THE DESIGN AND CONSTRUCTION OF A MICROPROCESSOR-CONTROLLED ELECTROPHORESIS POWER SUPPLY

by

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ABSTRACT

THE DESIGN AND CONSTRUCTION OF A MICROPROCESSOR-CONTROLLED ELECTROPHORESIS POWER SUPPLY

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This study deals with the design, construction, and application of a microprocessor-controlled power supply and the use of an electrophoresis/electrochemical cell as a detector of serum proteins during agarose gel electrophoresis.

The LNW 80 microcomputer was assembled as outlined in the LNW 80 Technical Reference Manual. The various electronic components were placed on the front side of the Main Computer Printed Circuit Board and soldered on the back side of the board. The same procedure was followed for the Expansion Interface Printed Circuit Board.

The electrophoresis/electrochemical detector cell was designed and constructed.

A cyclic voltammetry study was conducted on the cell containing 0.001 M potassium ferriccyanide. A platinum electrode was used as the working electrode and as the auxillary electrode. A Ag/AgCl electrode was used as the reference electrode. A similar study was carried out on a DNP-albumin (albumin-FDNB) complex using the same

electrode system. These studies illustrate the oxidation-reduction reaction of the electrophoresis/electrochemical detector cell.

Finally, a study using a microprocessor-controlled power supply together with an electrophoresis/electrochemical detector cell as a detector of serum proteins, during agarose gel electrophoresis, was conducted. This study illustrated that serum proteins, if made electrochemically active, could be detected using this method.

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LIST OF ABBREVIATIONS

ABBREVIATIONS	DEFINITION
A	amperes
ADC	analog-to-digital converter
Ag/AgCl	silver/silver chloride electrode
amp	amplifier
$cm^2/v\text{-}sec$	centimeters squared/volt-second
CPU	Central Processing Unit
d	width of the electrophoretic medium
DAC	digital-to-analog converter
d.c.	direct current
E	applied potential
E/cm	unit field strength
F	force exerted on an ion
F'	the counter force
FDNB	1-Fluoro-2,4-dinitrobenzene
I	current
IR	internal resistance
Kῼ	kiloohm
LDH	Lactate dehydrogenase
LSI	Large-Scale Integration
mA	milliamperes
mA/v	milliampere/volt
μ	electrophoretic mobility
μf	microfarad
η	viscosity of the buffer solution

op-amp	operational amplifier
pI	isoelectric point
Q	the net charge on the ion
r	the ionic radius of the solute
R	resistance
RAM	random-access memory
ROM	read-only memory
SCE	saturated calomel electrode
α_1	alpha one — serum protein fraction
α_2	alpha two — serum protein fraction
β	beta — serum protein fraction
γ	gamma — serum protein fraction
t	time
v,V	volts
v/cm	volts/centimeter
v	velocity
v/X	rate of migration per unit field strength
W	watts
X	the current field strength

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Electrophoresis

Electrophoresis refers to the migration of charged solutes or particles in an electric field. While the expression electrophoresis applies to the migration of all species, iontophoresis specifically refers to the migration of small ions.[1]

The first electrophoretic method used in the study of proteins was the free solution or moving boundary method described by Tiselius in 1937. This technique is still used by many scientists for the measurement of electrophoretic mobility and the study of protein-protein interaction, but rarely by clinical chemists in routine work. A complex apparatus is needed, the technique is difficult, and samples of the order of 0.5 mL of serum are required. Results similar to those obtained with moving-boundary electrophoresis can be obtained by gel electrophoresis. This technique requires only a small sample size and a simple apparatus.[2] A representative electrophoresis system is shown in Figure 1.

The term electrochromatography is used to refer to the migration of charged macromolecules within the confines of a nonconvective (or solid) supporting medium (e.g., paper, cellulose acetate, agar gel, etc.).

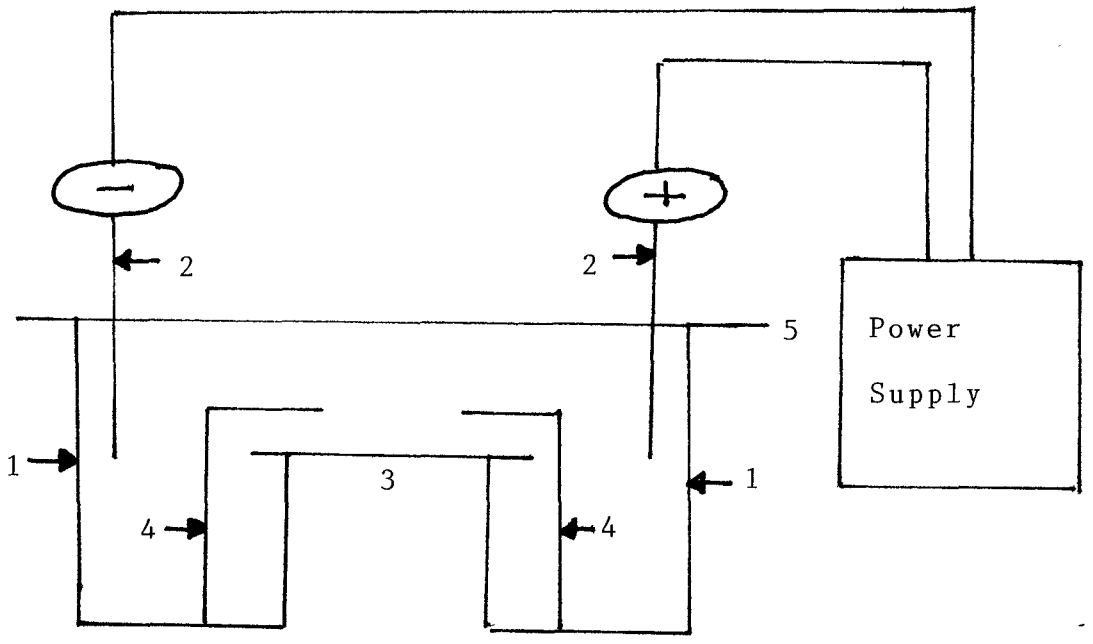


Figure 1. A Schematic of a Typical Electrophoresis Apparatus.[3]

Two buffer compartments (1) contain the buffer used in the process. In each buffer compartment is an electrode (2), either platinum or carbon, the polarity of which is fixed by the mode of connection to the power supply. The electrophoresis support (3) on which separation takes place is in contact with the buffer by means of wicks (4). The whole apparatus may be covered (5). Direct current power supply may be constant current (adjustable), constant voltage (adjustable), both, constant wattage, or pulsed.

The solutes of interest in clinical chemistry that can be studied employing electrophoresis, are mainly macromolecular in size and colloidal in nature. The samples include proteins in serum, erythrocytes, urine, cerebral spinal fluid, and other physiological fluids, as well as tissues.[4]

Basic Theory of Electrophoresis

Molecules carrying an electric charge by virtue of proton ionization will move either to the cathode or to the anode of an electrophoretic system depending on the nature of the charge. In a solution more acidic than the isoelectric point (pI) of the solute, an ampholyte (a molecule which can be either positively or negatively charged) takes on a positive charge and migrates toward the cathode. In the reverse situation, the ampholyte takes on a negative charge and migrates toward the anode. The rate of migration depends upon: (1) the net electric charge of the molecule, (2) the size and shape of the molecule, (3) the electric field strength, (4) the nature of the supporting medium, and (5) the temperature of operation. Equation (1) represents the driving force in any electrophoretic system:[5]

$$F = (X)(Q) = \frac{(E)(Q)}{(d)} \qquad (1)$$

where

F = the force exerted on an ion

X = the current field strength (v/cm)

Q = the net charge on the ion

E = the voltage applied (v)

d= the width of, or distance across, the electrophoretic medium (cm)

The steady acceleration of the ion is counteracted by a resisting force characteristic of the solution in which migration occurs. This force can be expressed by Stokes law as in Equation (2):[6]

$$F' = 6\pi r \eta v \tag{2}$$

where

F'= the counter force

r= the ionic radius of the solute

η= the viscosity of the buffer solution in which migration is occurring

π = 3.1416

v= the rate of migration of the solute = velocity (cm/sec)

The force F' counteracts the acceleration produced by F (as the ion approaches the electrode of opposite charge it tends to speed up) and the result of the two forces is a constant velocity. Therefore, when[7]

$$F = F' \tag{3}$$

$$6\pi r \eta v = (X)\ (Q) \tag{4}$$

or

$$\frac{v}{X} = \frac{Q}{6\pi r \eta} = \mu \tag{5}$$

where

$\frac{v}{X}$ = the rate of migration (cm/sec) per unit field strength (E/cm), and is defined as the electrophoretic mobility. It is expressed by the symbol

. The units of μ are $cm^2/v\text{-}sec$.

Aearose Gel Electrophoresis (AGE)

Agar (or Agarose) gel electrophoresis has been successfully applied to the analysis of serum proteins, hemoglobin, lactate dehydrogenase isoenzymes, lipoproteins, and many other substances. This gel medium parallels cellulose acetate in versatility and convenience and generally competes with other media for applicability to routine clinical laboratory applications.[8]

Agar is extracted from the cell membranes of various algae. The purity and chemical composition of different agar preparations show significant differences depending on the origin and purification methods. The precise structure of agar is not known, but it contains at least two poly-saccharides, one with a high content of sulfate and carboxyl groups (called agaropectin) and the other an almost neutral fraction (called agarose). The two fractions can be separated from each other after acetylation, as only agarose acetate is soluble in chloroform. The sulfate content of agar is mainly due to the presence of agaropectin (about 6% sulfate). Agarose only contains about 0.04% sulfate. Agar and agarose can be solubilized in aqueous solutions by heating in a boiling water bath, and the solution remains fluid down to about 40°C, at which temperature the viscosity of the solution abruptly increases. The solution gels at about 38°C. Once gelled, agar can be solubilized again only in a boiling

water bath.[9]

Agar gel is mechanically stable. After drying it forms a transparent film which allows optical evaluation, and can be easily stored without damage. Agar itself is an inexpensive and nontoxic material. On the basis of the advantageous properties of agar, and especially of agarose, these substances have become widely used as supporting media for electrophoresis.[10]

Power Supplies

Commercially available power supplies allow operation at constant current, constant voltage, and constant wattage.[11,12] The flow of current through a medium which offers electrical resistance involves the production of heat:

$$\text{heat} = (E)\ (I)\ (t) \qquad (6)$$

where

E = voltage (V)

I = current (A)

t = time (sec)

The heat evolved during electrophoresis increases the conductance of the system and decreases its resistance. With constant voltage power sources, the resulting rise in current causes an increase in the migration rate of proteins and an increase in the evaporation of water solvent from the stationary support medium. The water loss causes an increase in ion concentration and thus a further decrease in resistance (R). To minimize these various effects on

the migration rate, it is best to apply a constant current. According to Ohm's law:

$$E = (I)\ (R) \qquad (7)$$

Therefore, if R decreases, the voltage, E, also decreases. This in turn decreases the heat effect and thus keeps the migration rate fairly constant. If a constant-voltage source is used, the current, and therefore the migration rate will progressively increase.[13]

An example of a constant-power power supply is the Bio-Rad Model 1420B constant-power power supply. To set the power supply for the condition of constant power, the voltage must be set. This is accomplished by setting the current limit control at the desired voltage.[14]

Power supplies delivering a constant power output are independent of changes in the electrical resistance and are probably to be recommended for analytical work of the highest accuracy. These only ensure that heat generation is constant throughout the run and do not overcome the problems associated with heat production or permit the removal of it. So it seems likely that the extra expense of such units is not justifiable in most cases.[15]

Microprocessors and Microcomputers

Microprocessors are new and fascinating logic devices that are having pronounced effects upon our lives. Microprocessors can be found in pocket calculators, checkout terminals in stores, home appliances, office equipment,

scientific instruments, medical equipment, and video games, to name a few. Furthermore, new applications are being found and new microprocessor-based products are being developed every day.[16]

Digital computers have had a great deal of influence upon our society since 1951, when the first commercial digital computer (Univac I) became available. A new technology had emerged. Such terms as "digital computation," "logic design," and "programming" became concepts integral to science and engineering. However, the diversity of these concepts frequently caused a split in interest. Although this dichotomy in interest may have real foundations in the case of large-scale computers, the problems facing application programmers and computer designers became more interrelated with the introduction of minicomputers in 1965. These computers were no longer intended solely for data processing and problem solving, but were to become a part of a system requiring immediate computer decisions, called real-time systems.[17]

The introduction of the microprocessor in 1971 closed this gap. An era of software logic design, or programmed logic, has resulted. In this era, programming concepts and logic design principles have merged to the point that their interactions require scientists and engineers to have complete familiarity with both the software and hardware principles of computers to utilize fully the potential of the microprocessor.[18]

One of the results of the advancement in solid-state technology is the capability of fabricating very large numbers of transistors (i.e. 1000 and over) within a single silicon chip. This is known as large-scale integration (LSI). A direct consequence of large-scale integration is the microprocessor. In general, a microprocessor is a programmable logic device fabricated according to the concept of LSI. A microprocessor has a high degree of flexibility built into it. By itself it cannot perform a given task, but must be programmed and connected to a set of additional system devices. These devices usually include memory elements and input/output devices. In general, a set of system devices, including the microprocessor, memory, and input/output elements, interconnected for the purpose of performing some well-defined function, is known as a microcomputer or microprocessor system.[19]

A computer is an electronic machine capable of quickly performing complex calculations by means of stored instructions and data. There are two fundamental types of computers, the analog computer and the digital computer. The analog computer accomplishes its tasks by simulating physical situations with voltage levels. It is used to solve differential equations and is programmed by connecting together an array of circuits in such a way that the output voltage patterns simulate the variations of the physical quantities of interest. The digital computer, although it uses voltage levels to carry out its calculations,

internally represents numbers and its own instructions in terms of combinations of distinct states. Digital computers are capable of performing a much wider variety of tasks than are analog computers. They can be used to compile, correlate, sort, merge, and store data as well as perform calculations.[20]

A basic computer system consists of five functional units: the input unit, the memory unit, the arithmetic unit, the control unit, and the output unit.[21] A basic computer system is illustrated in Figure 2 and a flowchart depicting its operation is illustrated in Figure 3.

The physical components and circuits that comprise a computer system are called its hardware. These circuits are able to be used to perform only a small number of different operations. Any additional operational capabilities of the computer must be accomplished by programming. A program is an organized collection of elementary computer operations, called instructions, that manipulate information, called data. The programs that are written for a computer are called its software.[24]

The program and data are first stored in the memory unit via the input unit. The individual instructions of the program are then automatically entered, one at a time, into the control unit, where they are interpreted and executed. The execution usually requires data to be entered into the arithmetic unit, where the circuitry necessary for manipulating the data is contained. During the course of

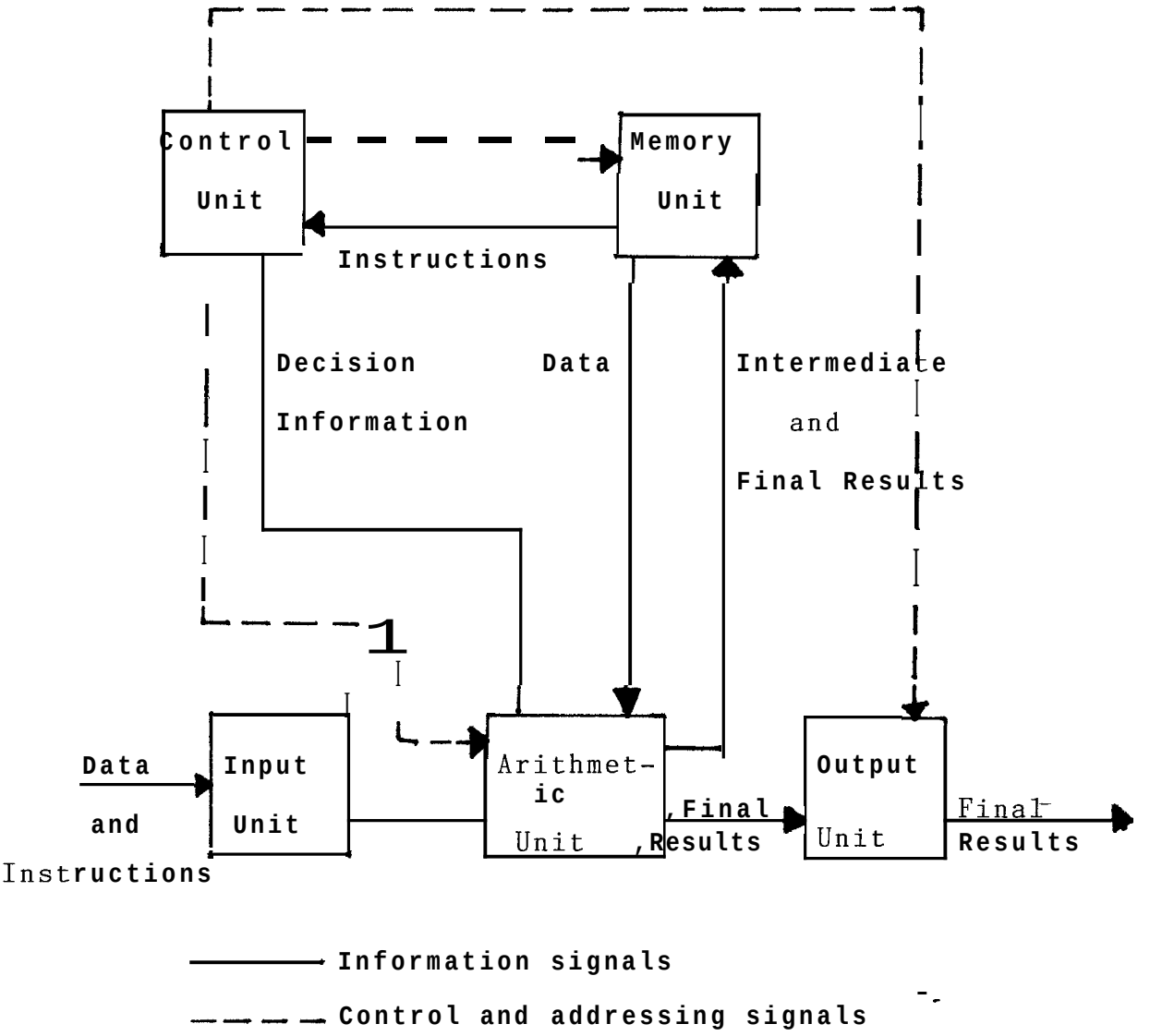


Figure 2. An Organization of a Basic Computer System.[22]

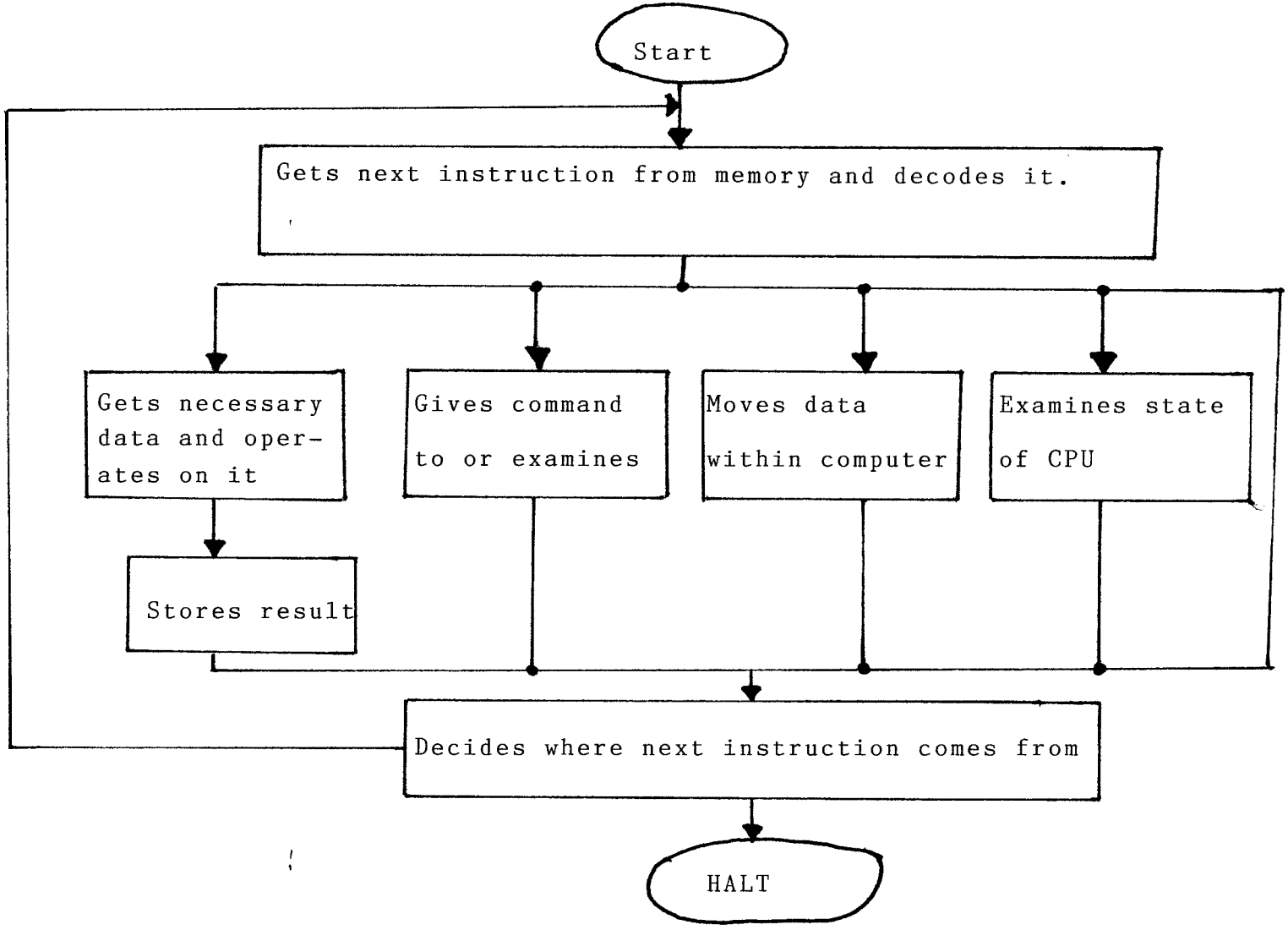


Figure 3. Flowchart of the Operations of a Basic Computer System.[23]

computation or at its completion, the derived results are sent to the output unit. The arithmetic unit and control unit together are usually called the Central Processing Unit (CPU). The CPU of a microcomputer is the microprocessor.[25]

In addition to the memory unit, the other computer units are capable of storing information. Information is stored as groups of binary digits (called bits) in storage devices called registers. Each register is capable of holding one computer word. A word is the group of binary digits handled all at the same time by the computer. The place in the memory where each substorage unit appears is known as a location. Each of these locations is identified by an address. The address is simply an integral number that uniquely designates a substorage unit.[26]

Microcomputers frequently have two different forms of memory within the memory unit. There is the read-only memory (ROM) and the random-access memory (RAM). Once information is placed into the read-only memory it cannot be modified easily, if at all. The read-only memory is used to hold the operating program and any constant data, while the random-access memory is used for holding information that is subject to change. Random access refers to the fact that all locations within the memory are accessible in the same amount of time.[27]

The arithmetic unit is where most of the data manipulations occur. These manipulations involve both

arithmetic and logic computations. The most important register in the arithmetic unit is the accumulator. This register normally contains one of the operands prior to a computation and the result after the computation. In addition, several auxiliary registers, called scratchpad registers, frequently appear in the arithmetic unit to facilitate the writing of programs.[28]

The function of the control unit is to oversee the operation of the computer. It automatically receives the instructions, one at a time, from the memory unit. Then it decodes each instruction and generates the necessary signals to provide for its execution. For the control unit to obtain an instruction, it must first know the instruction's location in the memory. Normally the instructions are in sequential order, and their location is indicated by a program counter within the control unit. It is the instruction register that stores the instruction within the control unit for the purpose of decoding and providing for instruction execution.[29]

In order for the control unit to interpret an instruction correctly, the instruction must have a definite organization, called the instruction format. The exact instruction format depends on the particular microprocessor. However, certain information must be included within an instruction. Most significant are the operation code and an address. The operation code is a set of binary digits that uniquely define what operation is to be performed

during the execution of the instruction. The address portion of an instruction indicates the location (e.g., in memory) that must be accessed to execute the instruction.[30]

Maintaining synchronization between the various computer units is another function of the control unit. This is achieved by means of a clock. Several clock periods are needed to handle an instruction. In general, an instruction must be fetched from memory, decoded, and then executed. The fetching, decoding, and executing of an instruction is broken down into several time intervals. Each of these intervals, involving one or more clock periods, is called a machine cycle. The entire period associated with the fetching, decoding, and executing of an instruction is called an instruction cycle.[31]

The final two units of a computer are the input and output units. These units are the contact between the computer and its surroundings. They act as buffers, translating information between the different speeds and languages with which computers and humans, or other systems, operate. The input unit receives data and instructions from the outside world, which eventually are entered into the memory unit. The output unit receives the computed results and communicates them to the operator or to another system. The input and output devices are known as peripherals. The points of contact between the input/output devices and the microprocessor are called the input/output ports. The input and output ports are also addressable so that several

input and output devices can be connected to the microprocessor.[32]

It is characteristic of digital computer operation that all information is handled in discrete form, i.e., with finite numbers. Frequently a digital computer must communicate with another system not capable of handling discrete information. Nondiscrete information is termed analog or continuous. The input and output devices capable of performing a transformation between continuous and discrete information are called analog-to-digital converters (ADC) and digital-to-analog converters (DAC).[33]

A digital-to-analog converter (DAC), as the name suggests, is a circuit that converts a digital signal into an electrical analog quantity directly related to the digitally encoded number. The digital domain input signal may be in either parallel or serial form, but the parallel form is by far the most common. The analog output signal is generally in the current or voltage domain. Since the input quantity is a number, the basis of the conversion techniques is to convert the number to a corresponding number of units of current, voltage, or charge, and then to sum these units in an analog summing circuit.[34] (See Figure 4).

An analog-to-digital converter (ADC) is a circuit that converts an analog domain signal (current, voltage, or charge) to a digital signal that encodes a number proportional to the analog magnitude. There are three classes of converters: integrating ADC's, digital servo ADC's, and flash ADC's. Three types of integrating ADC's exist; the voltage-to-

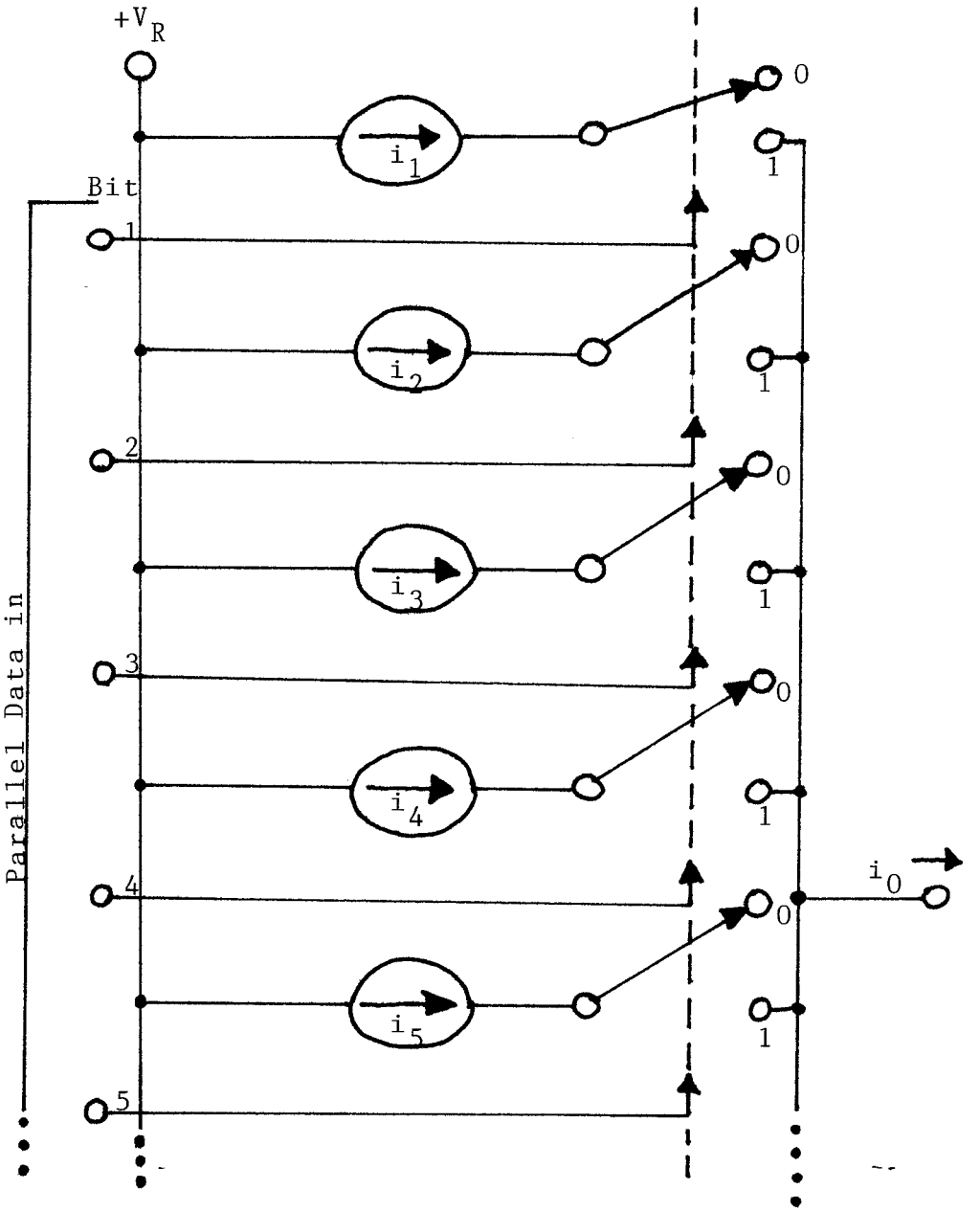


Figure 4. Basic DAC.[35]

Each bit of the input data word controls a switch that either connects a current generator or disconnects it from the output. When a switch is closed by a 1-level input bit, a current proportional to the weight value of its input bit is generated. The generated currents are then summed to produce a total output current, i_0, proportional-to the numerical value of the input word.

frequency converter, the dual-slope converter, and the charge-balance converter. The flash ADC uses parallel comparators and a decoder to digitize the signal. In the digital servo ADC a DAC is used to generate an analog signal for comparison with the signal to be digitized. The digital input to the DAC is changed in a direction determined by the results of the comparison until the input signal and the DAC output are equal.[36] (See Figure 5).

In a microcomputer the various units are connected by buses. A bus is a set of lines over which information is transferred from any of several destinations. One typical bus structure is shown in Figure 6.[38]

Serum Proteins

Blood is made up of particulate cell form suspended in a fluid medium called plasma, a very complicated mixture of inorganic and simple and complex organic materials dissolved in water. If blood is permitted to clot, the fluid which separates is referred to as serum. It lacks the protein fibrinogen present in plasma, the fibrinogen having been transformed into insoluble fibrin in the clotting process. Fibrinogen makes up only 3 to 6 per cent of the total plasma proteins. Since insoluble fibrin may interfere with many clinical chemical studies, it is therefore satisfactory and much more convenient to use serum rather than plasma.[40,41]

When serum proteins are separated on cellulose

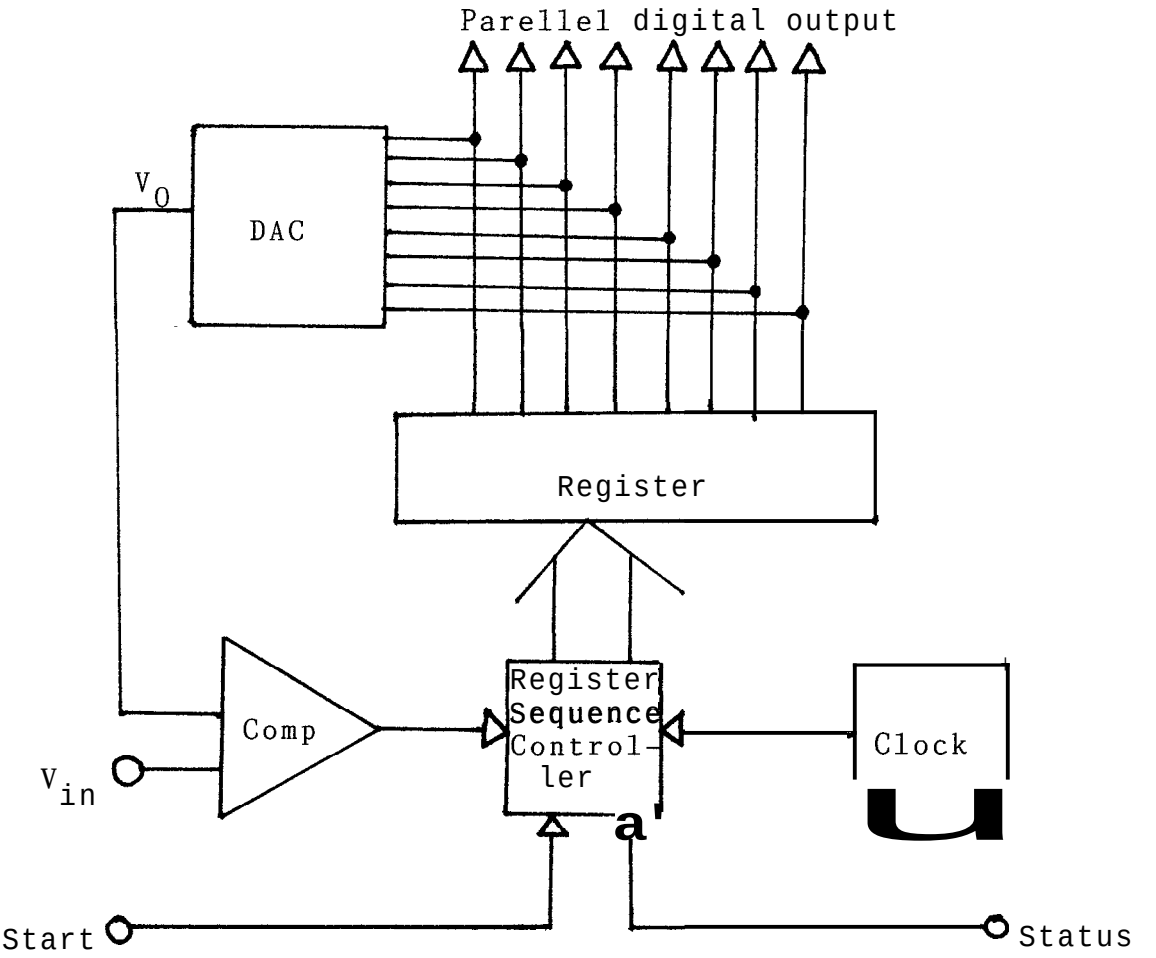


Figure 5. Digital Servo ADC.[37]

On application of a start signal, the register sequence controller alters the contents of the register until the DAC output is within one LSB (least significant bit) of the analog input voltage. The comparator determines whether the number in the register will be increased or decreased, and the clock determines the rate of change. The status line provides an appropriate logic-level signal when the converter is busy and a logic-level transition when the conversion is complete.

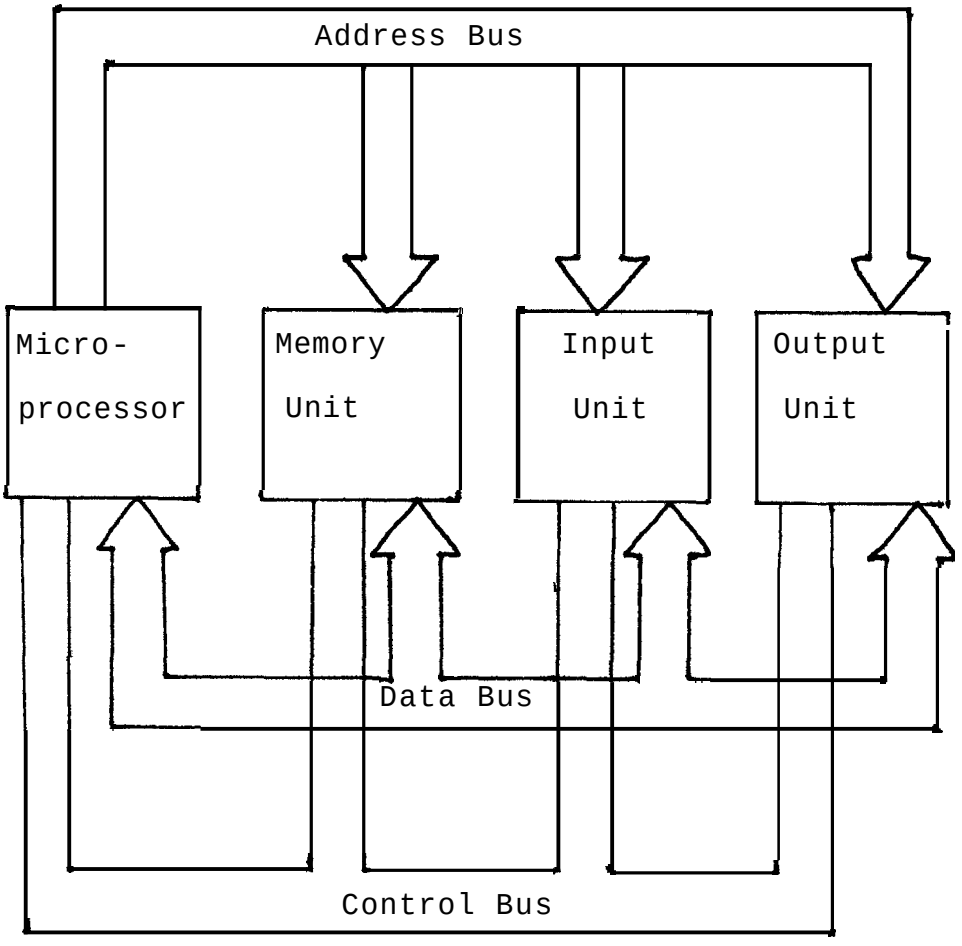


Figure 6. The Bus Structure of a Typical Microprocessor.[39]

This structure consists of three buses. The address bus is unidirectional; i.e., information flows in only one direction. This bus is used to transmit an address from the microprocessor to the memory, input, or output unit. The data bus is bidirectional; i.e., information can flow in either direction on these lines, and is the path for data flow. Finally, the control bus is the set of lines over which signals travel to maintain timing and status information. Some of these lines are bidirectional, while others are unidirectional.

acetate by conventional electrophoresis, five fractions or bands are usually obtained. (See Figure 7). These bands are known as albumin, alpha one (α_1), alpha two (α_2), beta (β), and gamma (γ) globulins, starting with the greatest migration toward the anode. The gamma band is usually displaced from the point of application slightly toward the cathode. The slowest, the beta fraction, usually is at the point of application, and all other fractions move toward the anode.[42]

Clinical Significance of Serum Proteins

Elevated Total Serum Proteins:

Elevated total serum protein levels may indicate states of dehydration. These levels may increase as much as 10 to 15 per cent. Dehydration may result from a decrease in water intake or in water deprivation. Dehydration may also result from excessive water loss as occurs in severe vomiting, diarrhea, Addison's disease, or diabetic acidosis. The absolute quantity of serum proteins is unaltered, but the concentration is increased because of the decreased volume of solvent water.[44]

Total serum protein levels may also increase in multiple myelomas. Multiple myeloma, or myelomatosis, is a malignant proliferation of plasma cells which results in an abnormally high concentration of serum immunoglobulins, usually IgG or IgA.[45] The total protein may increase to over 10 g/100 mL. The increase is due to the presence of

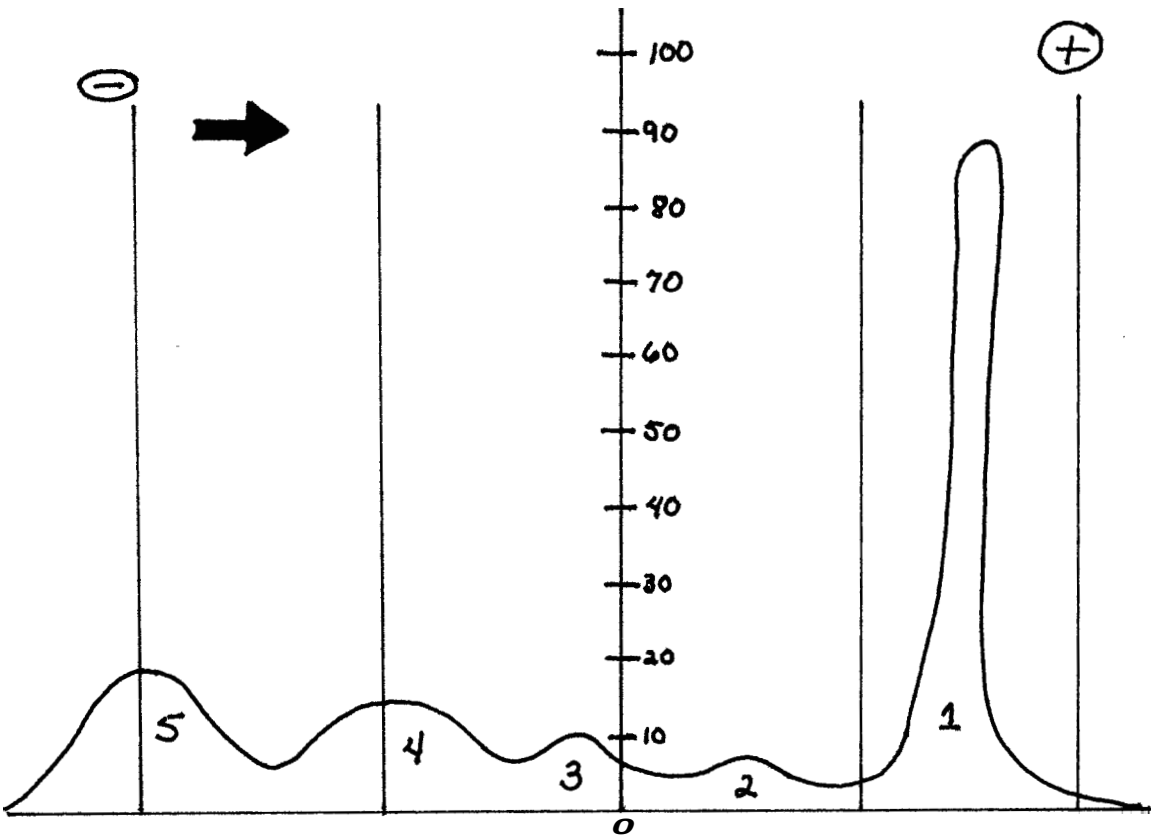


Figure 7. Typical Serum Protein Electrophoresis Pattern.[43]

Band 1 = albumin

Band 2 = α_1-globulin

Band 3 = α_2-globulin

Band 4 = β-globulin

Band 5 = γ-globulin

elevated levels of myeloma proteins (IgG, IgA, IgM, IgD, IgE, and Bence Jones Proteins).[46]

Decreaded Total Serum Proteins:

Decreased total serum protein levels are more commonly known as Hypoproteinemia. Hypoproteinemia is characterized by total serum protein levels below 6.0 g/100 mL. Hypoproteinemia is encountered in nephrotic syndrome, in salt retention syndromes, and in patients with severe burns, extensive bleeding, or open wounds. A long period of low intake or deficient absorption of protein may affect the level and composition of serum proteins, as in sprue and in other forms of intestinal malabsorption, as well as in acute protein starvation (Kwashiorkor). In these conditions the liver has inadequate raw material to synthesize serum proteins to replace those lost in the normal usage of proteins and amino acids.[47]

Statement of the Problem

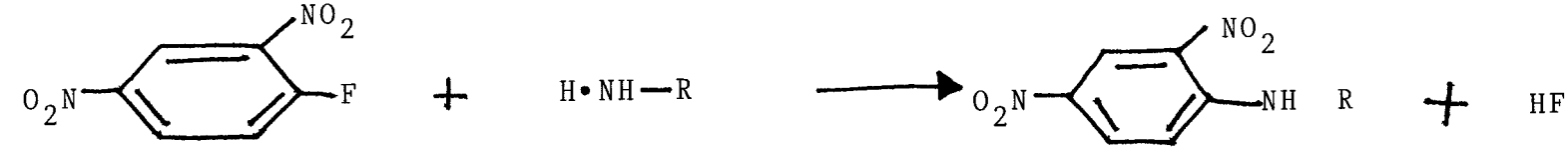
Currently, the most difficult aspect of gel electrophoresis is the detection of separated fractions. This is normally accomplished by staining the gel plates with one or a series of dyes, which hopefully will react with all of the components of the sample present. Many fractions may require additional reactions to produce a chromophore. Some techniques employ the use of ultraviolet radiation to cause the fragments to fluoresce before detection. Most procedures require both pre- and post-treatments to effect stability of

the gels and samples, decoloration of the gel, and preservation of the chromophores produced. Even after the effort to produce the chromophore, expensive instrumentation is required to quantitate the relative concentration of the fractions present. After the gel has been used once, it must be discarded and a new gel made. The preparation of the gel requires about one hour with a 24-hour wait for the gel to thicken.

A unique approach for the detection of separated fractions is believed to be the use of a three-electrode electrochemical cell to replace the staining and many of the pre- and post-treatment steps. Unlike conventional electrophoresis, which is a static detection scheme, this cell reacts to electroactive species passing between the working and auxillary electrode while the electrophoretic process is occurring.

Any component that is or could be made electroactive should elicit a response from the detector system. An example would be the clinically significant fractionation of human serum proteins by electrophoresis. Although serum proteins for the most part are not electroactive, they can easily be converted to the DNP (1-Fluoro-2,4-dinitrobenzene (FDNB)) derivatives which are quite active due to the two nitro groups. (See Figure 8).

Due to the slowness of the electrophoretic migration, the components arrive at the working electrode at such low instantaneous concentrations that detection becomes quite



1-Fluoro-2,4-dinitrobenzene (FDNB)

protein

DNP labelled protein

Figure 8. The Conversion of Human Serum Protein to its DNP (FDNB) Derivative [48]

difficult. One way to compensate for this effect would be to operate the electrochemical detector in pulses rather than continuously. This can be achieved by programming a digital microprocessor to cause the electrochemical potentiostat to operate in pulses rather than continuously. Therefore, the effective concentration at the electrode would be drastically increased producing a current which is much easier to detect.

Another possible difficulty may be the interference of the electric field, needed to produce electrophoresis, with the electrochemical detection. With the same microprocessor controlling the electrophoresis power supply, it could be programmed to turn off the electrophoretic power supply while an electrochemical measurement was being performed.

CHAPTER II

LITERATURE REVIEW

Power Supplies

Electrophoresis power supplies have been known for some 40 years and various types of supplies have been utilized for this purpose. Initially, most power supplies were constant voltage with some being constant current. Recently, with the advent of gel electrofocusing and the rapidly changing characteristics of the electrophoretic media conductivity, there has been much discussion in the literature concerning the design, construction, and use of constant-power power supplies. Several of these power supplies are discussed below.

Constant-Power Power Supplies

The reason conventional power supplies (i.e. constant voltage and constant current) are not adequate for today's uses of electrophoresis is because changes in resistance often occur within the electrophoretic system during a run. As a result the power level initially established also changes. Migration rate and resolution are proportional to voltage so long as the heat produced can be dissipated sufficiently fast by the cooling apparatus. Thus, if power is brought to and held at the maximum tolerable

amount —— called the optimum power —— electrophoretic patterns should show maximum resolution. Since the typical power supply does not hold power constant as resistance changes, the actual power delivered by such sources will come to deviate from any set optimum amount.[49]

A new method developed by Schaffer and Johnson, which achieves constant power and which has no apparent practical limitations, is a d.c. power supply with feedback regulation. (See Figure 9). The regulation is based on the instantaneous power (volts x amperes) dissipated as heat in the electrophoretic apparatus, and it is controlled by a circuit which includes an integrated circuit analog multiplier. (See Figure 10). A slightly modified Heathkit High Voltage Power Supply, Model IP-17 (Heath Co., Benton Harbor, Michigan) can be used as a convenient and inexpensive source of unregulated d.c. and a pass element for the regulator.[50]

Another type of power supply developed by Williams and Catsimpoolas is a versatile high voltage power supply that combines the advantages of linear and switching technologies to produce a precision output at high current. This supply is adjustable from 50 to 1000 V at up to 100-W output to better than 0.01% regulation. It will operate as a current source with up to 100 mA output with 0.01% stability. Finally, it will regulate power to 0.01% stability at up to 100-W.[53]

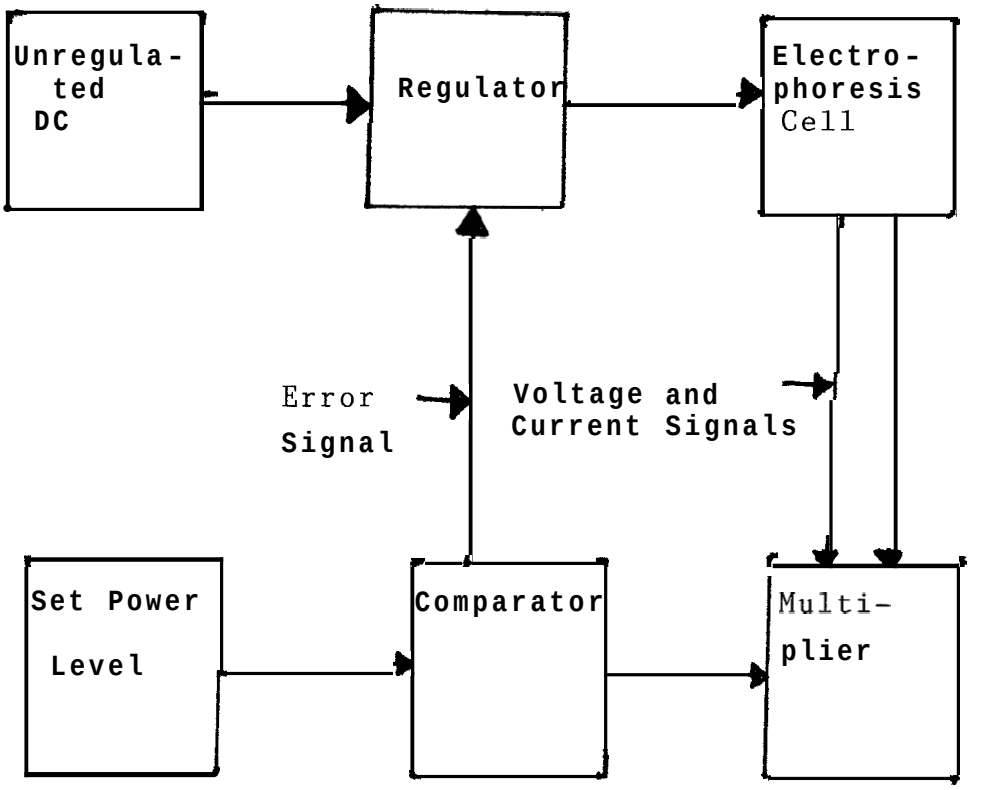


Figure 9. Constant Power Regulation for Optimum Electrophoretic Migration.[51]

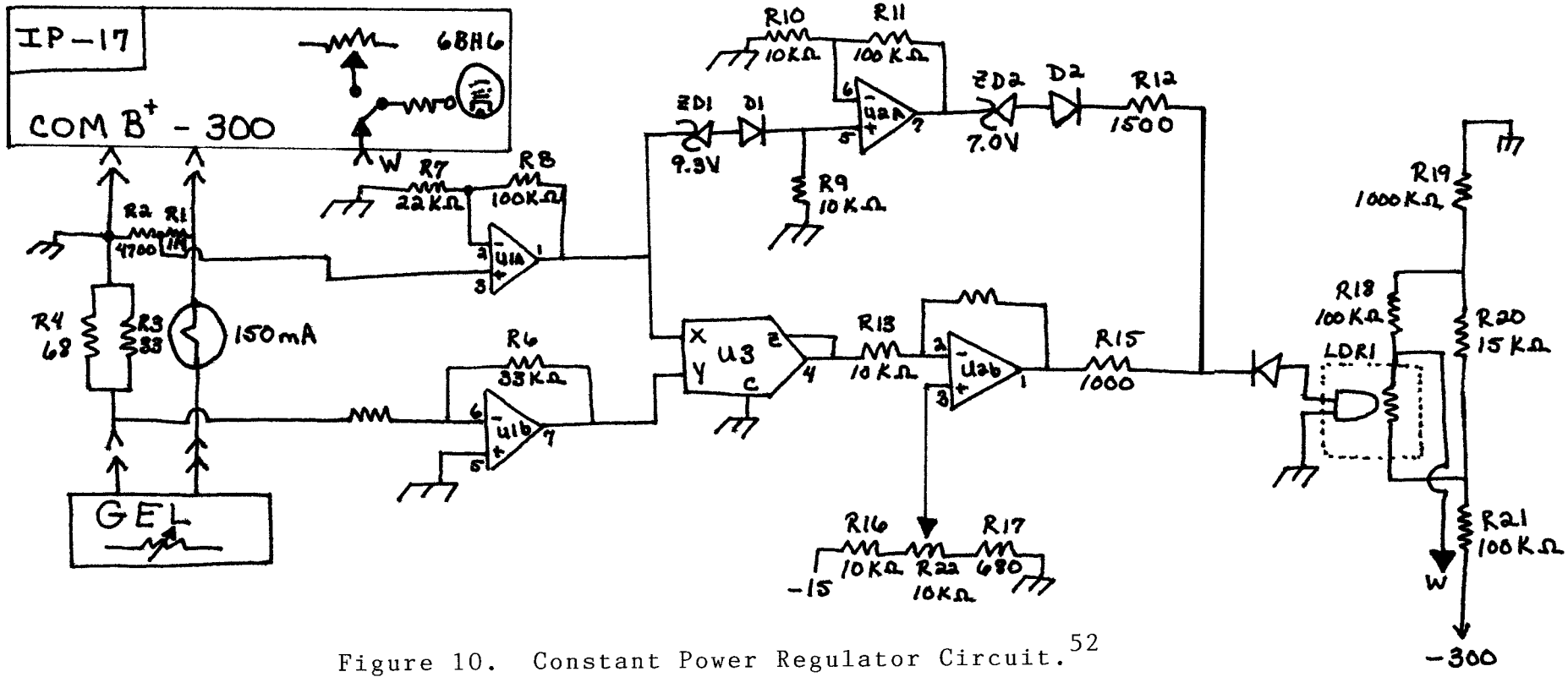


Figure 10. Constant Power Regulator Circuit.[52]

U 1-2, Motorola MC1458G (Dual "741" type op-amp); U3, Burr-Brown 4094-1EC (multiplier); D 1-3, 1N457A; ZD 1-2, Zener Diodes; R 1-21, ½W; R22, 10K potentiometer, LDR1, Raythion CK1122.

This instrument functions by controlling the input power to a toroidal d.c.–d.c. converter with an operational amplifier. (See Figure 11). One of the amplifier inputs is referenced to a precision variable voltage source. The other input is connected to the output of the converter. The pass regulator and converter function as an amplifier within the op-amp's feedback loop. When the feedback is taken from the "voltage sense" network, a constant voltage output is produced. When the feedback comes from the "current sense" network, a constant current through the load results. When the "V" and "I" loops are multiplied by the multiplier module, the load receives constant power, especially at low voltage output settings.[54]

The overvoltage/overcurrent protection circuit prevents an overload by shutting down the supply when it senses too much current flowing in the load. If the supply is either shorted out or a load dropout occurs, the instrument will shut down due to the overvoltage/overcurrent protection circuit.[55]

A third type of power supply is a handmade power supply developed by Kazuo Yoshida. Figure 12 shows a rectifying circuit for the simple power supply which generates pulse d.c. The circuit consists of a plug (a), a bridge-diode rectifier (1.8 A, 600 V) (b), a switch (c), a fuse (1 A) (d), d.c. output terminals (e-f), and an electrolytic capacitor (160 V, 100 μf) (g).[57]

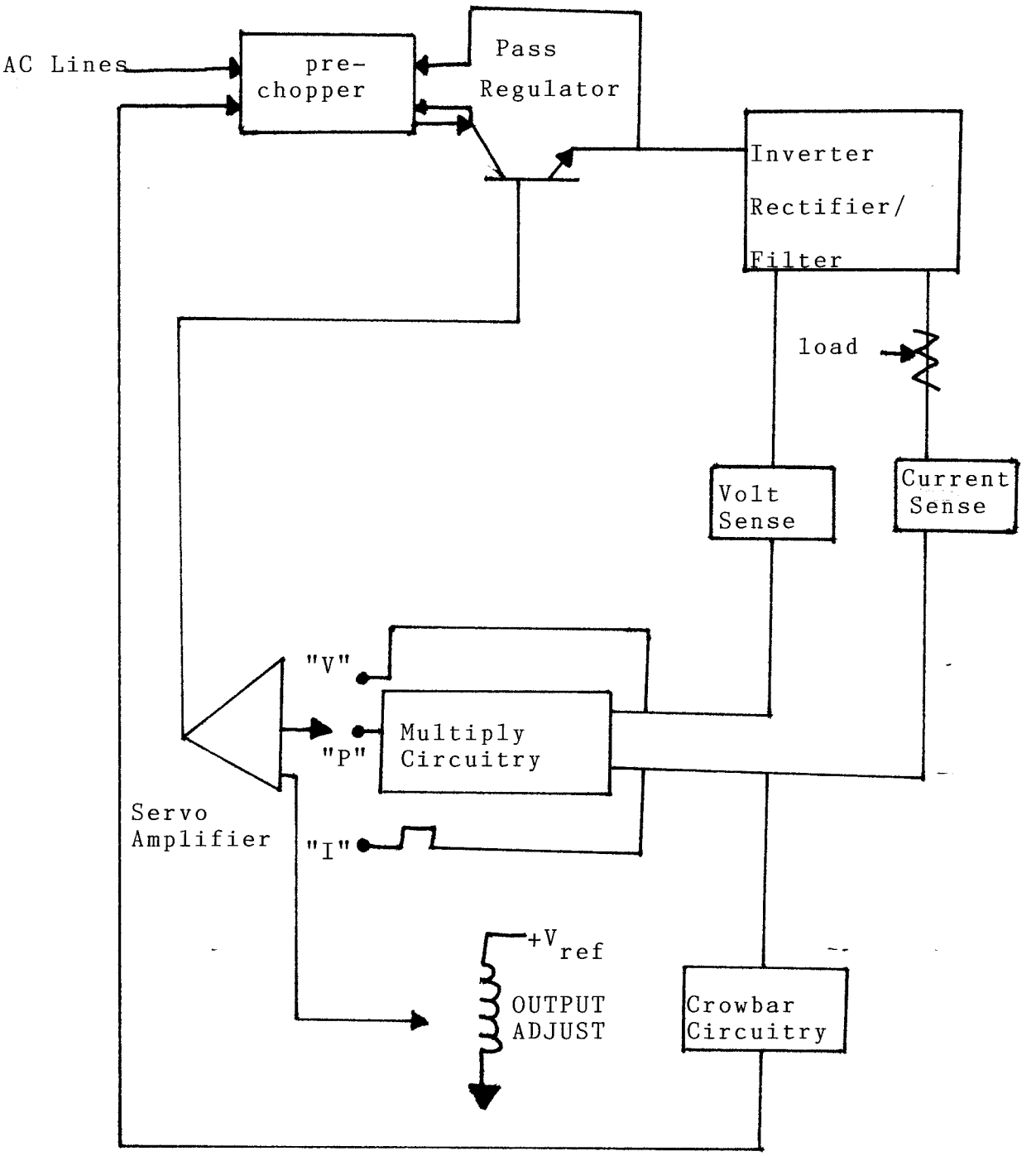


Figure 11. Block Diagram of the Main Power Supply Components.

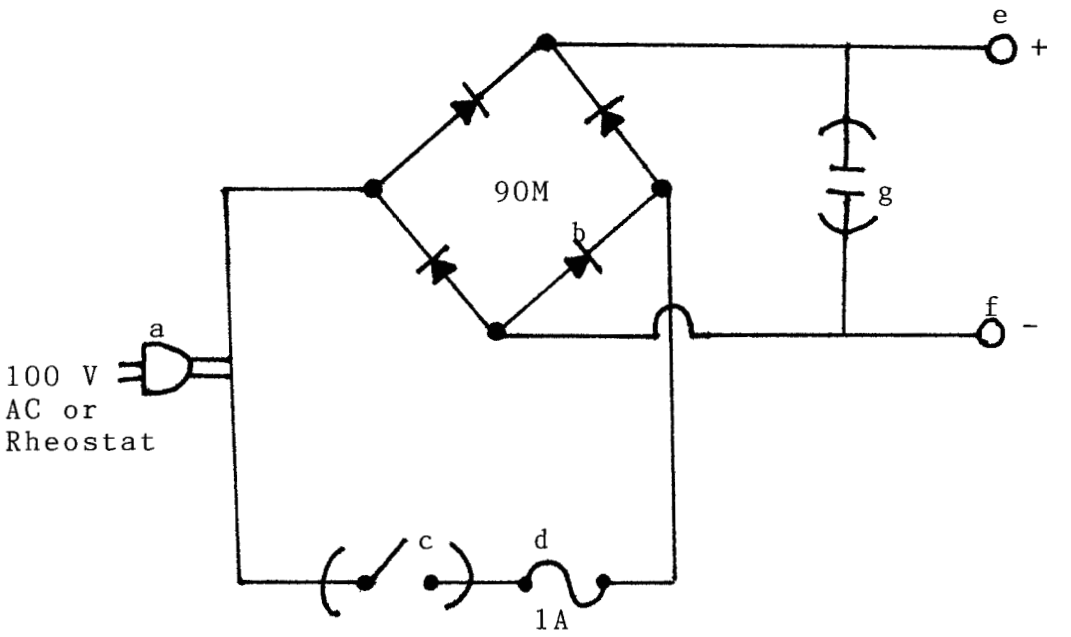


Figure 12. Simple Handmade Power Supply Which Generates Pulse DC.[58]

Variability of output current and/or potential is indispensable for an ordinary electrophoretic constant power supply. However, the rectifying.electric circuit of Figure 12 generates only constant 90-V pulse d.c. (in the absence of a capacitor) or 140 V (in the presence of a capacitor). This is an inconvenience of this power supply in spite of its compactness and low cost.[59]

CHAPTER III

MATERIALS AND APPARATUS

Materials

The LNW 80 microcomputer was obtained as a kit from the LNW Research Corporation of Tustin, California. The kit included everything necessary to build a computer with the exception of the electronic components. The electronic components such as resistors, capacitors, chip sockets, integrated circuits, transistors, diodes, zener diodes, operational amplifiers, switches, potentiostats, wire ribbon, and connectors were obtained from the electronics room #214 in Ward Beecher Science Hall and from Mike Repetsky, Electronics Technician, at Youngstown State University.

All of the reagents used were reagent grade. These reagents included powdered egg albumin (Matheson, Coleman, and Bell, Norwood, Ohio, CB951), sodium bicarbonate (J.T. Baker Chemical Co., Lot No. 36352), 1-Fluoro-2,4-dinitrobenzene (Eastman Organic Chemicals, Rochester, N.Y., Lot No. 6587), agarose (J.T. Baker Chemical Co., Phillipsburg, N.J., Lot No. 320103), 100% ethanol (AAPER Alcohol and Chemical Co., Louisville, Kentucky, Lot No. 10482), diethyl ether (Matheson, Coleman, and Bell, Norwood, Ohio, CB864), and potassium ferriccyanide (Mallinckrodt, St. Louis, Missouri, Lot No. 6912).

The Electra HR Buffer (Tris-barbiturate buffer pH=8.6) used for the preparation of the agarose gel and in the electrophoresis chamber was purchased from Helena Laboratories, Beaumont, Texas. The buffer was 0.05 ionic strength when diluted with the specified amount of deionized water as outlined in Chapter IV, stored in the refrigerator when not being used, and prepared when necessary.

All of the water used in either the preparation of the buffer or the solutions, was deionized water.

Apparatus

An LNW 80 microcomputer was built and used to control the constant power electrophoresis power supply. The power supply was a Bio-Rad Constant Power Power Supply, Model No. 1420B-150.

A Thermolyne 1000 heat-stir apparatus was used in the preparation of the agarose gel. The electrophoresis cell was originally designed and constructed by my thesis advisor Dr. Daryl Mincey at Youngstown State University. The electrophoresis chamber was from Gelman Instrument Company. A Heath Binary Information Module Model EU801-12 was used for the relays connecting the LNW 80 to the electrophoresis chamber and the electrochemical detector. The potentiostat used to apply the potential was a CV-1B Cyclic Voltammograph Model No. CV-1B-120 from Bioanalytical Systems Inc. A Houston Instrument's, a division of Bausch and Lomb, Model No. 200 X-Y recorder was used to monitor the current.

Three-Electrode Potentiostat

An electrochemical experiment such as "The Design and Construction of a Microprocessor-Controlled Electrophoresis Power Supply" requires the use of a three-electrode potentiostat. (Figure 13 represents such a system). The first electrode, the platinum auxillary electrode, is controlled by the elements in the lower left half of the figure. The second electrode, the Ag/AgCl reference electrode, is controlled by the elements in the upper left half of the figure. And the third electrode, the carbon paste working electrode, is controlled by the elements in the right half of the figure. One can find an electrometer connected to the auxillary electrode and a scanner. The electrometer allows for the rigorous control of the applied potential, E, to the platinum auxillary electrode. The power booster, summing amp, bias 1, and bias 2 are used to control the auxillary electrode. The power boosters are added to increase the voltage-current range of the instrument and bias 1 and 2 set the voltage. The IR Compensation Circuit is added to account for the high internal resistance (between the working and reference electrodes) of the gel.

The three-electrode potentiostat was obtained from Bioanalytical Systems, Inc.

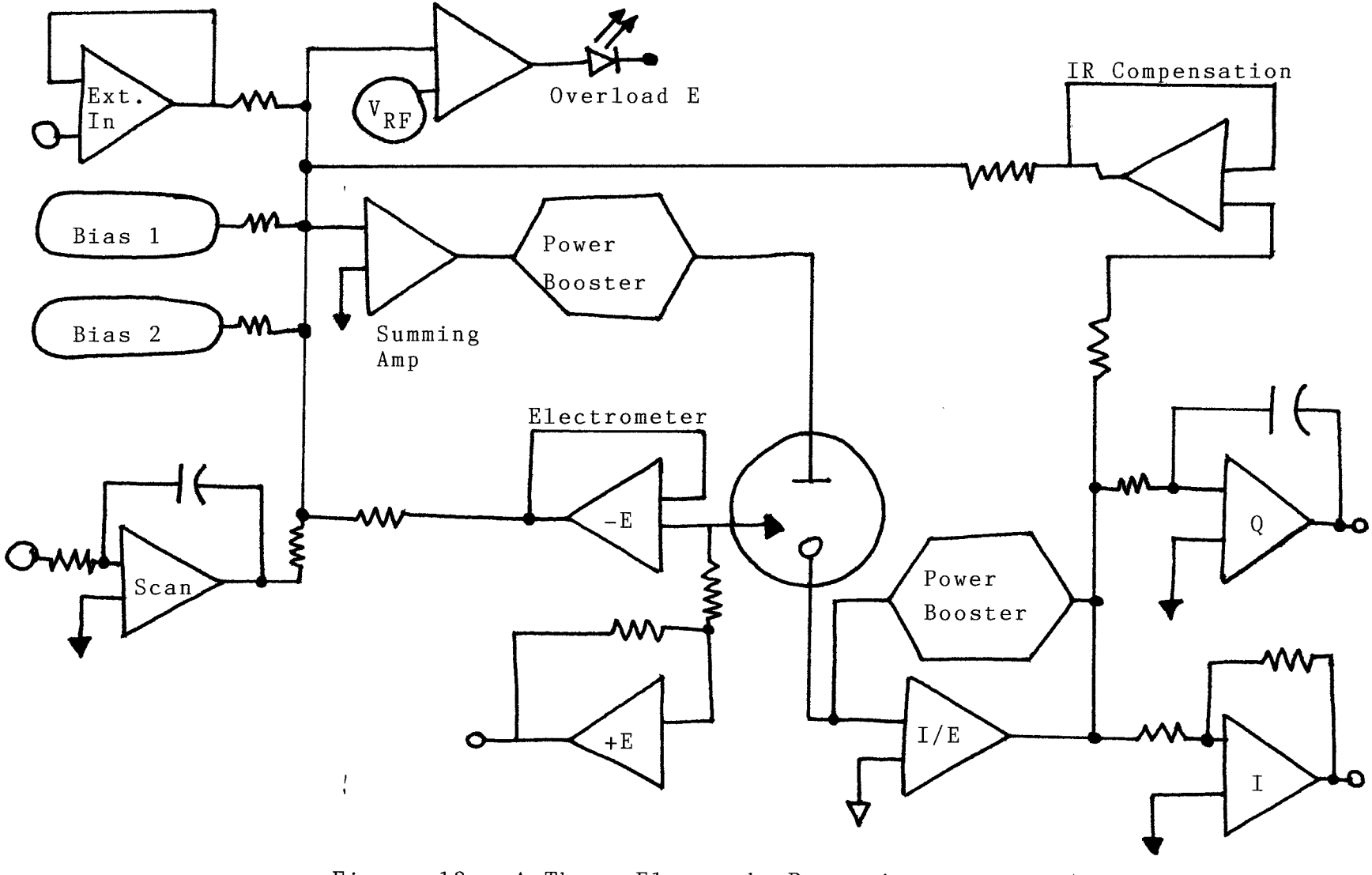


Figure 13. A Three-Electrode Potentiostat.

CHAPTER IV

EXPERIMENTAL

Assembly of the LNW 80 Main Computer Printed Circuit Board

Figure 14 is a block diagram of the LNW 80 Main Computer Printed Circuit Board.

The chip sockets were placed into their appropriate pin holes on the front side of the circuit board and soldered on the back side of the board. Then the resistors, capacitors, transistors, diodes, zener diodes, operational amplifiers, switches, and potentiostats were placed into their respective places on the front side of the board and soldered on the back side of the board. Then the cables used to connect the main board with the expansion board were made from the wire ribbon and the connectors. Finally, the integrated circuit chips were placed one by one into their appropriate sockets.

An illustration of each side of the main computer board is in Appendix A. The negative transparencies of each side are in Appendix B.

Assembly of the LNW 80 Expansion Interface Printed Circuit Board

Figure 15 is a block diagram of the LNW 80 Expansion Interface Printed Circuit Board.

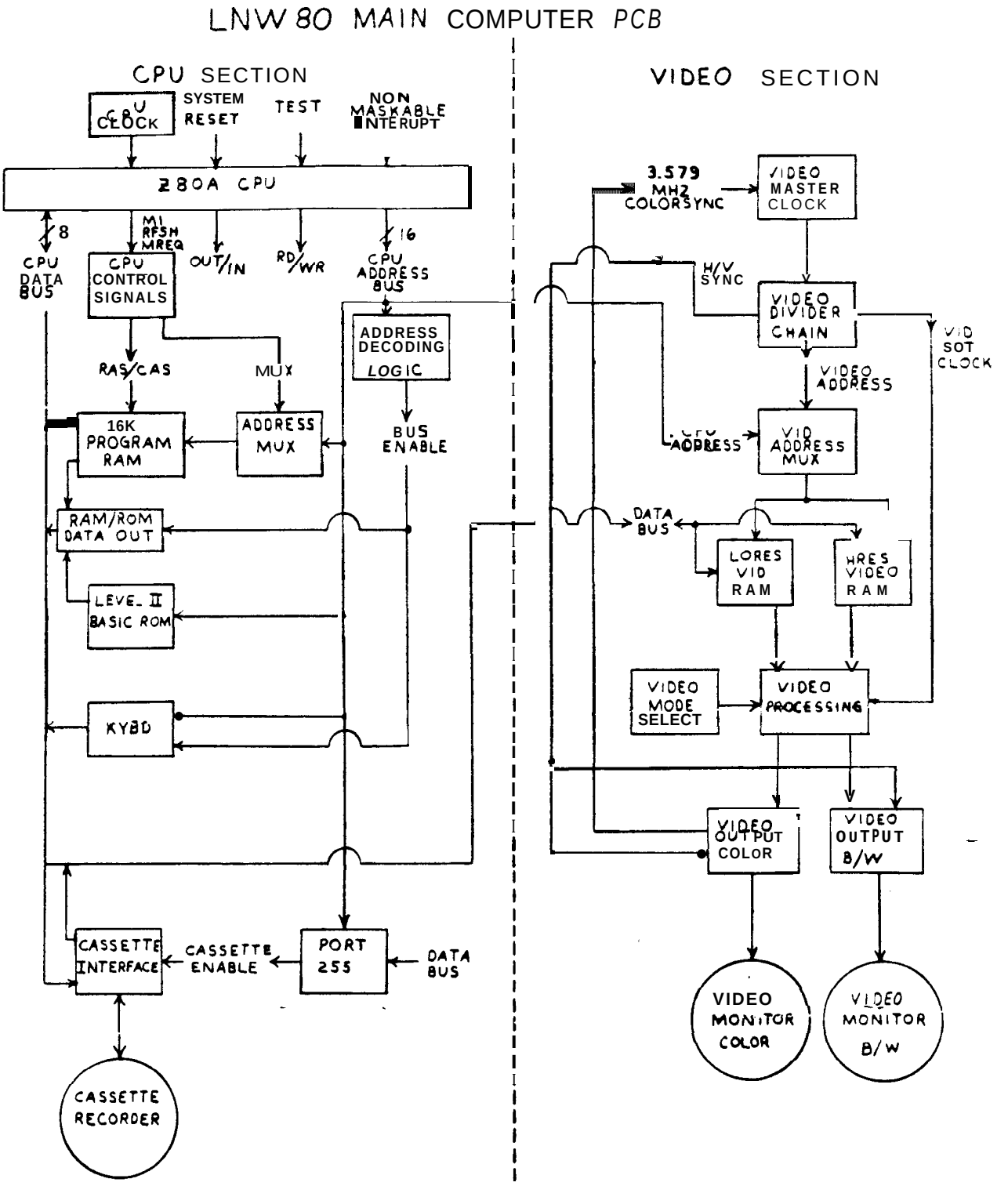


Figure 14. Block Diagram of the LNW 80 Main Computer Circuit Board.

EXPANSION INTERFACE PCB

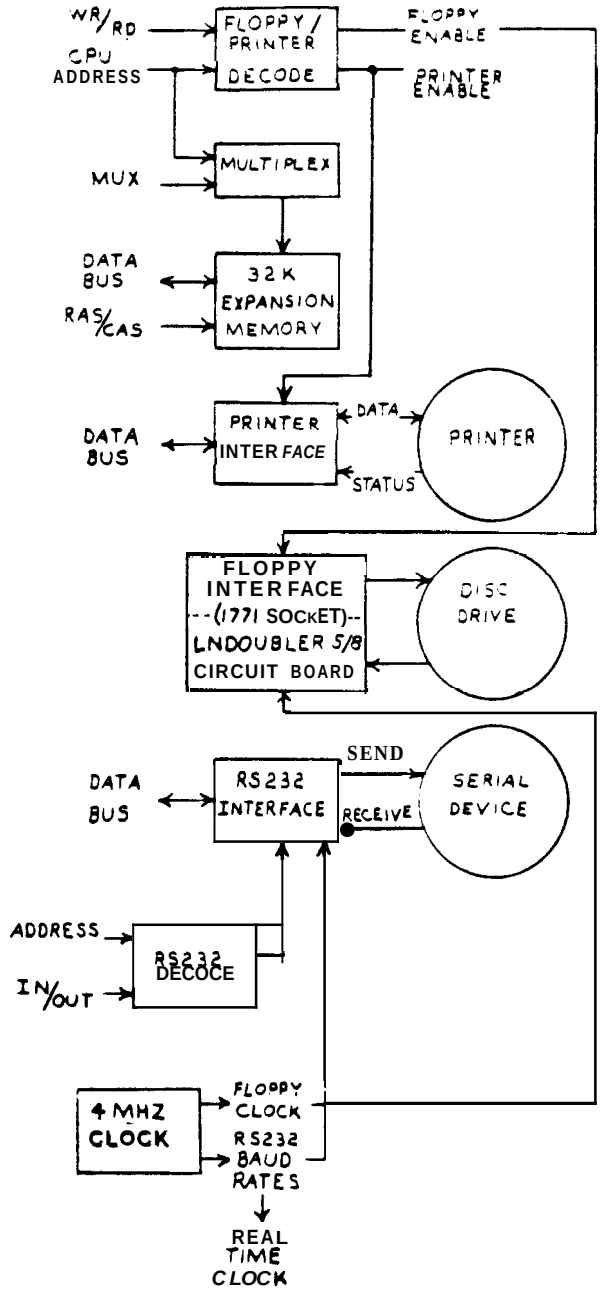


Figure 15. Block Diagram of the LNW 80 Expansion Interface Printed Circuit Board.

The Expansion Interface Board was assembled in the same manner as the Main Computer Board.

An illustration of each side of the expansion board is in Appendix C. The negative transparencies of each side are in Appendix D.

Final Assembly of the LNW 80 Microcomputer

The main computer board was placed right side up inside the bottom of the metal computer cabinet and screwed into place. The expansion board was then placed upside down over the main board and screwed into place. The power supply and several capacitors and fuses were also secured to the bottom of the cabinet. Then the top of the cabinet was placed over the boards and keyboard and screwed into place. Finally, the monitor and the disc drives were connected and the entire system was trouble-shot.

Electrophoresis/Electrochemical Detector Cell Design and Construction

The electrophoresis/electrochemical detector cell was designed and constructed. The design of the electrophoresis/electrochemical detector cell can be seen in Figures 16, 17, and 18.

Controlling the Microcomputer

A BASIC program was written to control the microcomputer. The program requirements were to control the power supply voltage, the time of the electrophoresis period,

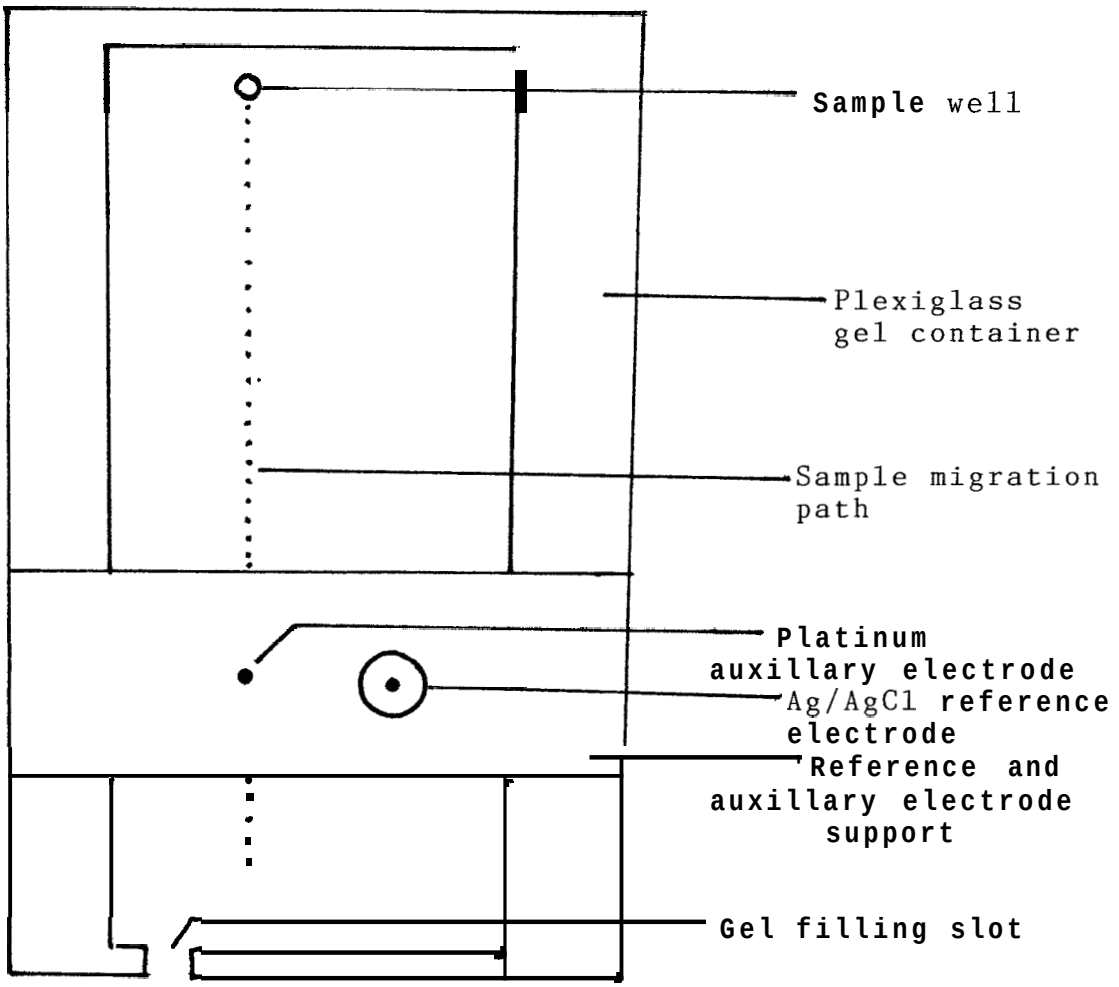


Figure 16. Top View of the Electrophoresis/Electrochemical Detector Cell.

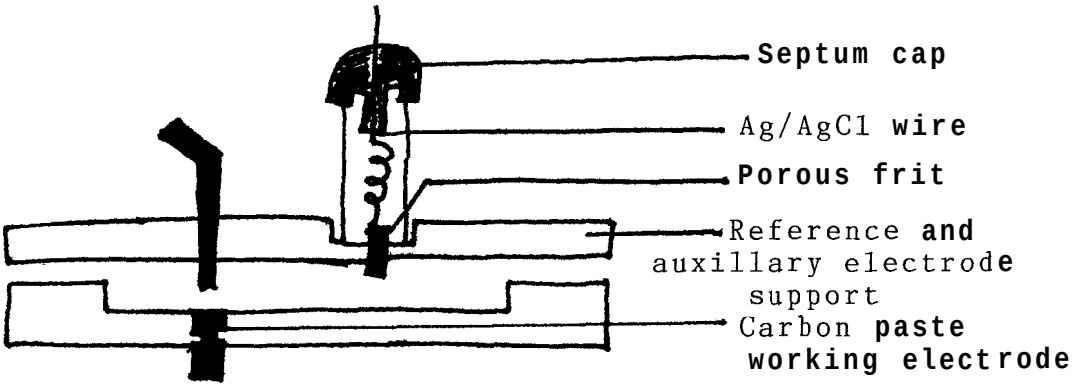


Figure 17. Short Side View of the Electrophoresis/Electrochemical Detector Cell.

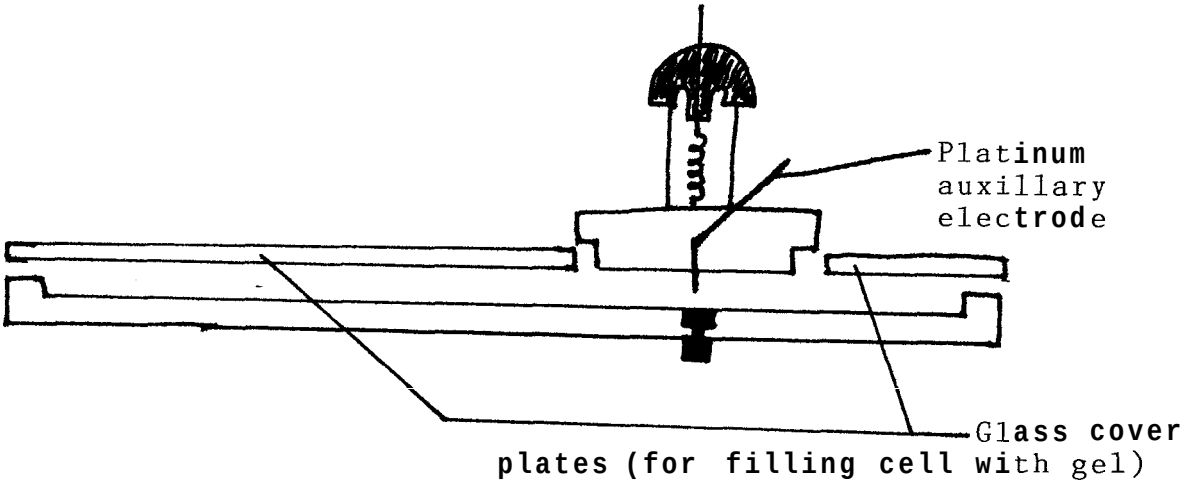


Figure 18. Long Side View of the Electrophoresis/Electrochemical Detector Cell.

the working electrode deactivation time, and the detection time.

Ken Kuzior, a fellow graduate student, helped write and debug the program. The final program is in Appendix E.

Preparation of the Solutions

The buffer used in the electrophoresis and in the preparation of the agarose gel was the first solution prepared. The buffer was an Electra HR Buffer, Tris-Barbiturate Buffer, pH=8.6. One packet of buffer, 18.0 g/packet, was reconstituted with 1.0 liter of deionized water to yield an ionic strength of 0.05. Once the buffer was prepared it was stored in the refrigerator and prepared monthly or as needed.

The second solution prepared was a 0.1 M $K_3Fe(CN)_6$ solution. The ferriccyanide solution was prepared as follows:

1. 16.4625g of $K_3Fe(CN)_6$ was weighed out on an analytical balance.
2. The $K_3Fe(CN)_6$ powder was placed in a 500-mL volumetric flask.
3. The flask was then filled to the mark with deionized water and mixed completely by shaking.

The third and final solution was a DNP-protein complex. This solution was prepared as follows:[62]

1. 0.5 g of the protein and 1 g of sodium bicarbonate were dissolved in a little deionized water.
2. 1 g of 1-Fluoro-2,4-dinitrobenzene (FDNB) was dissolved in 25 mL of ethanol and added to the above solution.

3. This solution was then shaken for 2 hours at room temperature.

4. The solution was then concentrated by heating to remove the ethanol, dissolved in deionized water, and the excess FDNB was extracted with ether.

5. The solution that remained was the DNP-protein complex.

The proteins used in this study were egg albumin and Hemoglobin.

Preparation of the Agarose Gel

The preparation of the agarose gel was as follows:[63]

1. 1.00 g of agarose was weighed out on an analytical balance.

2. 100 mL of a buffer was pipetted into a 125-mL erlenmeyer flask that contained the agarose.

3. The solution was heated to 90 °C in a hot water bath implementing a Thermolyne-1000, heat/stir apparatus.

4. After melting, the solution was cooled under tap water until it felt comfortably warm.

5. The gel was then poured into the electrophoresis cell and placed in the refrigerator.

The agarose gel was ready for electrophoresis 24 hours after it was prepared.

Cyclic Voltammetry Studies

A cyclic voltammetry study was conducted on the cell alone. A platinum electrode was used as the working electrode and a platinum electrode was used as the auxillary electrode. A saturated calomel electrode (S.C.E.) was used as the reference electrode. Figure 19 is the cyclic voltammogram of the cell alone.

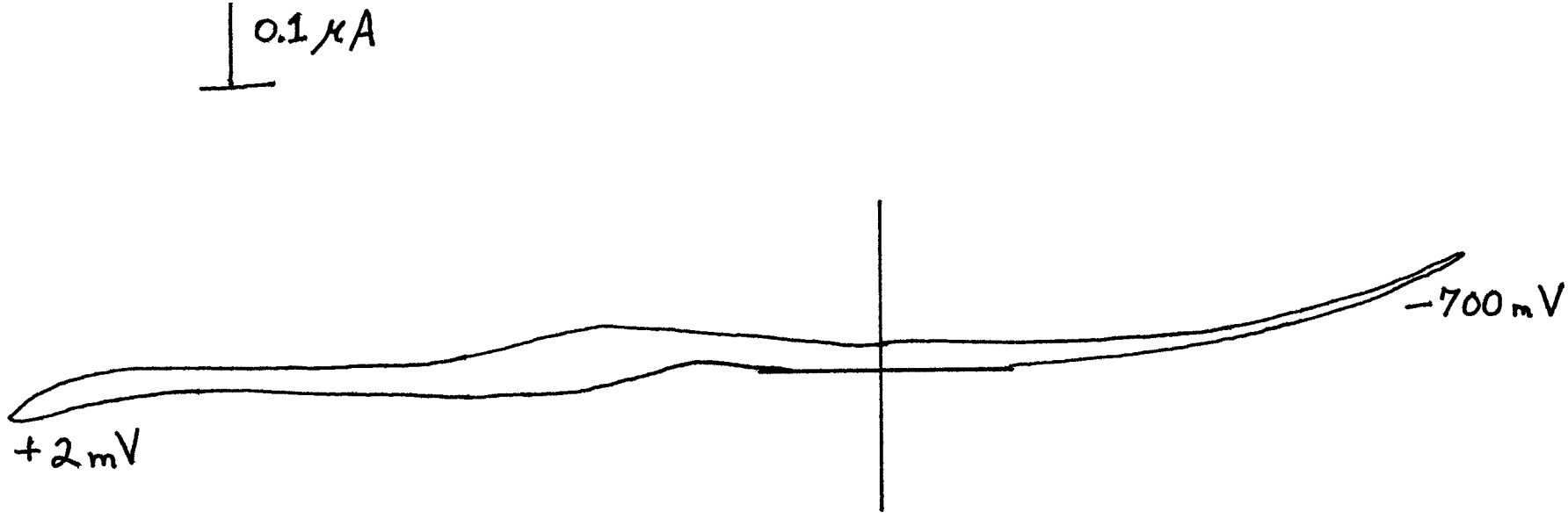


Figure 19. Cyclic Voltammogram of the Electrophoresis/Electrochemical Detector Cell.

A study of an albumin-DNP(FDNB) complex was also carried out using the same electrode system as described above. Figure 20 is the cyclic voltammogram of the albumin-DNP(FDNB) complex.

Agarose Gel Electrophoresis

The agarose gel electrophoresis was carried out as follows:

1. The agarose gel was prepared as outlined in "Preparation of the Agarose Gel" in Chapter IV.
2. The agarose gel was pipetted into the cell so as not to create air bubbles.
3. The electrophoresis/electrochemical cell was connected as illustrated in Figure 21.
4. Then the power supply voltage, the time of the electrophoresis period, the working electrode deactivation time, and the detection time were programmed into the microprocessor.
5. A slit was made in the agarose gel. A strip of filter paper containing the sample to be run was placed in the slit.
6. The microprocessor was then programmed to run.

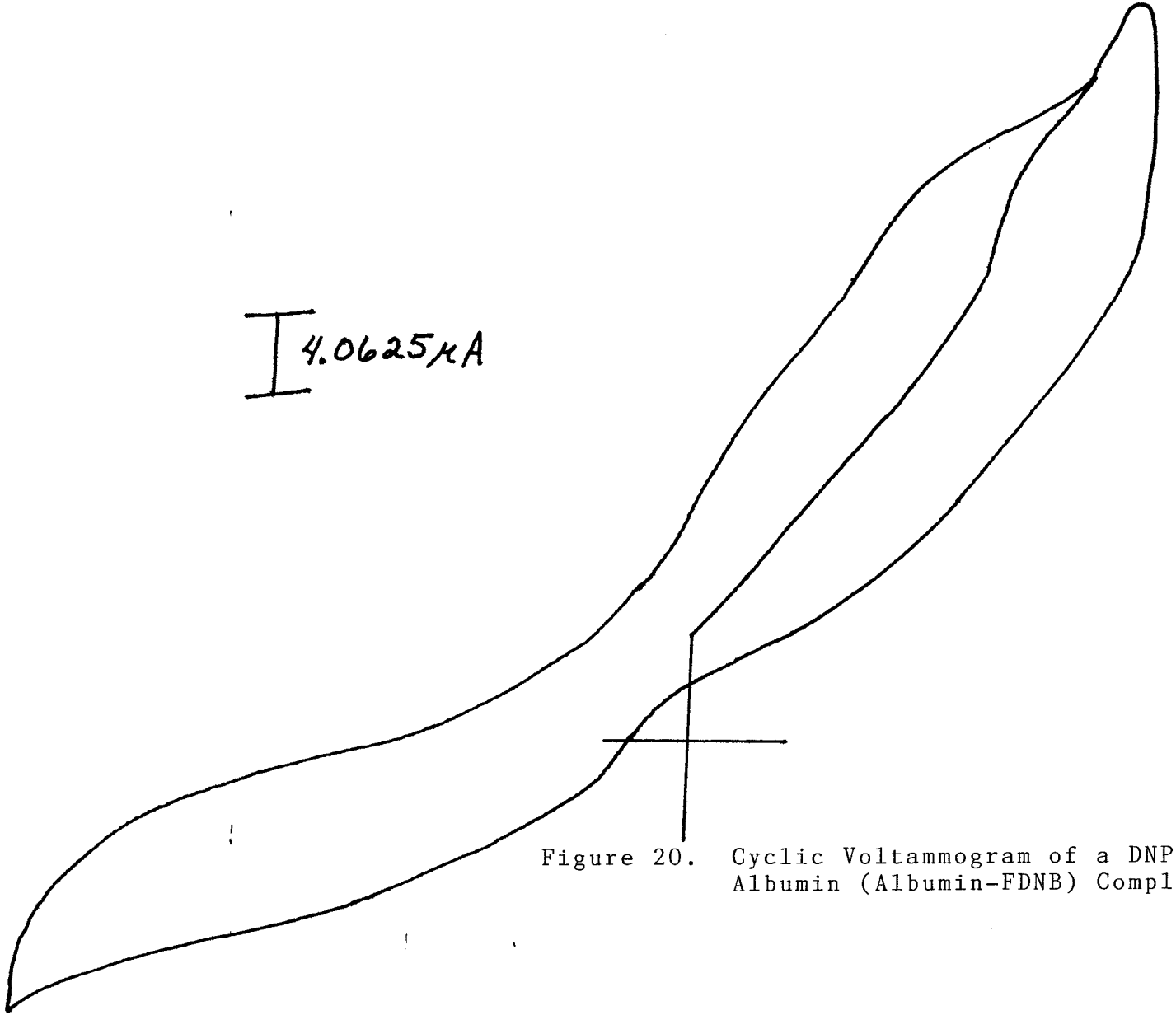


Figure 20. Cyclic Voltammogram of a DNP-Albumin (Albumin-FDNB) Complex.

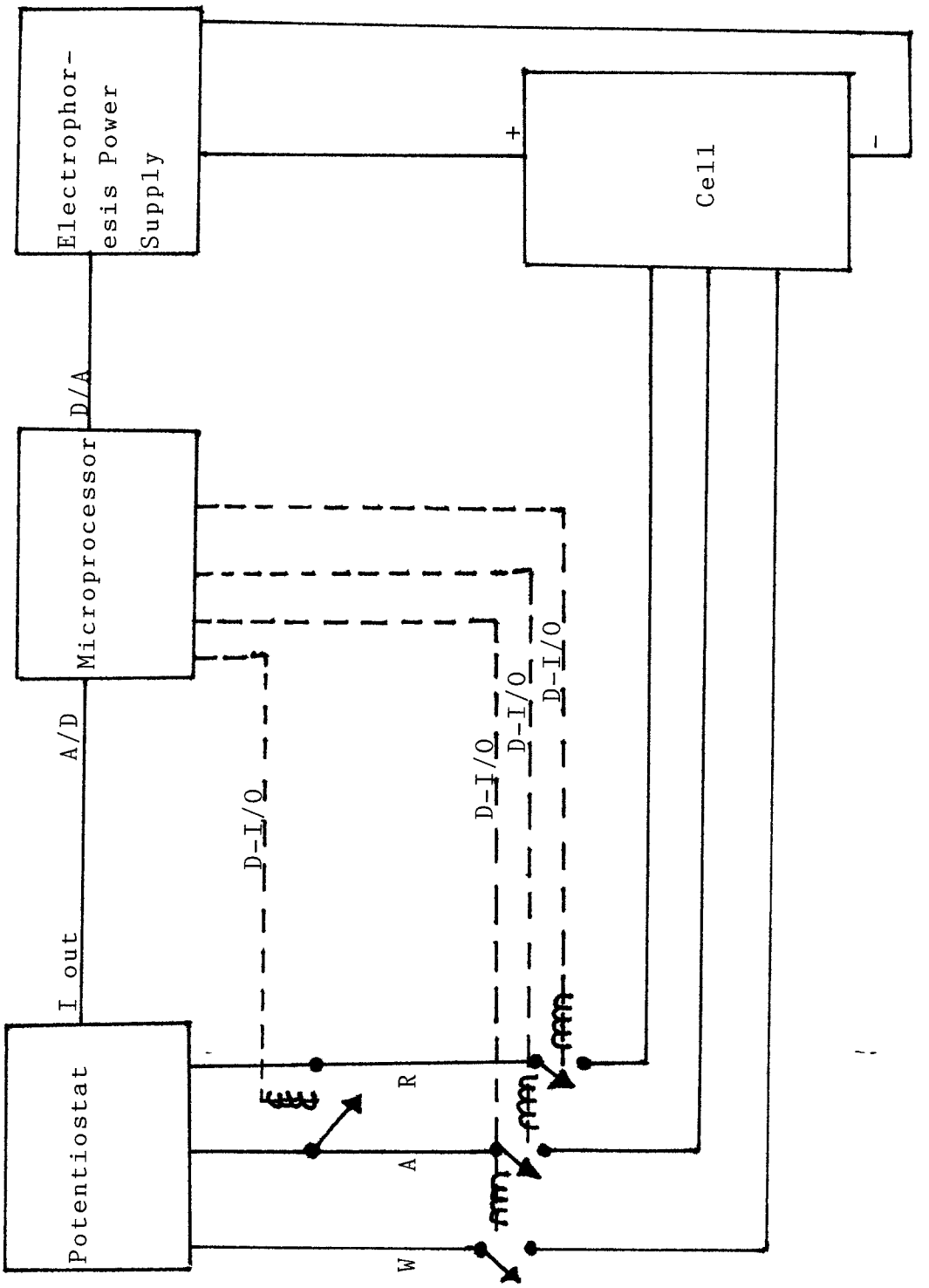


Figure 21. Block Diagram of the Electrophoresis/Electrochemical Cell Instrumentation.

CHAPTER V

RESULTS AND DISCUSSION

Background of the Electrophoresis/Electrochemical Detector Cell

A cyclic voltammetry study of an albumin-DNP(FDNB) complex illustrates the proper function of the Electrophoresis/Electrochemical Detector Cell as the detector system. (See Figure 20).

LNW 80 Microcomputer

The LNW 80 Microcomputer's main printed circuit board and expansion interface printed circuit board were assembled as described in Chapter IV.

The schematics of the circuitry and components of the main printed circuit board can be seen in Appendix A.

The schematics of the circuitry and components of the expansion interface printed circuit board can be seen in Appendix C.

Verification of the Electrophoresis/Electrochemical Cell as a Detector System

As a test of the system, $K_3Fe(CN)_6$ was applied to the agarose gel in the detector cell and electrophoresed at 20 mA/V and 60-second pulse durations. As the yellow-colored $K_3Fe(CN)_6$ passed through the electrode system of the

cell, an increase in current was noted. This is due to the detection of the $Fe_2(CN)_6^{2-}$ ion which is highly electroactive. (See Figure 22).

Agarose Gel Electrophoresis

0.5 µL serum was placed in the agarose gel and electrophoresed at 5 mA/V, 400 V, and 1-minute pulse durations. The serum proteins were detected by this agarose gel electrophoresis procedure. (See Figure 23).

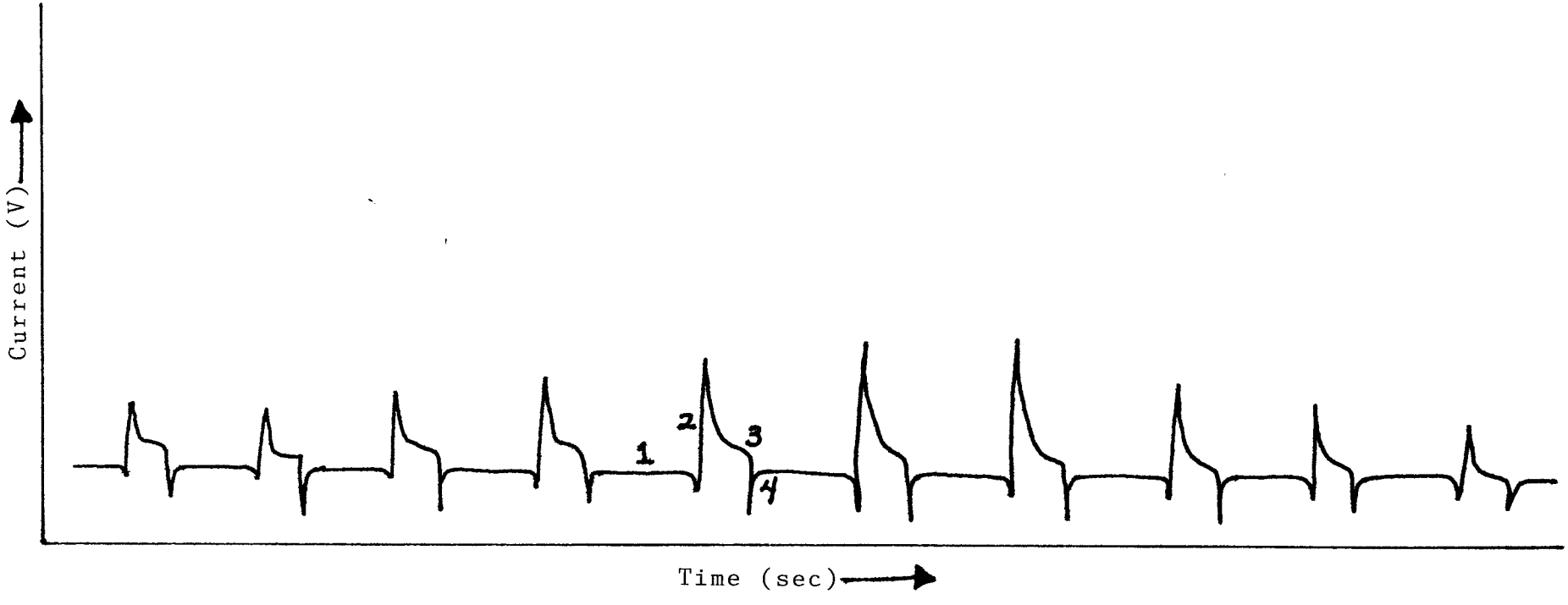


Figure 22. Agarose Gel Electrophoresis of $K_3Fe(CN)_6$.

1. Electrophoresis time interval.
2. Detection of sample.
3. Deactivation time —— power supply is turned off
4. Power supply is turned on.

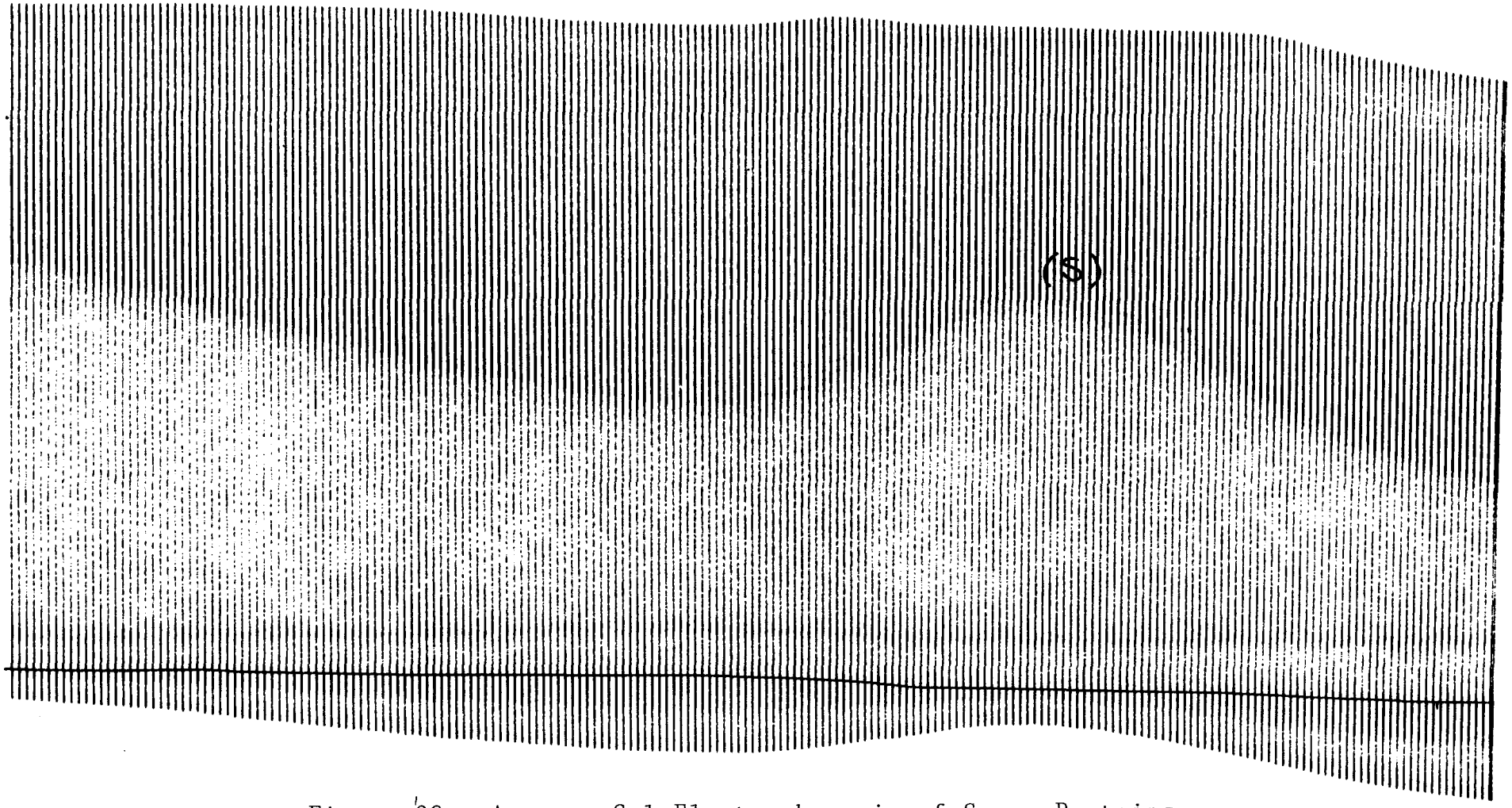


Figure 23. Agarose Gel Electrophoresis of Serum Proteins.

The peaks as represented in Figure 22 are condensed in this figure to show the separation peak of the serum proteins (S).

CHAPTER VI

CONCLUSIONS

The design, construction, and application of a microprocessor-controlled electrophoresis power supply implementing an electrophoresis/electrochemical cell as a detector of serum proteins by agarose gel electrophoresis was carried out in this study.

The electrophoresis/electrochemical detector cell proved to be a valuable means of detecting serum proteins — although separation was not accomplished. The lack of separation is believed to be due to the N-terminus groups being destroyed by the dye FDNB. The destruction of the N-terminus groups apparently causes all of the proteins to look alike in an electric field. Therefore, the separation of the proteins is impossible.

By programming the microprocessor to cause the electrochemical potentiostat to operate in pulses rather than continuously, the effective concentration of the proteins was drastically increased producing a current which was much easier to detect. Thus, the detection of the serum proteins is possible.

The microprocessor-controlled power supply performed as expected. The microprocessor turned the power supply on and off as programmed. Without the use of the micro-

processor to control the power supply, this experiment would have been impossible.

This method can also be employed to detect isoenzymes such as LDH as was noted in Gary Boano's thesis entitled "Electrochemical Detection of LDH Isoenzymes".

Cytochromes, oxidases, and many other redox enzymes which are already electroactive, can also be detected by this method. Since these redox enzymes are already electroactive, the process of making them electroactive can be omitted. Thus, the experimental work can be completed in a much shorter time period and therefore, more runs done.

Various electrophoretic techniques, in addition to agarose gel, could be carried out using this electrophoresis/electrochemical detector cell. Such techniques include polyacrylamide gel electrophoresis, starch gel electrophoresis, and cellulose acetate gel electrophoresis.

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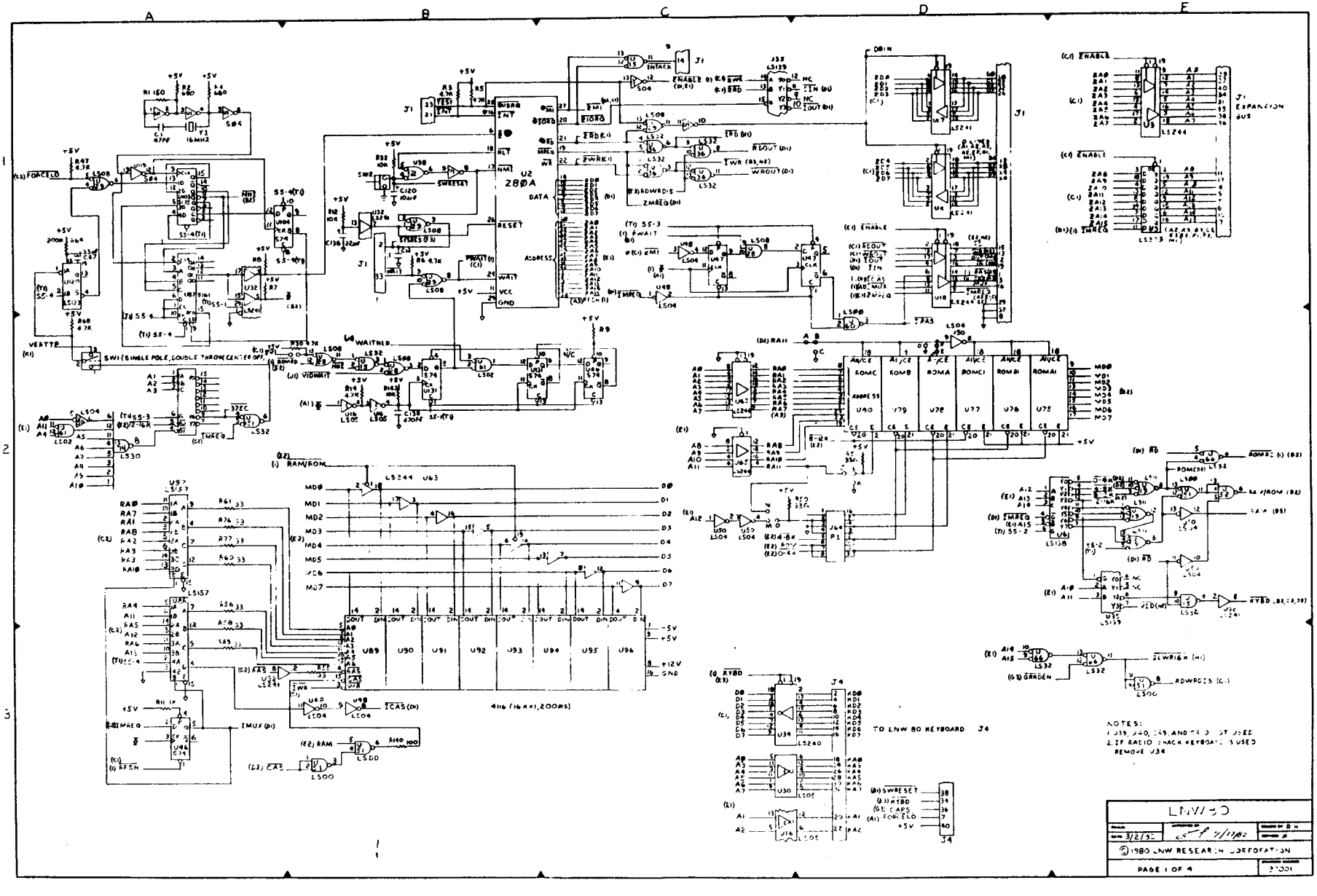
APPENDIX A

Schematics of the Circuitry and Components of the Main Printed Circuit Board[64]


LNW80
3/2/82
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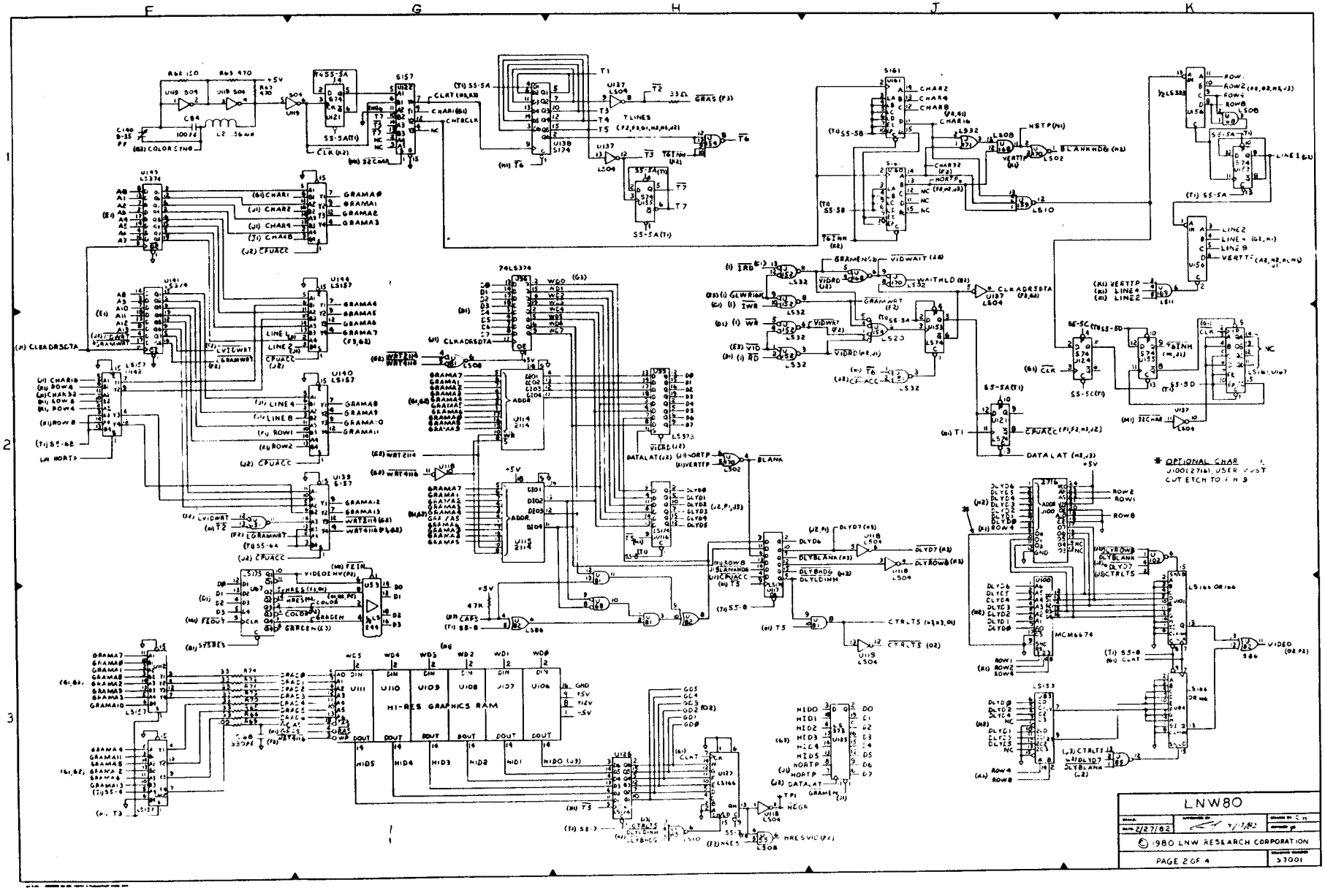
PAGE 1 OF 4
U2
280A
TO LNW 80 KEYBOARD J4
NOTES:
1. U39, U40, U29, AND U30 ARE NOT USED
2. IF RADIO SHACK KEYBOARD IS USED REMOVE U39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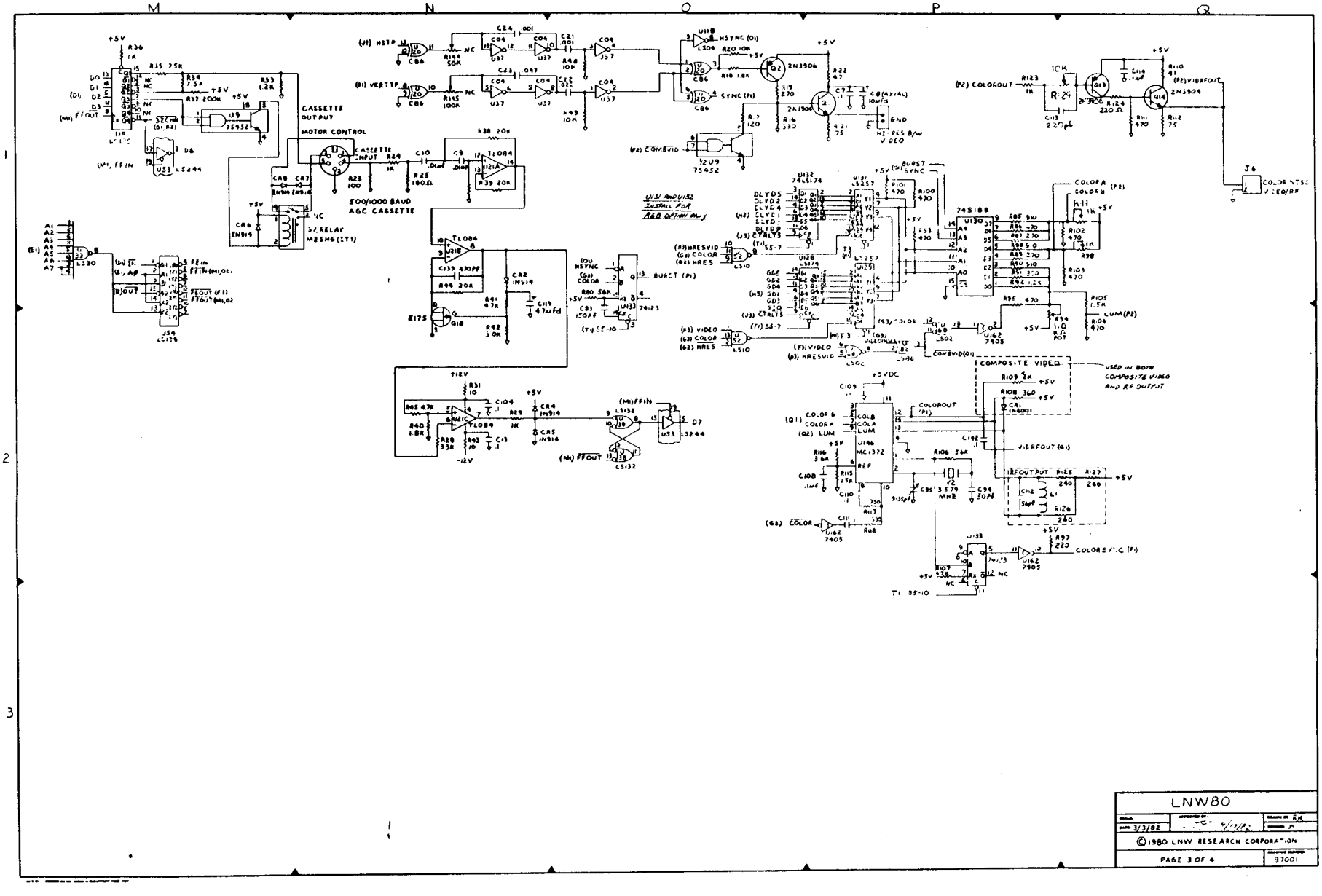
57001
HI-RES GRAPHICS RAM
* OPTIONAL CHAR GEN 2716 (2716) USER MUST CUT ETCH TO PIN 9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HSYNC (01)
SYNC (P1)
VERTTP
HSTP
COMPOSITE VIDEO
USED IN BOTH COMPOSITE VIDEO AND RF OUTPUT
COLOR NTSC VIDEO/RF
COLOR A (P2)
COLOR B
LUM (P2)
COLORES NTSC (F)
U131 AND U132 INSTALL FOR R&D OPTION ONLY
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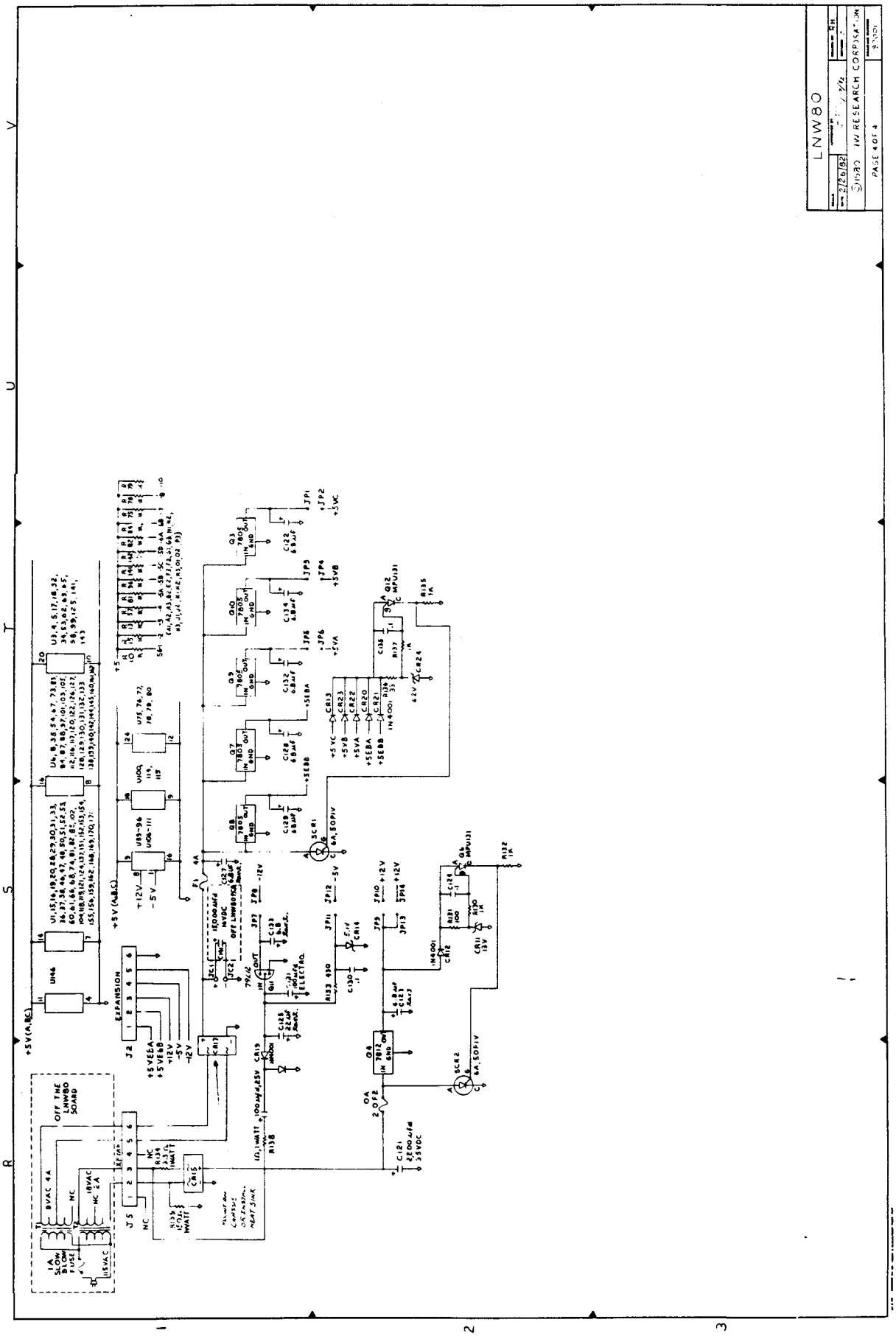
LNW80
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LNW RESEARCH CORPORATION
PAGE 4 OF 4
EXPANSION
OFF THE LNW80 BOARD
+5V(A,B,C)
+12V
-5V
-12V
+5VEBA
+5VEBB
+5VC
+5VB
+5VA
+5EBA
+5EBB
+42V
1A SLOW BLOW FUSE
8VAC 4A
18VAC 2A
NC
J2
J5
U146
U89-96
U100, 115, 118
U97, 74, 77, 78, 79, 80
U1, 15, 16, 19, 20, 28, 29, 30, 31, 33, 34, 37, 38, 46, 47, 48, 50, 51, 52, 53, 60, 61, 66, 68, 74, 81, 82, 85, 102, 104, 118, 119, 121, 124, 137, 151, 152, 153, 154, 155, 156, 159, 162, 168, 169, 170, 171
U3, 4, 5, 17, 18, 32, 34, 53, 62, 63, 95, 58, 59, 125, 141, 143
U6, 8, 35, 54, 67, 73, 83, 84, 87, 88, 97, 101, 103, 105, 112, 116, 117, 120, 122, 126, 127, 128, 129, 130, 131, 132, 133, 138, 139, 140, 142, 144, 145, 149, 167
SW1
F1 4A
C127 1500uFd 40VDC
C121 2,200 ufd 35VDC
C123 6.8 Tant.
C124 1 Tant.
C125 22uFd Tant.
C126 6.8 Tant.
C128 68uFd
C129 68uFd
C130 .1
C131 100uFd ELECTRO.
C132 68uFd
C133 6.8 Tant.
C134 68uFd
C135 1
C136 1
C122 68uFd
Q1 79L12 IN GND OUT
Q2 79L05 IN GND OUT
Q3 79L05 IN GND OUT
Q4 79L12 IN GND OUT
Q5 79M05 IN GND OUT
Q6 79M05 IN GND OUT
Q7 79M05 IN GND OUT
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Q10 79M05 IN GND OUT
Q11 MPU131
Q12 MPU131
Q13 MPU131
SCR1 C6,50PIV
SCR2 C6,50PIV
CR1 1N4001
CR2 1N4001
CR3 1N4001
CR4 1N4001
CR5 1N4001
CR6 1N4001
CR7 1N4001
CR8 1N4001
CR9 1N4001
CR10 1N4001
CR11 15V
CR12 1N4001
CR13 1N4001
CR14 1N4001
CR15 1N4001
CR16 5.1V
CR17 1N4001
CR18 1N4001
CR19 1N4001
CR20 1N4001
CR21 1N4001
CR22 1N4001
CR23 1N4001
CR24 42V
R131 100
R132 1K
R133 430
R134 1K
R135 1K
R136 1K
R137 1K
R138 10,1WATT
R139 2.2K 1WATT
R140 2.2K 1WATT
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R142 330
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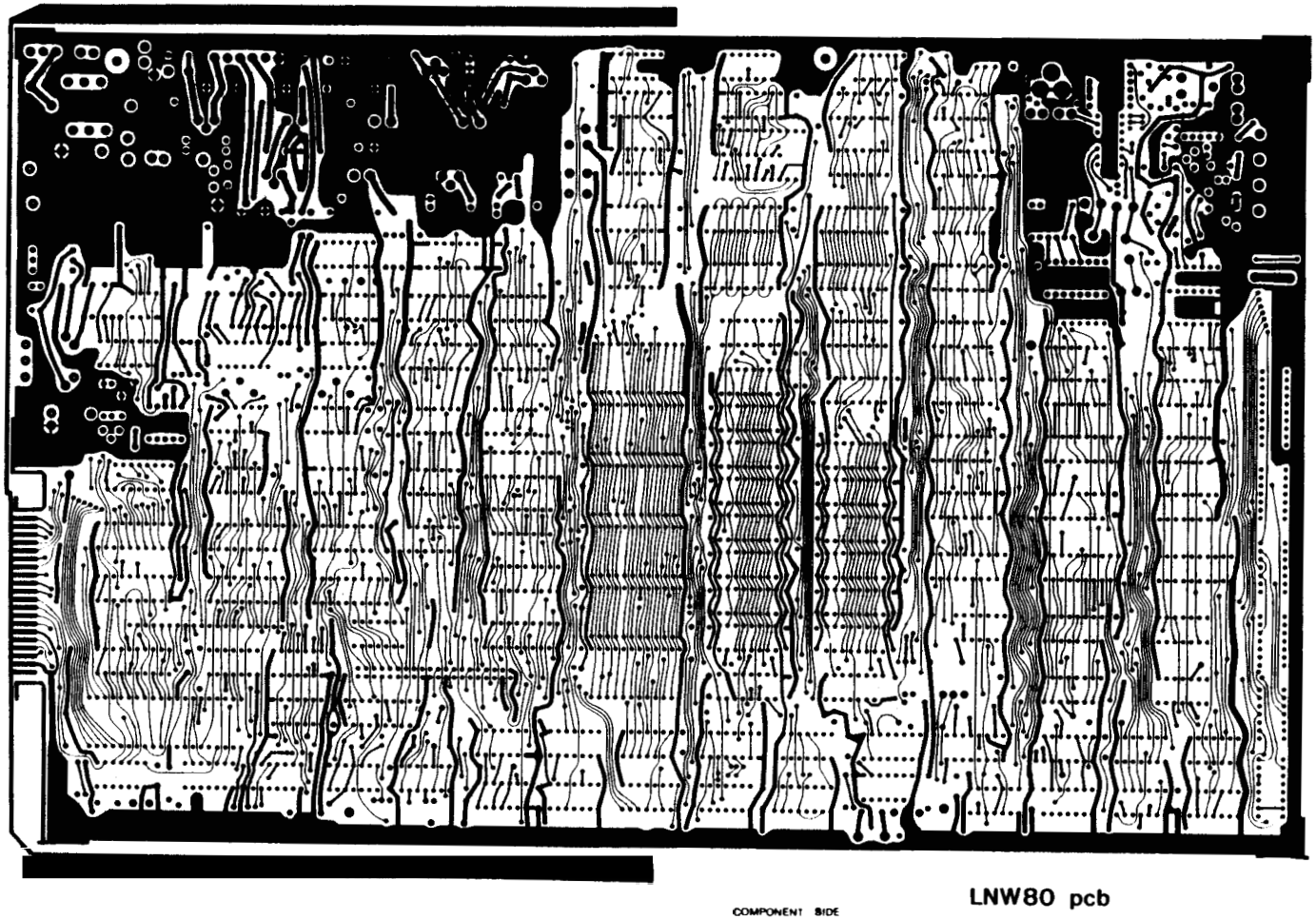
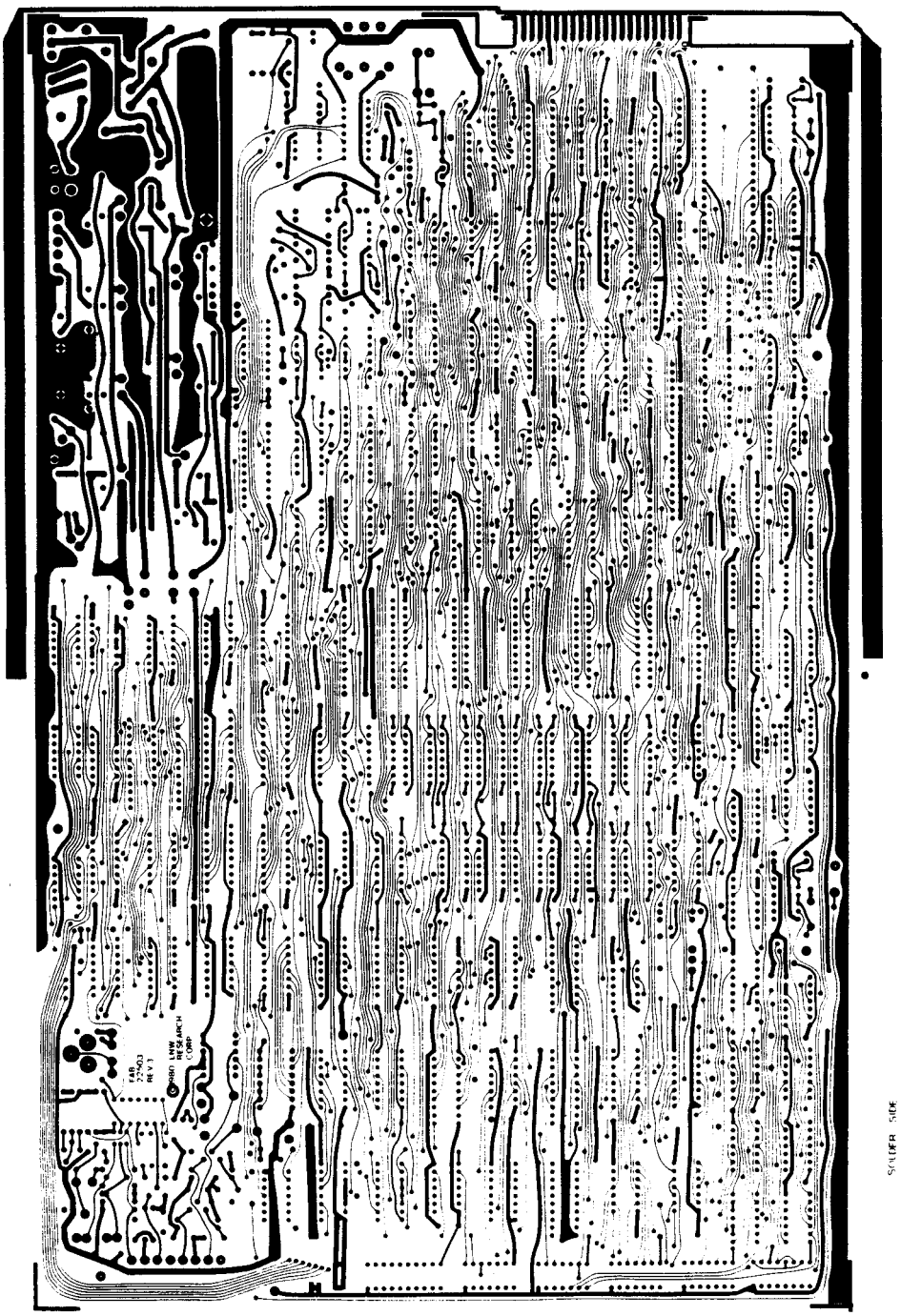
APPENDIX B

The Negative Transparencies of Each Side of the LNW 80 Main Computer Printed Circuit Board[65]



COMPONENT SIDE

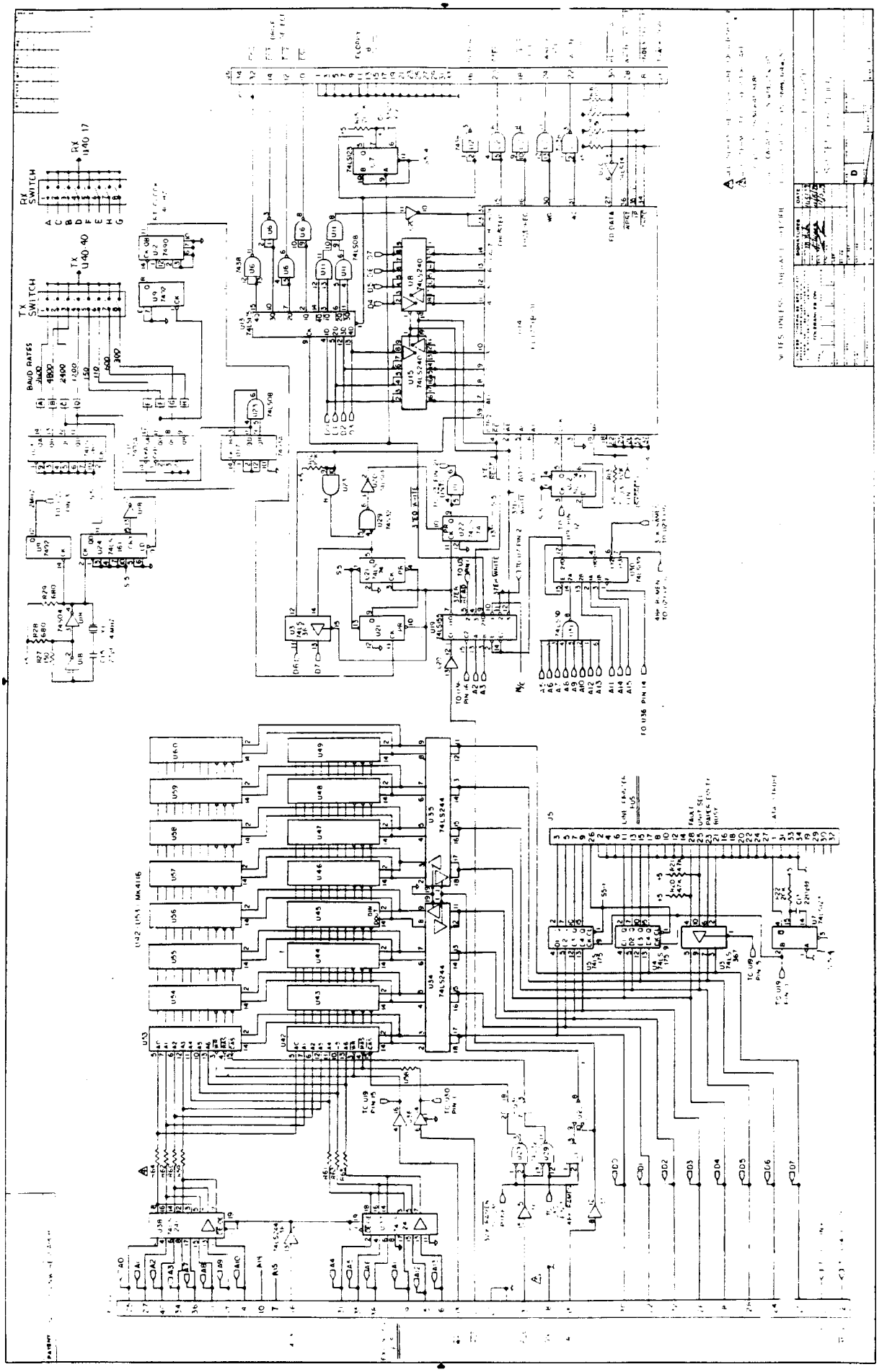
LNW80 pcb

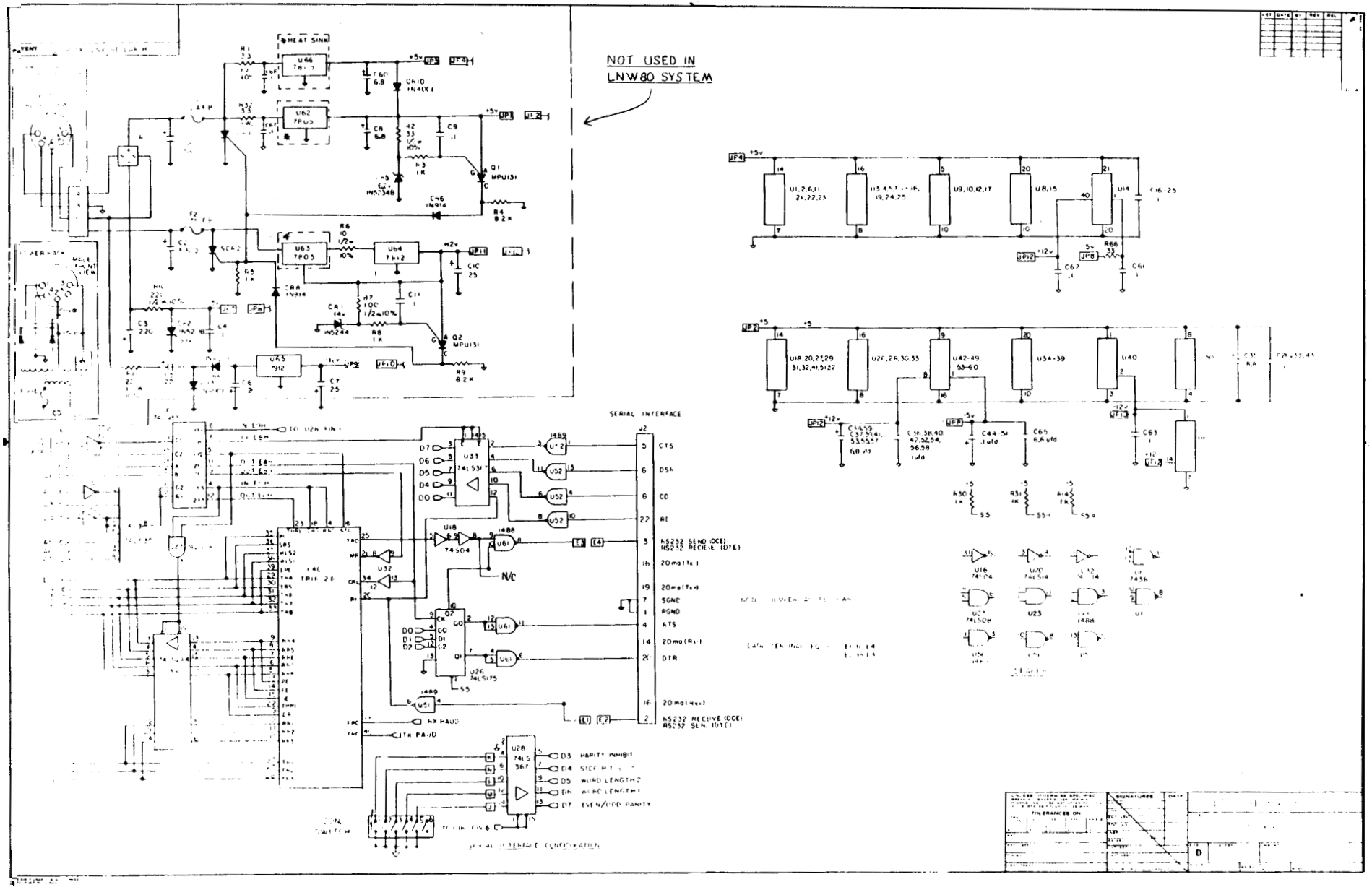


LNW80 pcb

APPENDIX C

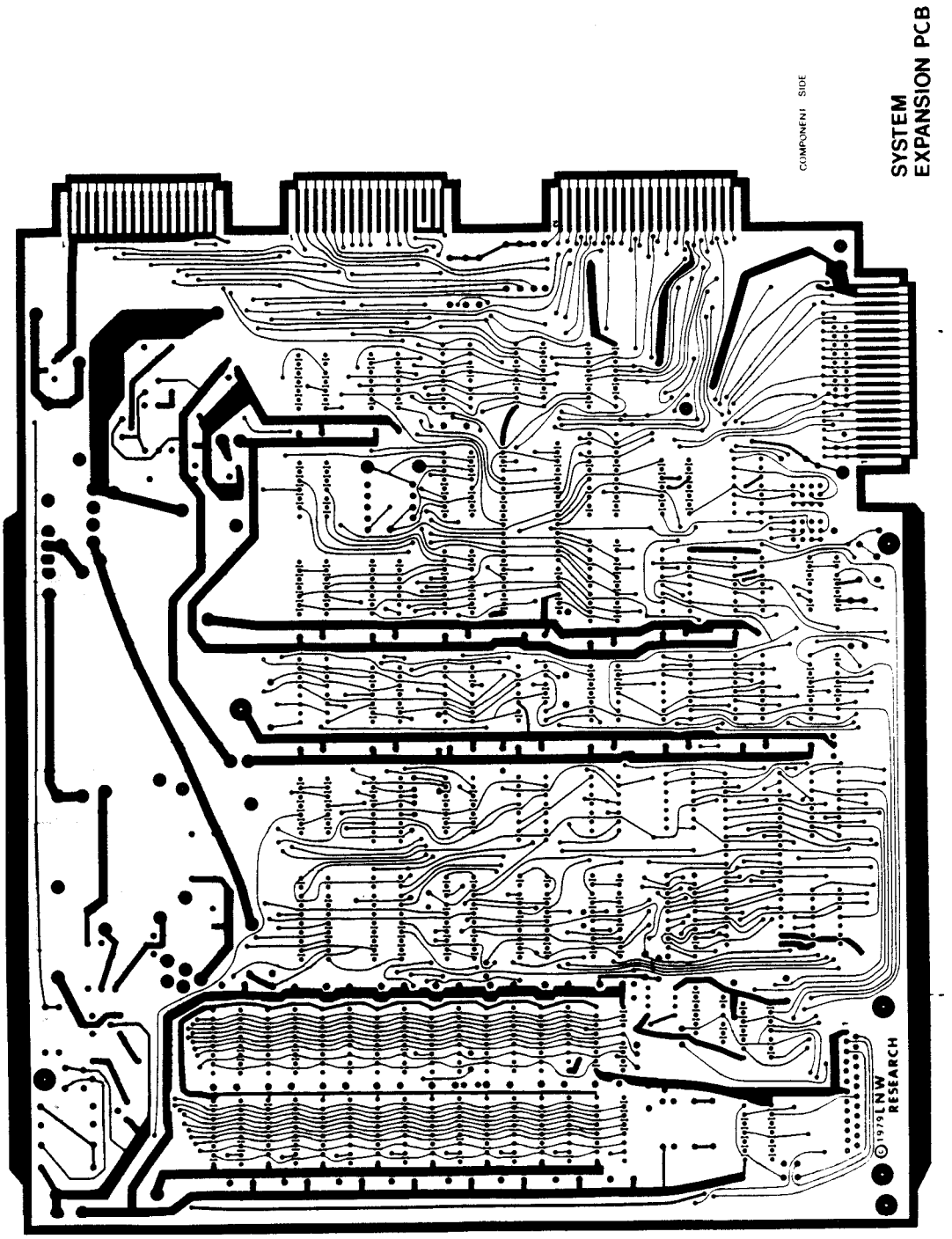
Schematics of the Components of the Expansion Interface Printed Circuit Board66



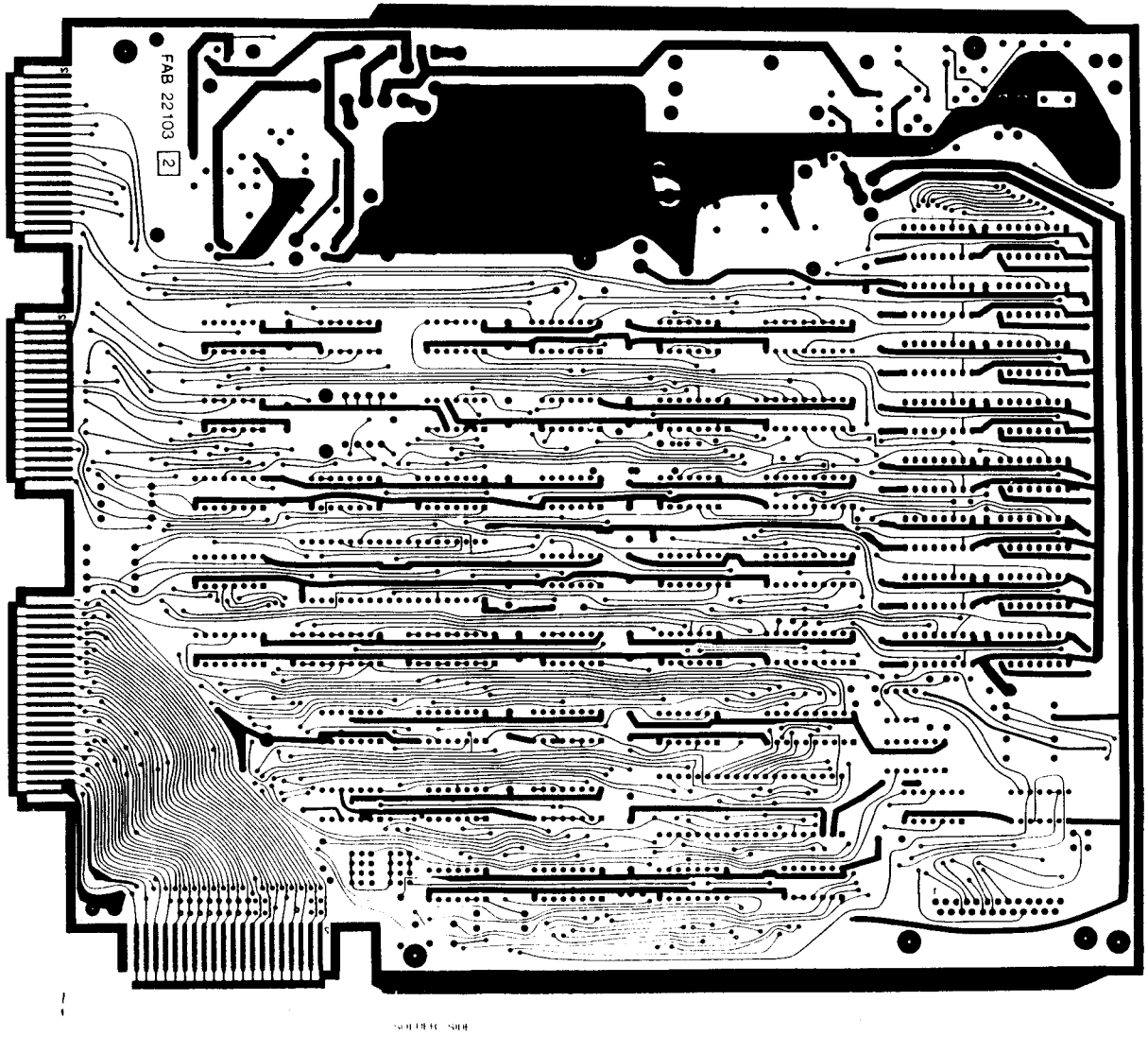

NOT USED IN
LNW80 SYSTEM
HEAT SINK
SERIAL INTERFACE
RS232 SEND (DCE)
RS232 RECEIVE (DTE)
20ma TX
20ma TX
SGND
PGND
CTS
20ma RX
DTR
RS232 RECEIVE (DCE)
RS232 SEND (DTE)
N/C
RX BAUD
TX BAUD
PARITY INHIBIT
WORD LENGTH 2
WORD LENGTH 1
EVEN/ODD PARITY

APPENDIX D

The Negative Transparencies of Each Side of the LNW 80 Expansion Interface Printed Circuit Board[67]


SYSTEM
EXPANSION PCB
COMPONENT SIDE
©1979 L N W
RESEARCH

SYSTEM EXPANSION PCB



APPENDIX E

Final Computer Program

This is the program for an Apple IIe with Addalab. The Apple IIe computer was used for the completion of this project instead of the LNW 80 because the programming of the Apple IIe is easier than the LNW 80 computer.

```
1.  HIMEM:  36095  D%=0:  DIM C%(5), Q%(5), D%(DESIRED SIZE):
    PRINT CHR$(4) "BRUN QUICK I/O"
20.  INPUT "POWER SUPPLY VOLTAGE?"; V
21.  IF V< 800 THEN GOTO 29
22.  IF V> 800 THEN PRINT
23.  PRINT "YOUR POWER SUPPLY VOLTAGE IS TOO HIGH FOR THIS
     SYSTEM PRESENTLY!"
24.  PRINT "WOULD YOU LIKE TO CHOOSE ANOTHER VOLTAGE? (Y  OR N)":
     INPUT YN$
25.  IF YN$="Y" THEN GOTO 20
26.  IF YN$="N" THEN GOTO 260
29.  PRINT
30.  INPUT "TIME OF ELECTROPHORESIS PERIOD IN SECONDS?"; D1
39.  PRINT
40.  INPUT "WORKING ELECTRODE DEACTIVATION TIME IN SECONDS?"; D2
41.  IF D2> D1 THEN GOTO 49
42.  IF D2< D1 THEN PRINT
43.  PRINT
44.  PRINT "WARNING!!!  THE DEACTIVATION TIME PERIOD SHOULD
     EXCEED THE ELECTROPHORESIS TIME PERIOD BY A SUFFICIENT
     MARGIN TO ALLOW THE POWER SUPPLY TO ACHIEVE ZERO OUTPUT
     VOLTAGE."
45.  PRINT
46.  PRINT "WOULD YOU LIKE TO CHOOSE A NEW DEACTIVATION TIME?
    (Y  OR N)":  INPUT YN$
47.  IF YN$= "Y" THEN GOTO 40
48.  IF YN$= "N" THEN GOTO 260
49.  PRINT
50.  INPUT "DETECTION TIME IN SECONDS?": D3
51.  IF D3> D2 THEN GOTO 100
52.  IF D3< D2 THEN PRINT
53.  PRINT
54.  PRINT "DETECTION TIME PERIOD SHOULD BE A TIME FOLLOWING
     THE DEACTIVATION TIME PERIOD!"
55.  PRINT
56.  PRINT "WOULD YOU LIKE TO CHOOSE A NEW DETECTION TIME?
    (Y  OR N)":  INPUT YN$
57.  IF YN$= "Y" THEN GOTO 50
58.  IF YN$= "N" THEN GOTO 260
100.  D%= 32767: & TO 1
120.  & TI1:  T= D%
140.  D%= 0:  & DOO
143.  FOR X= 1 TO 50:  NEXT
145.  D%= 0:  & DO1:  & D02:  & DO3
150.  T1= T - (D1 * 10)
155.  GOSUB 2000
160.  D%= ((2048  / 893) * V) + (FUG  / 10) ; &AOO
170.  & TI1:  IF D%> T1 GOTO 170
180.  D%= 0:  &AOO
190.  T2= T - (D2  * 10)
200.  & TI1:  IF D%> T2 GOTO 200
205.  D%= 1:  & DO1:  & D02:  & DO3
207.  FOR X= 1 TO 50:  NEXT
```

```
210.  D%= 1:  & DOO
220.  T3= T - (D3  * 10)
230.  & TI1:  IF D%> T3 GOTO 230
240.  & AIO:  FOR I= 1 TO 50:  NEXT I:  & AIO
245.  PRINT "ANALOG INPUT = "D% / 2000" VOLTS"
250.  GOTO 100
260.  PRINT "THANK YOU":  END
2000.  REM SUBROUTINE
2010.  IF 0< V AND V< 101 THEN FUG = 1866
2020.  IF 101< V AND V< 201 THEN FUG = 1666
2030.  IF 201< V AND V< 301 THEN FUG = 1466
2040.  IF 301<V AND V< 401 THEN FUG = 1266
2050.  IF 401< V AND V< 501 THEN FUG = 1066
2060.  IF 501< V AND V< 601 THEN FUG = 866
2070.  IF 601< V AND V< 701 THEN FUG = 666
2080.  IF 701< V AND V< 801 THEN FUG = 466
2100.  RETURN
```