPE "FILE DOES NOT EXIST"
CALL RESET
GO TO 10

TYPE "DATA FILE OPEN ERROR. IER = ", IER
STOP ABORT

CONTINUE
C
IF SWITCHES = 377 BYPASS FOLLOWING TABLE
CALL RSIVITCH(NSW)
IF(NSW .EQ. 377K) GO TO 50
TYPE "WHAT DO YOU WANT TO DO?"
TYPE "PLOT INDIVIDUAL SPECTRA(1)"
TYPE "PLOT SERIES OF SPECTRA(2)"
TYPE "PLOT A BASELINE(3)"
TYPE "PLOT A Z AXIS SERIES(4)"
TYPE "PLOT A Z AXIS BASELINE CORRECTED SERIES(5)"

TYPE "PLOT A BASELINE CORRECTED SERIES(6)"
TYPE "PLOT ABS. VS. CHARGE INCREMENT(7)"
TYPE "PLOT POTENTIAL VS CHARGE INCREMENT(8)"
TYPE "TYPE A TABLE OF CHARGE VS POTENTIAL(9)"
TYPE "TYPE FILE INFORMATION(10)"
TYPE "SELECT POINT FOR ABS. VS. CHARGE PLOT(11)"
TYPE "SELECT POINT FOR DIFFERENCE SPECTRA(12)"
TYPE "PLOT A SERIES OF DIFFERENCE SPECTRA(13)"
TYPE "PLOT A BOX(14)"
TYPE "CALIBRATE PLOTTER(15)"
TYPE "GO DRINK BEER(16)"
TYPE "NEW DATA FILE(17)"
TYPE "TAKE NEW DATA(18)"
TYPE "DONE(19)"
TYPE "TYPE WHAT DO YOU WANT TO DO TABLE(20)"
TYPE "<15><15><15>" INPUT NEXT PROGRAM
C
50
ACCEPT "NEXT?..", NEXT
TYPE "<15><15><15><15>" GO TO (101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111,
112, 113, 114, 115, 116, 117, 118, 119, 120, 121) NEXT

101
INDI = 1
C
LOAD CORRECT OVERLAY
C
CHECK FOR OVERLAY LOAD ERROR
CALL FOVL(9, OVISP, 0, IER)
IF (IER NE. 1) CALL OVERR(INDI, IER)
C
ACTIVATE OVERLAY PROG. TASK
C
CHECK FOR TASK ERROR
CALL ITASK(ISPEC, 1, 0, IER)
IF (IER NE. 1) CALL TKERR(INDI, IER)
C
WAIT FOR NONZERO MESSAGE FROM TASK
C
INDICATING COMPLETION OF TASK
CALL REC(KEY1, MSG1)
C
RELEASE OVERLAY
C
CHECK FOR ERROR
CALL FOVRL(OVISP, IER)
IF (IER NE. 1) CALL REERR(INDI, IER)
C
RETURN FOR NEXT PROGRAM
GO TO 50

102
INDI = 2
C
CALL FOVL(9, OVSPT, 0, IER)
IF (IER NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(SPEC, 2, 0, IER)
IF (IER NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY2, MSG2)
CALL FOVRL(OVSPT, IER)
IF (IER NE. 1) CALL REERR(INDI, IER)
GO TO 50

103
INDI = 3
C
CALL FOVL(9, OVLN, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(9LINE, 3, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY3, MSG3)
CALL FOVRL(OVBLN, IER)
IF (IER .NE. 1) CALL REERR(INDI, IER)
GO TO 50

104 INDI=4
CALL FOVLDC9(9, OVZAX, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(7AXIS, 4, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY4, MSG4)
CALL FOVRL(OVZAX, IER)
IF (IER .NE. 1) CALL REERR(INDI, IER)
GO TO 50

105 INDI=5
CALL FOVLDC9(9, OVEAC, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(ZAXCO, 5, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY5, MSG5)
CALL FOVRL(OVEAC, IER)
IF (IER .NE. 1) CALL REERR(INDI, IER)
GO TO 50

106 INDI=6
CALL FOVLDC9(9, OVSPC, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(CSPEC, 6, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY6, MSG6)
CALL FOVRL(OVSPC, IER)
IF (IER .NE. 1) CALL REERR(INDI, IER)
GO TO 50

107 INDI=7
CALL FOVLDC9(9, OVWVC, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(WAVSC, 7, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY7, MSG7)
CALL FOVRL(OVWVC, IER)
IF (IER .NE. 1) CALL REERR(INDI, IER)
GO TO 50

108 INDI=8
CALL FOVLDC9(9, OVPCH, 0, IER)
IF (IER .NE. 1) CALL OVERR(INDI, IER)
CALL ITASK(PTCHG, 8, 0, IER)
IF (IER .NE. 1) CALL TKERR(INDI, IER)
CALL REC(KEY8, MSG8)
CALL FOVRL(OWPCH,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

109 INDI=9
CALL FOVLD(9,OWTCH,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(TYCHG,9,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY9,MSG9)
CALL FOVRL(OWTCH,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

110 INDI=10
CALL FOVL(9,OWINF,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(INF0,10,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY10,MSG10)
CALL FOVRL(OWINF,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

111 INDI=11
CALL FOVL(9,OPTN,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(POINT,11,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY11,MSG11)
CALL FOVRL(OPTN,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

112 INDI=12
CALL FOVL(9,OWAL,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(DUAL,12,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY12,MSG12)
CALL FOVRL(OWAL,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

113 INDI=13
CALL FOVL(9,ODFNS,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(DIFSP,13,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY13,MSG13)
CALL FOVRL(ODFNS,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

114 INDI=14
CALL FOVLD(9,OVBOX,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(BOXPT,14,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY14,MSG14)
CALL FOVRL(OVBOX,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

INDI=15
CALL FOVLD(9,OVCP,T,0,IER)
IF(IER .NE. 1) CALL OVERR(INDI,IER)
CALL ITASK(CALPT,15,0,IER)
IF(IER .NE. 1) CALL TKERR(INDI,IER)
CALL REC(KEY15,MSG15)
CALL FOVRL(OVCP,IER)
IF(IER .NE. 1) CALL REERR(INDI,IER)
GO TO 50

115 CALL RESET
TYPE"GOOD BYE"
CALL RLSEC("DP0",IER)
IF(IER .NE. 1) TYPE "RELEASE ERROR. IER = ",IER
STOP

116 CALL RESET
GO TO 10

C RELOAD TITCONTROL FOR NEW DATA
117 CALL CHAIN("TITCONTROL.SV",IER)
IF(IER .NE. 1) TYPE "CHAIN ERROR. IER = ",IER
STOP

120 GO TO 30
119 CONTINUE
121 CONTINUE
END
Program 9

Program, TITA, used to plot any individual spectrum.
OVERLAY OVISP
TASK ISPEC
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
! KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MSG1=-1
C PLOT ANY SPECTRUM IN DATA SET
ACCEPT"PLOT WHICH SPECTRA? ",NSPECTRA
30 CALL TITR(4*(NSPECTRA-1)+1)
PAUSE PLOT DATA
CALL TITPLOT
ACCEPT"NEXT SPECTRA? DONE =-1 ",NSPECTRA
IF(NSPECTRA .EQ. -1) GO TO 99
GO TO 30
99 KEY1=0
C TRANSMIT NONZERO MESSAGE TO MAIN
CALL XMT(KEY1,MSG1,999)
999 END
Program 10

Program, TITB, used to plot a series of spectra.
C PLOT A SERIES OF SPECTRA
OVERLAY OVSPT
TASK SPECT
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MSG2=-2
10 ACCEPT"FIRST SPECTRA AND LAST SPECTRA TO BE PLOTTED? ",NFIRST,NLAST
ACCEPT"SPECTRA INTERVAL(1,2,5 ETC..)? ",NSTEP
15 PAUSE PLOT DATA
DO 20 I=NFIRST,NLAST,NSTEP
CALL TITR(4*(I-1)+1)
CALL TITPLOT
20 CONTINUE
ACCEPT"PLOT AGAIN? YES = 1 ",NPA
IF(NPA .EQ. 1) GO TO 15
ACCEPT"REPEAT PROCEDURE? YES =1 ",NA
IF(NA .EQ. 1) GO TO 10
KEY2=0
CALL XMT(KEY2,MSG2,$999)
999 END
Program 11

Program, TITC, used to plot any spectrum as a baseline spectrum.
C DETERMINE A SPECTRUM TO BE USED AS A BASELINE
OVERLAY OVBLN
TASK BLIN
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVE1,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MS33=-3

10 ACCEPT "WHICH SPECTRA FOR BASELINE? DONE=-1 "
   NBLINE
   IF(NBLINE .EQ. -1) GO TO 99
   CALL TITR(4*(NBLINE-1)+1)
   PAUSE PLOT BASELINE
   CALL TITPLOT
C PUT DATA IN BASELINE COMMON AREA
DO 20 I=1,1000
20 NBASE(I)=NDATA(I)
   GO TO 10
99 KEY3=0
   CALL XMT(KEY3,MSG3,$999)
999 END
Program 12

Program, TITD, used to plot a series of spectra on a Z axis projection.
PLOT A SERIES OF SPECTRA USING A Z AXIS PROJECTION

OVERLAY OVSAX

TASK RAXIS

COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20

COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT

COMMON NDATA(1024),NBASE(1024)

MSG4=-4

10 ACCEPT"FIRST AND LAST SPECTRA TO BE PLOTTED? "

,NFIRST,NLAST

ACCEPT"BIAS VALUE TO BE ADDED TO ABS.? "NBIAS

C X AND Y DISPLACEMENTS

ACCEPT"X & Y AXIS DISPLACEMENTS? ",NX,NY

ACCEPT"SPECTRA SCAN INTERVAL(1,2,5 ETC.)? ",NSTEP

15 NSCAN=0

CALL PEN(0,500)

PAUSE PLOT DATA

DO 23 I=NFIRST,NLAST,NSTEP

NEY=NSCAN*NY

AXIS=NSCAN*NX

NSCAN=NSCAN+1

NDEL=500

CALL TITR(4*(I-1)+1)

DO 30 N1=1,1000

NP01NT=ND4TA(N1)+N2Y+NBIAS

IAXIS=INT(AXIS)

CALL PLOT(NP01NT,IAXIS,NDEL)

CALL PEN(1,NDEL)

AXIS = AXIS + 4096.0/2000.0

30 NDEL=35

CALL PEN(0,500)

NDEL=500

ACCEPT"PLOT AGAIN? YES=1 ",NPA

IF(NPA .EQ. 1 ) GO TO 15

ACCEPT"REPEAT PROCEDURE? YES=1 ",NPA

IF(NPA .EQ. 1 ) GO TO 10

KEY4=0

CALL XMT(KEY4,MSG4,999)

999 END
Program 13

Program, TITE, used to plot a series of baseline corrected spectra on a Z axis projection.
C PLOT A SERIES OF BASELINE CORRECTED SPECTRA
C ON A 3 AXIS PROJECTION
OVERLAY OVKAC
TASK TATEAC
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/KEYS/NMBLKS,NMBLKS,NMBLKS,NNDIFPNT
COMMON NDATAM(1024),NBASEM(1024)
MSG5=-5
10 ACCEPT"FIRST AND LAST SPECTRA TO BE PLOTTED? ",NFIRST,NLAST
ACCEPT"BIAS VALUE TO BE ADDED TO ABS.? ",NBIAS
ACCEPT"X & Y AXIS DISPLACEMENTS? ",NX,NY
ACCEPT"SPECTRA SCAN INTERVAL (1, 2, 5 ETC.)? ",NSTEP
ACCEPT"USE PREVIOUSLY DETERMINED BASELINE? YES=1 ",NUPDB
IF(NUPDB .EQ. 1) GO TO 15
ACCEPT"WHICH SPECTRA FOR BASELINE",NBLINE
CALL TITE(4*(NBLINE-1)+1)
DO 16 I=1,1000
16 NBASEM(I)=NDATAM(I)
15 NSCAN=0
CALL PEN(0,500)
PAUSE PLOT DATA
DO 20 I=NFIRST,NLAST,NSTEP
NEY=NSCAN+NY
AXIX=NSCAN+NX
NSCAN=NSCAN+1
NDEL=500
CALL TITE(4*(I-1)+1)
DO 30 NI=1,1000
NPOIN=NDATAM(NI)+NEY+NBIAS
AXIX=NAXI
CALL PLOT(NPOIN,AXIX,NDEL)
CALL PEN(1,NDEL)
AXIX = AXIX+4096.0/(2.0*1000.0)
30 NDEL=35
CALL PEN(0,500)
20 NDEL=500
ACCEPT"PLOT AGAIN? YES=1 ",NPA
IF(NPA .EQ. 1) GO TO 15
ACCEPT"REPEAT PROCEDURE? YES=1 ",NPA
IF(NPA .EQ. 1) GO TO 10
KEYS=0
CALL XMT(KEY5,MSG5,S999)
999 END
Program 14

Program, TITF, used to plot a series of baseline corrected spectra.
C PLOT A SERIES OF BASELINE CORRECTED SPECTRA
OVERLAY OVSPC
TASK CSPEC
COMMON/KEYS/KEY1, KEY2, KEY3, KEY4, KEY5, KEY6, KEY7, KEY8, KEY9, KEY10, KEY11, KEY12, KEY13, KEY14, KEY15, KEY16, KEY17, KEY18, KEY19, KEY20
COMMON/XINFO/NNDLSKS, NWAVEL, NNDIFPNT
COMMON NDATA(1024), NBASE(1024)
MSG6=-6
10 ACCEPT "FIRST AND LAST SPECTRA TO BE PLOTTED? " , NFIRST, NLAST
ACCEPT "SPECTRA INTERVAL (1, 2, 5 ETC.)? ", NSTEP
ACCEPT "USE PREVIOUSLY DETERMINED BASELINE YES = 1? ", NUPDB
IF(NUPDB .EQ. 1) GO TO 20
ACCEPT "WHICH SPECTRA FOR NEW BASELINE? ", NBLINE
CALL IITRC4*(NBLINE-1)+1)
DO 30 I=1, 1000
20 ACCEPT "SCALE DATA? YES = 1 " , NSCALE
IF(NSCALE .EQ. 1) GO TO 40
SCALE=1.0
GO TO 50
C INCREASE PLOTTING SIZE WITH A MULTIPLIER
40 ACCEPT "SCALE MULTIPLIER ", SCALE
50 ACCEPT "BIAS FOR ABSORBANCE? ", NBIAS
PAUSE PLOT DATA
DO 50 NJ=NFIRST, NLAST, NSTEP
CALL IITRC4*(NJ-1)+1)
AXIS=1
NDEL=500
DO 70 I=1, 1000
POINT=(NDATA(I)-NBASE(I)+NBIAS)*SCALE
NPOINT=INT(POINT)
IAXIS=INT(IAXIS)
CALL PLOT(NPOINT, IAXIS, NDEL)
CALL PEN(1, NDEL)
AXIS = AXIS+4096.0/1000.0
NDEL=50
CALL PEN(0, 500)
NDEL=500
CONTINUE
CALL IITRC4*(NJ-1)+1)
AXIS=1
NDEL=500
DO 70 I=1, 1000
POINT=(NDATA(I)-NBASE(I)+NBIAS)*SCALE
NPOINT=INT(POINT)
IAXIS=INT(IAXIS)
CALL PLOT(NPOINT, IAXIS, NDEL)
CALL PEN(1, NDEL)
AXIS = AXIS+4096.0/1000.0
NDEL=50
CALL PEN(0, 500)
NDEL=500
CONTINUE
ACCEPT "PLOT AGAIN? YES = 1 " , NPA
IF(NPA .EQ. 1) GO TO 55
ACCEPT "REPEAT PROCEDURE? YES = 1 " , NPA
IF(NPA .EQ. 1) GO TO 10
KEY6=0
CALL XMT(KEY6, MSG6, $999)
999  END
Program 15

Program, TITG, used to produce an absorbance versus charge plot.
PLOT ABSORBANCE AT A WAVELENGTH VERSUS CHARGE INJECTION NUMBER

OVERLAY OVWVC

TASK WAVSC

COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10

COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT

COMMON NDATA(1024),NBASE(1024)

MSG7=-7

ACCEPT "FIRST AND LAST SPECTRA TO BE PLOTTED? ", NFIRST, NLAST

ACCEPT "POINT IN EACH SCAN TO BE PLOTTED? ", NPNT

ACCEPT "SCALE FACTOR AND ABSORBANCE BIAS? ", SCALE, NBIAS

AXIS=0

PAUSE PLOT DATA

DO 20 I=NFIRST,NLAST

CALL TITR(C*(I-1)+1)

NPOINT=IFIX((NDATA(NPNT)+NBIAS)*SCALE)

NAXIS=INT(Axis)

CALL PLOT(NPOINT,NAXIS,500)

CALL PEN(1,500)

CALL PEN(0,500)

AXIS=AXIS+4095.0/(NLAST-NFIRST)

CONTINUE

ACCEPT "PLOT AGAIN? YES=1 ", NPA

IF (NPA .EQ. 1) GO TO 15

ACCEPT "REPEAT PROCEDURE? YES=1 ", NPA

IF (NPA .EQ. 1) GO TO 10

KEY7=0

CALL XMTCKEY7,MSG7,999)

999 END
Program 16

Program, TITH, used to produce potential versus charge plots.
C PLOT POTENTIAL VERSUS CHARGE INCREMENT
C FOR A SERIES OF CHARGE INCREMENTS
OVERLAY OVPCH
TASK PTCHG
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10
COMMON/XINFO/NDBLKS
COMMON NDATA(1024),NBASE(1024)
DIMENSION NCHAR(256)
MSG8=-8
10 ACCEPT"PLOT CHARGE VS POT. FOR SPEC # TO SPEC # ? ",NFIRST,NLAST
IBLK=0
ACCEPT"SCALE MULTIPLIER AND POTENTIAL BIAS? ",SCALE,NBIAS
PAUSE READY
AXIS=0
DO 20 I=NFIRST,NLAST
CALL RD3LKO(I,4*1,NCHAR(I),1,IER,IBLK)
IF(IER .NE. 1)TYPE "READ ERROR IER = ",IER
IF(IBLK .NE. 0) TYPE"READ ERROR IBLK = ",IBLK
POINT=(NCHAR(235)-NBIAS)+SCALE/10
IAXIS=INT(AXIS)
CALL PLOT(INT(POINT)+2048,IAXIS,500)
CALL PEN(1,500)
CALL PEN(0,500)
AXIS=AXIS+4996.0/(NLAST-NFIRST+1)
20 CONTINUE
ACCEPT"DO AGAIN? YES = 1 ",NPA
IF(NPA .EQ.1) GO TO 10
KEY8=0
CALL XMT(KEY8,MSG8,$999)
999 END
Program 17

Program, TITI, used to type a table of charge increment, potential, and time.
TYPE A TABLE OF CHARGE INCREMENT,
COUNTING TIME, POTENTIAL, AND TIME OF DAY
SPECTRA WERE TAKEN
OVERLAY OVTCH
TASK TYCHG
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10
COMMON/XINFO/NDBLKS
COMMON NDATA(1024),NBASEC(1024)
DIMENSION NCHAR(256)
MS9=-9

ACCEPT"POTENTIAL INFO FOR SPEC# TO SPEC #? ",NFIRST,NLAST

IBLK=0
PAUSE READY
TYPE"<15><15><15><15><15>>"
WRITE (10,1000)
DO 20 I=NFIRST,NLAST
CALL RDBLKC1,4*I,NCHARC1),1,IER,IBLK)
IF (IER .NE. 1) TYPE"READ ERROR. IER = ",IER
IF (IBLK .NE. 0) TYPE"READ ERROR. IBLK = ",IBLK
WRITE (10,1001) (NCHAR(NI),NI=233,238)
CONTINUE

TYPE"<15><15><15><15><15>>"
ACCEPT"AGAIN? YES=1 ",NPA
IF (NPA .EQ. 1) GO TO 10
KEY9=0
CALL XMTCKEY9,MSG9,S999)

FORMAT(3X,"SPECTRA NO.",3X,"COUNT TIME",3X,"POTENTIAL(.1MV)",
! 3X,"TIME COUNTS TAKEN")
FORMAT(5X,13,12X,13,9X,15,15X,12,":",12,"":"",12)
END
(10) TITU (Program #18) types a table of the time and count parameters input to TITCONTROL. (11) TITK (Program #19) determines a point (wavelength) for an absorbance versus charge plot. (12) TITL (Program #20) determines a wavelength for use in dual wavelength plots. (13) TITM (Program #21) plots a series of baseline corrected, dual wavelength spectra. Dual wavelength means one wavelength used as a reference wavelength. The absorbance at the reference wavelength is forced to be constant. (14) TITN (Program #22) plots a box around plotted data. And (15) TITO (Program #23) is used to calibrate the computer plotter. All of the programs described above (TITA through TITO) are contained in the TITREAD overlay file.

Several Fortran subroutines were also used. These are 1. TITPLOT (Program #24) plots a series of data points. 2. OVERR, TKERR, and REERR (all listed in Program #25) are used if a RDOS system error occurred. 3. TITX (Program #26) initializes the labeled common variables. And 4. TITR (Program #27) which reads the data from the disk.

The relocatable loader commands (RLDR) for use after all programs are Fortran compiled are given in Program #28.

The program used to acquire the electrochemically generated intermediate spectrum is called SECAV and is shown in Program #29. SECAV initially requests some time information (scan time, delay time) and number of averages. Note that SECAV employs the high-speed interface and the ADC calls employ a different format. The ADC parameters are then initialized. A keyboard interrupt program (KBI, Program #30) is then initialized. KBI allows a keyboard
Program 18

Program, TITJ, used to type time and count parameters.
C TYPE A TABLE OF EXPERIMENTAL
C PARAMETERS INPUT TO TITCONTROL
C FOR DATA SET
OVERLAY OVINF
TASK INFO
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
COMMON IFNAME(40),NJUNK(256)
MSG10=-10
TYPE"<15><15><15><15><15>
PAUSE READY
IBLK=0
WRITE (10,1000) IFNAME
CALL RDBLK(I,0,NJUNK(I),1,IER,IBLK)
IF(IER .NE. 1) TYPE "READ ERROR. IER = ",IER
IF(IBLK .NE. 0) TYPE "READ ERROR. IBLK = ",IBLK
WRITE (10,1001) (NJUNK(I),I=7,9)
TYPE"COUNTER DIVIDER AND NUMBER OF COUNTS = ",NJUNK(1),NJUNK(2)
TYPE"STIRRER START TIME = ",NJUNK(3)
TYPE"DELAY AFTER COUNTS = ",NJUNK(4)
TYPE"STIRRER STOP TIME = ",NJUNK(5)
TYPE"PLOTTING/DATA TIME = ",NJUNK(6)
TYPE"TOTAL NUMBER OF SPECTRA TAKEN = ",NJUNK(10)

TYPE"<15><15><15><15><15>
KEY10=0
CALL XMTCKEY10,MSG10,$999)
1000 FORMAT(1X,"DATA FOR FILE, FILENAME = ",1X,40S 2)
1001 FORMAT(1X,"DATE OF EXPERIMENT ",5X,I2,"/",I2,
"/",I2)
999 END
Program 19

Program, TITK, used to determine a point for an absorbance versus charge plot.
C DETERMINE A WAVELENGTH IN A SPECTRUM
C FOR THE ABSORBANCE VERSUS CHARGE PLOT
OVERLAY OVPNT
TASK POINT
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
! KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,K
KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MSG11=-11
Iblk=0
ACCEPT""WHICH SPECTRA DO YOU WANT DISPLAYED? ",NSPECT
CALL TITR(4*(NSPECT-1)+1)
CALL TITPLOT
20 ACCEPT""WHICH POINT? DONE=-1 ",N
IF(N .EQ. -1) GO TO 99
NWAVEL=N
CALL PLOT(NDATA(NWAVEL),INT(4.996*NWAVEL),1)
GO TO 99
KEY11=0
99 CALL XMT(KEY11,MSG11,$999)
999 END
Program 20

Program, TITL, used to determine a wavelength for use in dual wavelength plots.
DETERMINE A WAVELENGTH IN A SPECTRUM FOR USE IN A DUAL WAVELENGTH PLOT OVERLAY OVAL

COMMON/KEYS/KEY1, KEY2, KEY3, KEY4, KEY5, KEY6, KEY7, KEY8, KEY9, KEY10,
KEY11, KEY12, KEY13, KEY14, KEY15, KEY16, KEY17, KEY18, KEY19, KEY20
COMMON/XINFO/NDRLKS, NWAVEL, NDIFPNT
COMMON NDATA(1024), NBASE(1024)
MSG12=-12
IBLK=0
ACCEPT"WHICH SPECTRA DO YOU WANT DISPLAYED? ", NSPEC
CALL TITR(4*(NSPEC-1)+1)
CALL TITPLOT
20 ACCEPT"WHICH POINT? DONE=-1 ", N
IF(N .EQ. -1) GO TO 99
NDIFPNT=N
CALL PLOT(NDATA(NDIFPNT), INT(4.096*NDIFPNT),1)
GO TO 20
KEY12=0
99 CALL XMT(KEY12, MSG12, $999)
999 END
Program 21

Program, TITM, used to plot a series of baseline corrected, dual wavelength spectra.
C PLOT A SERIES OF DUAL WAVELENGTH DIFFERENCE
C SPECTRA
OVERLAY ODFS
TASK DIFSP
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
! KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MSG13=-13
IBLK=0
10 ACCEPT"PLOT SPECTRA # TO # ",NFIRST,NLAST
ACCEPT"USE PREVIOUSLY DETERMINED REFERENCE WAVELENGTH ENGIH? YES = 1 ",NPA
IF(NPA .NE. 1) ACCEPT"INPUT POINT NO. (1 TO 1000)? ",NDIFPNT
ACCEPT"USE PREVIOUSLY DETERMINED BASELINE? YES = 1 ",NPA
IF(NPA .NE. 1) GO TO 20
ACCEPT"USE WHICH SPECTRA FOR BASELINE? ",NBLINE
CALL TITR(4*(NBLINE-1)+1)
DO 30 I=1,1000
30 NBASE(I)=NDATA(I)
CONTINUE
20 ACCEPT"SCALE FACTOR AND BIAS FOR ABSORBANCE? ",SCALE,NBIAS
CALL PENC0,53)
PAUSE READY
NDEL=500
DO 50 I=NFIRST,NLAST
CALL TITR(4*(I-1)+1)
AXIS=0
DO 60 NI=1,1000
NP0INT=NDATA(I)-NDATA(NDIFPNT)-(NBASE(I)-NBASE(NDIFPNT))
NP0INT=IFIX((NP0INT-NBIAS)*SCALE)
IAXIS=INT(AXIS)
CALL PLOT(NP0INT,IAXIS,NDEL)
AXIS=AXIS+4996.9/1000.0
CALL PENC1,NDEL)
60 NDEL=35
CALL PEN0(0,500)
NDEL=500
CONTINUE
50 ACCEPT"PLOT AGAIN? YES = 1 ",NPA
IF(NPA .NE. 1) GO TO 40
ACCEPT"REPEAT PROCEEDURE? YES = 1 ",NPA
IF(NPA .NE. 1) GO TO 10
KEY13=0
CALL XMT(KEY13, MSG13, 999)
999 END
Program 22

Program, TITN, used to plot a box around the data.
C PLOT A BOX AND TICKS ON BOX
OVERLAY OVOX
TASK BOXPT
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20
COMMON/XINFO/NDELKS,NXAVEL,NDIFPNT
COMMON NDATA(1024),NBASE(1024)
MS314=-14
10 ACCEPT"LOWER LEFT X & Y COORDINATES ",NLX,NLY
ACCEPT"UPPER RIGHT X & Y COORDINATES ",NUX,NUY
CALL PEN(0,300)
CALL PLOT(NLY,NLX,1)
15 PAUSE READY
CALL PEN(1,500)
DO 20 I=NLX,NUX
20 CALL PLOT(NLY,I,15)
CALL FDELY(3)
DO 30 I=NLY,NUY
30 CALL PLOT(I,NUX,15)
CALL FDELY(3)
NN=NUX-NLX
DO 40 I=1,NN
40 CALL PLOT(NUY,NUX-I,15)
NN=NUY-NLY
CALL FDELY(3)
DO 50 I=1,NN
50 CALL PLOT(NUY-I,NLX,15)
CALL FDELY(3)
CALL PEN(0,200)
ACCEPT"PLOT AGAIN? YES = 1 ",NPA
IF(NPA .EQ. 1) GO TO 15
ACCEPT"TICKS ON BOX? YES=1 ",NPA
IF(NPA .NE. 1) GO TO 99
ACCEPT"NO OF TICKS ON X AND Y AXIS? ",NXTICKS,NYTICKS
ACCEPT"SIZE OF X AND Y TICKS(1 TO 5) ",NXSIZE,NYSIZE
NXSIZE=NXSIZE*100
NYSIZE=NYSIZE*100
NX=(NUX-NLX)/NXTICKS
NY=(NUY-NLY)/NYTICKS
NX1=NXTICKS-1
NY1=NYTICKS-1
NX11=NUY-NXSIZE
NY11=NLX+NYSIZE
NX12=NLY+NXSIZE
NY12=NUY-NYSIZE
PAUSE READY
NXAXIS=NX+NLX
DO 60 I=1,NXI
NDEL=400
CALL PLOT(NLY,NXAXIS,NDEL)
DO 70 II=NLY,NXI2
CALL PLOT(II,NXAXIS,NDEL)
CALL PEN(1,NDEL)
70 NDEL=00
NXAXIS=NXAXIS+NX
CALL PEN(0,500)
NYAXIS=NY+NLY
DO 80 I=1,NYI
NDEL=400
CALL PLOT(NYAXIS,NUY,NYAXIS*500)
DO 90 II=NYI2,NUX
CALL PLOT(NYAXIS,II,NDEL)
CALL PEN(1,NDEL)
80 NDEL=00
NYAXIS=NYAXIS+NY
CALL PEN(0,500)
NXAXIS=NUX-NX
DO 100 I=1,NXI
NDEL=400
CALL PLOT(NUY-NX,HAXIS,NXAXIS,NDEL)
DO 110 II=NXI2,NUX
CALL PLOT(NYAXIS,II,NDEL)
CALL PEN(1,NDEL)
100 NDEL=00
NXAXIS=NXAXIS-NX
CALL PEN(0,500)
NYAXIS=NUY-NY
DO 120 I=1,NYI
NDEL=400
CALL PLOT(NYAXIS,NLY,NDEL)
DO 130 II=NLX,NYI1
CALL PLOT(NYAXIS,II,NDEL)
CALL PEN(1,NDEL)
130 NDEL=00
NYAXIS=NYAXIS-NY
CALL PEN(0,500)
99 ACCEPT"REPEAT PROCEEDURE? YES=1 "*NPA
IF(NPA .EQ. 1 ) GO TO 10
KEY14=0
CALL XMT(KEY14,MSG14,$999)
999 END
Program 23

Program, TITO, used to calibrate the computer plotter.
CALIBRATE PLOTTER
OVERLAY OVCPT
TASK CALPT

COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,KEY18,KEY19,KEY20

COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON NDATA(1024),NRASE(1024)
MSG15=-15

ACCEPT "X & Y VALUES? DONE IF X OR Y =-1",NX,NY

IF(NY .EQ. -1) GO TO 99
IF(NX .EQ. -1) GO TO 99
CALL PEN(0,100)
CALL PLOT(NY,NX,1)
GO TO 10

99 KEY15=0
CALL XMT(KEY15,MSG15,$999)

999 END
Program 24

Program, TITPLOT, subroutine used to plot a series of data plots.
SUBROUTINE TITPLOT
COMMON NDATA(1024)
CALL PEN(0,500)
NDEL=500
C SUBROUTINE PLOIS A SPECTRUM
AXIS = 0.0
IAXIS=0
DO 10 I=1,1000
CALL PLOT(NDATA(I),IAXIS,NDEL)
AXIS=AXIS+4096.0/1024.0
IAXIS=INT(AXIS)
CALL PEN(1,NDEL)
10 NDEL=50
CALL PEN(0,500)
RETURN
END
Program 25

Programs, OVERR, TKERR, and REERR, used in case an RDOS system error occurred.
SUBROUTINE OVERR(INDI, IER)
C OVERLAY ERROR SUBROUTINE
TYPE "OVERLAY LOAD ERROR AT OV. # ",INDI
TYPE "ERROR # = ",IER
STOP ABORT
END

SUBROUTINE TKERR(INDI, IER)
C TASK ERROR SUBROUTINE
TYPE "TASK ERROR AT TASK # ",INDI
TYPE "ERROR # = ",IER
STOP ABORT
END

SUBROUTINE REERR(INDI, IER)
C OVERLAY RELEASE ERROR SUBROUTINE
TYPE "OVERLAY RELEASE ERROR. OV. # = ",INDI
TYPE "ERROR # = ",IER
STOP PROGRAM ABORTED
END
Program 26

Program, TITX, used to initialize labeled common variables.
BLOCK DATA

C BLOCK DATA INITIALIZES ALL LABELED COMMON
COMMON/KEYS/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,KEY10,
!    KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,K
    KEY18,KEY19,KEY20
COMMON/XINFO/NDBLKS,NWAVEL,NDIFPNT
COMMON/NDATA(1024),NBASE(1024)
COMMON/FILENAME(40),NJUNK(256)
DATA/NDBLKS,NWAVEL,NDIFPNT/4,0,0/
DATA/KEY1,KEY2,KEY3,KEY4,KEY5,KEY6,KEY7,KEY8,KEY9,
!    KEY10,
    KEY11,KEY12,KEY13,KEY14,KEY15,KEY16,KEY17,K
    KEY18,KEY19,KEY20/20*0/

END
Program 27

Program, TITR, used to read data from a disk file.
SUBROUTINE TITRCNDBLK
COMMON/XINFO/NDBLKS
COMMON NDATA(1024)
IBLK=0

C SUBROUTINE READS A SPECTRA FROM DATA SET
CALL RDBLK(1,NDBLK,NDATA(1),NDBLKS,IER,IBLK)
IF(IER .NE. 1) TYPE "READ ERROR SUB. READD IER = ",IER
IF(IBLK .NE. 0) TYPE "BLOCK ERROR IN READD IBLK = ",IBLK
RETURN
END
Program 28

Relocatable loader commands for .TITCONTROL and .TITREAD.
TITRLDRS

RLDR TITCONTROL REGS TRIGS ADI 
COUNTER RSWITCH PEN PLOT FORT.LB

RLDR TITREAD TITR TITPLOT RSWITCH 
PEN PLOT OVERR TKERR REERR 
TIX TITB, TITC, TITD, TITE, 
TITF, TITG, TITH, TITI, TITJ, 
TITK, TITL, TITM, TITN, TITO, 
FMT.LB FORT.LB 10/C 10/K
Program 29

Program, SEC AV, used to acquire the electrochemically generated intermediate spectrum.
SECAV

C PROGRAM ACQUIRES SEC SPECTRA FROM RSS
EXTERNAL KBI
COMMON KFLAGG
DIMENSION DAT(1000), BASE(1000)
CALL PEN(0,500)
1 ACCEPT "SCAN TIME IN MSEC ", NSCANTIME
ACCEPT "TIME BETWEEN START OF POT STEP AND START OF SCAN(1MSEC) ", NDTPS
ACCEPT "APPROXIMATE DELAY BETWEEN STEPS(IN 100MSEC) ", NDELAY
ACCEPT "NUMBER OF AVERAGES ", NSCAN
KFLAGG=0
NSCANS=0
C SET TIMES FOR FAST INTERFACE
CALL AD(1,6,NDTPS*10)
CALL AD(2,7,NSCANTIME*1000)
C ZERO AVERAGING ARRAYS
DO 10 I=1,1300
DAT(I)=0
10 BASE(I)=0
400 CONTINUE
PAUSE READY, STRIKE ANY KEY TO CONTINUE
C ACTIVATE KEY BOARD INTERRUPT TASK
CALL ITASKCKBI, 2, 0, IER)
IF (IER .NE. 1) TYPE "KBI TASK ERR...IER= ", IER
DO 20 J=1, NSCANS
C TRIGGER RSS, ACQUIRE BASELINE SPECTRUM
C AND ADD TO OLD DATA
CALL AD(6)
CALL AD(4,1,2)
DO 30 I=1,1000
CALL AD(7, I, NDATA)
30 BASE(I)=BASE(I)+NDATA
C TRIGGER POTSTAT AND THEN ACQUIRE
C DATA SPECTRUM
C THEN ADD TO OLD DATA
CALL AD(6)
CALL TRIGA
CALL AD(4,1,3)
DO 40 I=1,1000
CALL AD(7, I, NDATA)
40 DAT(I)=DAT(I)+NDATA
NSCANS=NSCANS+1
C CHECK FOR KEYBOARD INTERRUPT
IF (KFLAGG .NE. 0) GO TO 50
CALL FDELY(NDELAY)
20 CONTINUE
CALL ABORT(2, IER)
IF (IER .NE. 1) TYPE "KBI ABORT ERROR IER = ", IER
GO TO 60
CONTINUE

50 TYPE "NO. OF COMPLETED SCANS= ", NSCANS
CONTINUE

C DIVIDE ARRAYS BY NUMBER OF
C COMPLETED SCANS
DO 70 I = 1, 1000
DAT(I) = DAT(I) / NSCANS
BASE(I) = BASE(I) / NSCANS

70 C PLOT DATA
ACCEPT "PLOT BASELINE(1), DATA(2) OR DATA-BASELINE(3) ", NPLOT
GO TO (100, 101, 102) NPLOT

100 PAUSE PLOT BASELINE
NDEL = 500
DO 110 I = 1, 1000
CALL PLOT (IFIX (BASE(I)), 4*I, NDDEL)
CALL PENC(1, NDDEL)

110 NDDEL = 60
CALL PENC(0, 500)
GO TO 200

101 PAUSE PLOT DATA
NDDEL = 500
DO 120 I = 1, 1000
CALL PLOT (IFIX (DAT(I)), 4*I, NDDEL)
CALL PENC(1, NDDEL)

120 NDDEL = 60
CALL PENC(0, 500)
GO TO 200

102 ACCEPT "SCALE MULTIPLIER ", SCALE
ACCEPT "RECORDER BIAS? ", BIAS

125 PAUSE PLOT CORRECTED DATA
DO 130 I = 1, 1000
POINT = (DAT(I) - BASE(I) - BIAS) * SCALE
NPOINT = IFIX (POINT)
CALL PLOT (NPOINT, 4*I, NDDEL)
CALL PENC(1, NDDEL)

130 NDDEL = 50
ACCEPT "PLOT AGAINST(1), ENTER NEW SCALING AND REPELO
T(2) CONT(3) ", ND
GO TO (125, 102, 200) ND

200 TYPE "REPEAT CURVE(1) CONTINUE AVERAGE(2) NEW A
VERAGE(3)
ACCEPT "OR TERMINATE(4) ", NEXT
IF (NEXT .EQ. 1) GO TO (100, 101, 102) NPLOT
GO TO (999, 300, 1, 999) NEXT

300 CONTINUE
DO 310 I=1,1000
DAT(I)=DAT(I)*NSCANS
310 BASE(I)=BASE(I)*NSCANS
ACCEPT'NUMBER OF ADDITIONAL SCANS ',NSCAN
GO TO 400
999 STOP
END
Program 30

Program, KBI, used to handle teletype keyboard interrupts.
TASK KBI
COMMON KFLAGG
READ(11, 130) KFLAGG

130 FORMAT(I4)
END
interrupt (striking a teletype key) to stop the signal averaging process. SECAV then triggers the RSS for a baseline scan and upon completion of the baseline scan the new baseline data is averaged into the baseline averaging array. The next step is to trigger the potentiostat and delay several milliseconds and then trigger the RSS and take the data spectrum. The new data spectrum is read and added to the data averaging array. KBI is checked for an interrupt. If a keyboard interrupt has occurred then the baseline and data averaging arrays are divided by the number of completed scans and the resulting data can be plotted. If a keyboard interrupt has not occurred the acquisition cycle continues until a keyboard interrupt does occur of a preset number acquisition cycles is complete. After plotting the acquisition cycle can be restarted or a completely new acquisition can be done.
LIST OF REFERENCES


