DESIGN AND UTILIZATION OF A MICROPROCESSOR-CONTROLLED ELECTROPHORESIS POWER SUPPLY

by

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Submitted in Partial Fulfillment of the Requirements

for the Degree of

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TITLE DESIGN AND UTILIZATION OF A MICROPROCESSOR-CONTROLLED ELECTROPHORESIS POWER SUPPLY

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ABSTRACT

DESIGN AND UTILIZATION OF A MICROPROCESSOR-CONTROLLED ELECTROPHORESIS POWER SUPPLY

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MASTER OF SCIENCE

YOUNGSTOWN STATE UNIVERSITY, 1987

Electrophoresis has successfully been employed for many years by chemists to separate biologically active materials. It has proven to be an important clinical tool for the analysis of such components of blood as hemoglobin, proteins, enzymes and other biological material. The electrophoretic patterns resulting from these studies have been shown to be related to certain pathological conditions in the human body.

Many variations in the electrophoretic technique to suit different purposes have been suggested. Considerable changes in such areas as supporting media, buffer systems, geometric arrangements, dimensions and cooling techniques have been used. However, relatively little attention has been given to the power supply.

In the past, the typical power supplies were totally analog in nature. They provided output variation only in power range and regulation such as constant voltage, current and wattage. These types of regulation could not be maintained with any accuracy. Therefore, the development of

a digitally-controlled power supply would suggest a means to achieve better resolution.

In this study, a microprocessor equipped with analog interface circuitry is substituted for the typical electronic feedback loop encountered in commercial electrophoresis power supplies. Voltage and current data are sent to analog-to-digital converters and a digital-to-analog converter is employed to impress an input signal on the power supply. Simple BASIC programs are employed to maintain constant voltage or current and a multiply subroutine is utilized to produce constant power. Other subroutines enable various waveforms, such as square and saw tooth waves, with different DC offsets, to be put out by the power supply. A non-invasive infrared (IR) temperature probe is used to monitor the heat generated in the electrophoresis medium, either cellulose acetate or agarose gel, during all procedures. The voltage signal originating from the IR temperature probe is additionally employed to perform constant-temperature electrophoresis.

The electrophoresis patterns of several hemoglobin-variant controls and serum proteins were compared under constant voltage, current, wattage and temperature conditions. The migration rates and resolutions of these variants were also examined in experiments employing different pulse techniques. It was indicated from this investigation that constant-temperature, non-pulsed electrophoresis can reduce experimental time with an increase in resolution.

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Finally, I would like to thank Linda for giving me the encouragement to finish writing this text.

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LIST OF SYMBOLS

SYMBOL	DEFINITION
AC	Alternating current
A/D	Analog-to-digital
°C	Degrees Celsius
cm	Centimeter
D/A	Digital-to-analog
DC	Direct current
g	Gram
H^+	Hydrogen ion
I	Current, amps
IR	Infrared
kΩ	Thousand-ohm
L	Liter
MΩ	Mega-ohm
µg	Microgram
µF	Microfarad
µL	Microliter
mA	Milliamp
mL	Milliliter
mm	Millimeter
mV	Millivolt
min	Minute
M	Molar
nm	Nanometer
OH^-	Hydroxide ion
op. amp.	Operational amplifier

P	Power, watts
pH	Hydrogen ion concentration
pI	Isoelectric point
R	Resistance, ohms
s	Second
V	Voltage, volts
v/v	Volume-to-volume
W	Wattage, watts
w/v	Weight-to-volume

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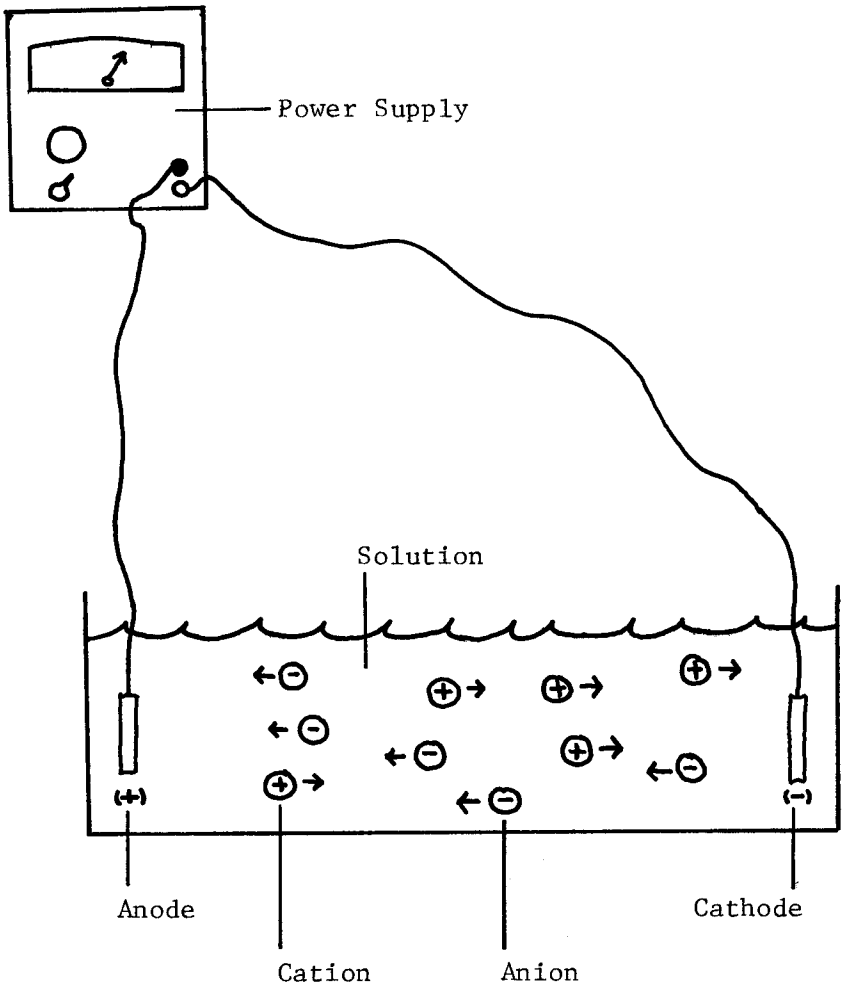
CHAPTER I

INTRODUCTION

Definition of Electrophoresis

In the study of a material of biological origin, or of a biological process, one is confronted with the problem of having to separate and examine the properties of molecules of high molecular weight such as proteins, enzymes, nucleic acids, complex lipids and carbohydrates. It is also necessary to accomplish this task without causing any damage that would affect the properties of these molecules (1). Several methods of separation are available, but this study will be limited to the technique known as electrophoresis. This technique is a powerful tool which a researcher or clinician can use to monitor biological processes or diagnose specific disease states.

The term electrophoresis, which is derived from Greek, means "borne by electricity". This is appropriate since electrophoresis involves the migration of charged particles under the influence of an electric field. An electric field is applied to the solution in Figure 1 (2). Here, two oppositely-charged electrodes are placed at opposing ends of the container. When the electric field is applied, a particular particle or ion travels through the solution toward the electrode of opposite charge depending



Electrophoresis of Ions

in Solution

Figure 1

on the sign of its charge.

In Figure 2, a representation of a typical contemporary electrophoresis chamber is shown (3). A solid support medium makes contact with the buffer by means of a paper wick. The charged particle is placed on the medium near the negative electrode because buffers of basic pH are generally used for electrophoresis. The basic buffer causes the protein to be ionized with a negative charge and thus migration is achieved from the cathode to the anode when a potential difference is applied.

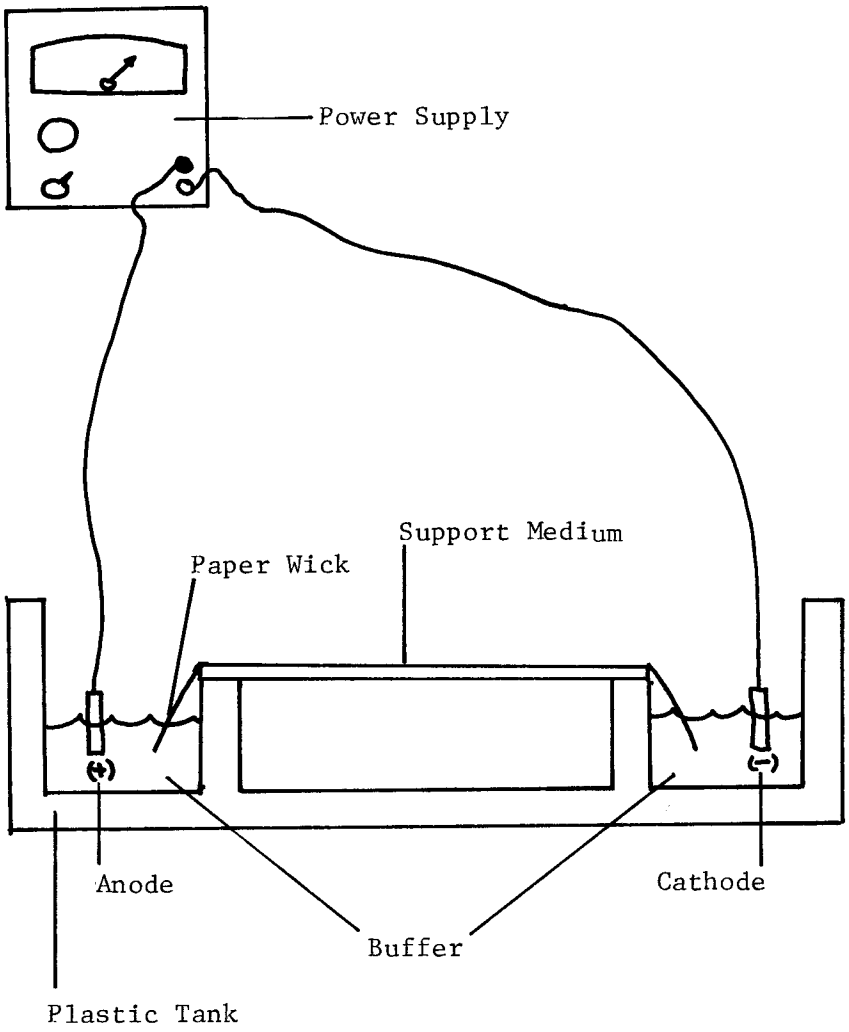
For example, a specific particle such as a protein is similar to an amino acid in that it may be charged positively or negatively depending on the presence of positive or negative ions in the buffer solution. Since a protein is basically a long chain of amino acids, the net charge of the protein is easily described by the following:

$$NH_3^+ \text{-R-}COO^-$$

This structure represents a species with no net charge, which is favored at a pH referred to as the isoelectric point. The net charge on the protein is made more positive or negative by simply adjusting the pH as shown:

$$NH_3^+ \text{-R-COOH} \xleftarrow{H^+} NH_3^+ \text{-R-}COO^- \xrightarrow{OH^-} NH_2\text{-R-}COO^-$$

However, negative charges are almost always preferred when electrophoresing since positively-charged proteins exhibit more adsorption to the medium than negatively charged proteins (4).



Electrophoresis Chamber

Figure 2

Electrophoresis works because charged particles migrate in the presence of an external electric field. It functions as a means of separation because physical properties such as molecular size and charge-to-mass ratios cause different molecules to have different velocities in an electric field. Thus, electrophoresis is used as a separation technique just as chromatography or ultracentrifugation.

Principles of Electrophoresis

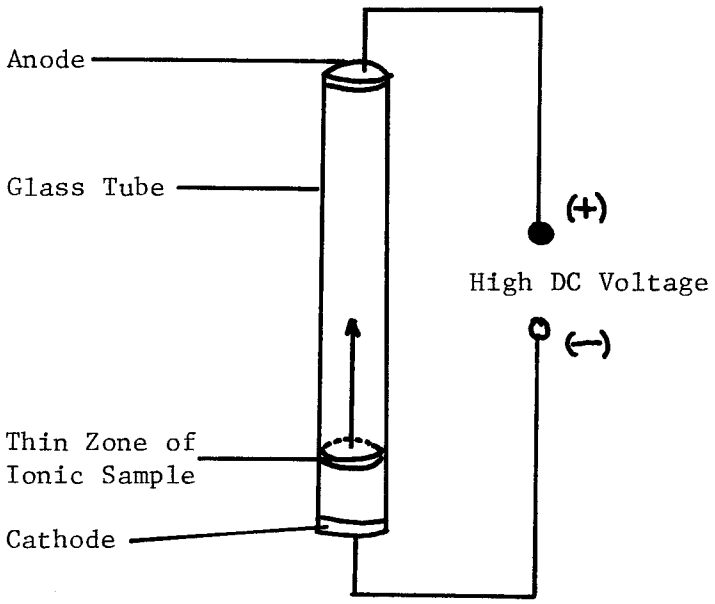
If the ionic band in Figure 3 did not diffuse during an electrophoretic experiment, the following equations would apply to the migration of the ions (3). Foremost, there exists a force causing the ions to accelerate up the tube. It is represented by the equation:

$$F = Q\ E \qquad (1)$$

where F is the force of electrostatic attraction, Q is the charge on the ion and E is the applied electrical potential. As the ions approach the electrode of attraction, the electrostatic force increases thus increasing the velocity of the ions. An opposing force caused by the viscosity of the solution is given by the equation:

$$Fs = 6\ \pi\ rnv \qquad (2)$$

where Fs is the opposing force, r is the ionic radius, n is the viscosity of the solution and v is the velocity of the ions (3). It is shown by this equation that an increase in velocity will increase the opposing force. Therefore, at



Ideal Electrophoresis Experiment

Figure 3

some point during the experiment, the electrostatic and opposing forces are equal, thus giving

$$E\ Q = 6\pi rnv \qquad (3)$$

as the combination of equations 1 and 2 (3).

The ability of a particle to move in an electric field is given as:

$$U = v / E \qquad (4)$$

where v is velocity in cm/sec, E is electrical potential in volts/cm and U represents the mobility of the ion with units of cm^2/volt-sec (2). Combination of equations 3 and 4 yields a basic electrophoretic principle:

$$U = Q / 6\pi rn \qquad (5)$$

in which $6\pi n$ is constant for a given experiment (2). The resulting equation is

$$U = Q / kr \qquad (6)$$

which relates mobility to a ratio of charge to size among the ions (2). Therefore, electrophoresis separates ions due to their size and charge or, in other words, mobility.

Supporting Medium

Several types of support media are avaliable for electrophoresis. They fall into two major groups as seen in Table 1 (5). Group 1 media are relatively inert. Separation with these media is dependent on the charge to mass ratio. Group 2 media influence separation because the pore size of the medium causes a sieving effect which allows molecules of similar charge-to-mass ratios to be separated

TABLE 1

SUPPORT MEDIA

GROUP NUMBER	MEDIA TYPE
1a	paper, cellulose acetate, glass fiber paper, thin layer materials, agar gel, single cellulose fibers. (analytical & preparative)
1b	pevikon, starch and gypsum blocks, sponge rubber. (preparative only)
2	starch gel, acrylamide gel. (analytical & preparative)

due to size.

Two media of common use and interest are cellulose acetate and agarose gel. The raw material for cellulose acetate is produced by treating the hydroxyl groups of cellulose with acetic anhydride. Eighty percent of the commercial material is composed of air pockets formed by interlacing fibers. The membrane is dry, opaque and brittle until moistened with buffer. Then the air pockets fill with liquid and the fiber becomes pliable. By treating the membrane with a series of solutions, the cellulose acetate is made transparent for densitometry scans. The advantages of this technique are speed of separation and unlimited storage of the transparent membrane (6).

Agar gel is composed of agaropectin and agarose, which is a purified form of agar. Agarose is the agar medium of choice and has fewer adsorption problems. It exhibits little endosmosis which is the ability of the sample to diffuse through the medium. Also, it is similar to cellulose acetate in versatility and convenience and has a natural clarity which enables excellent densitometer scans. One advantage that it has over cellulose acetate is the ability to be heated, poured and shaped at approximately 50 °C. This allows some proteins to be premixed without concern about denaturation (2).

The Buffer

In an electrophoretic system, a buffer maintains a constant pH and ensures that each component will maintain a

constant charge. This constant charge is what determines the direction of movement of the molecule. For example, proteins will migrate to the anode at pH's above their isoelectric point, pI, and in the reverse direction at pH's below their pI (2).

The ionic strength of the buffer affects the velocity of the molecule. This is due to the concentration of ions clustered about the charged molecule. As the buffer concentration increases, the ionic cloud increases which hinders the movement of the molecule. High- ionic-strength buffers reduce movement but hold bands more tightly. Low ionic strength buffers increase movement but allow diffusion of the components (6).

In protein electrophoresis, buffers with a pH over 8.0 are generally used. The buffers which afford the greastest utility are the barbital buffers and tris-boric acid-EDTA buffer. Minimal association effect occurs between the buffer and protein because of the monovalent base in these buffers (6).

Stains and Analysis

Stains are necessary in order to enhance the absorption of the sample. When using any stain, the quantitative accuracy is dependent upon dye uptake by the sample. The amount of dye uptake is affected by many factors such as the type of protein and the degree of denaturation of the protein by fixing agents.

Unfortunately, stains are often specific for one chemical group and many different stains need to be employed for various substances as seen in Table 2 (6).

Ponceau S is a popular stain for proteins. It is an anionic dye which reacts with the free basic amino groups of the proteins in an acid solution. It produces a vivid color at low protein concentrations on the order of 0.05 µg/L and can be readily washed from the background after staining the protein. Other advantages are its low cost, availability and ease of use (6).

Once the supporting medium is stained, analysis is best accomplished by densitometry. The electrophoretic strip is moved past the measuring optics of a densitometer. Each fraction is shown on a strip chart as a peak according to its location and absorbance. Figure 4 illustrates an electrophoretic strip after destaining of the background and the resultant densitometer scan for serum proteins (3). Determination of the peak areas is performed automatically by instrument integration. These scans are then used in the determination of disease states by comparison to known disorders. An illustration of the instrument is shown in Figure 5 (3).

The Power Supply

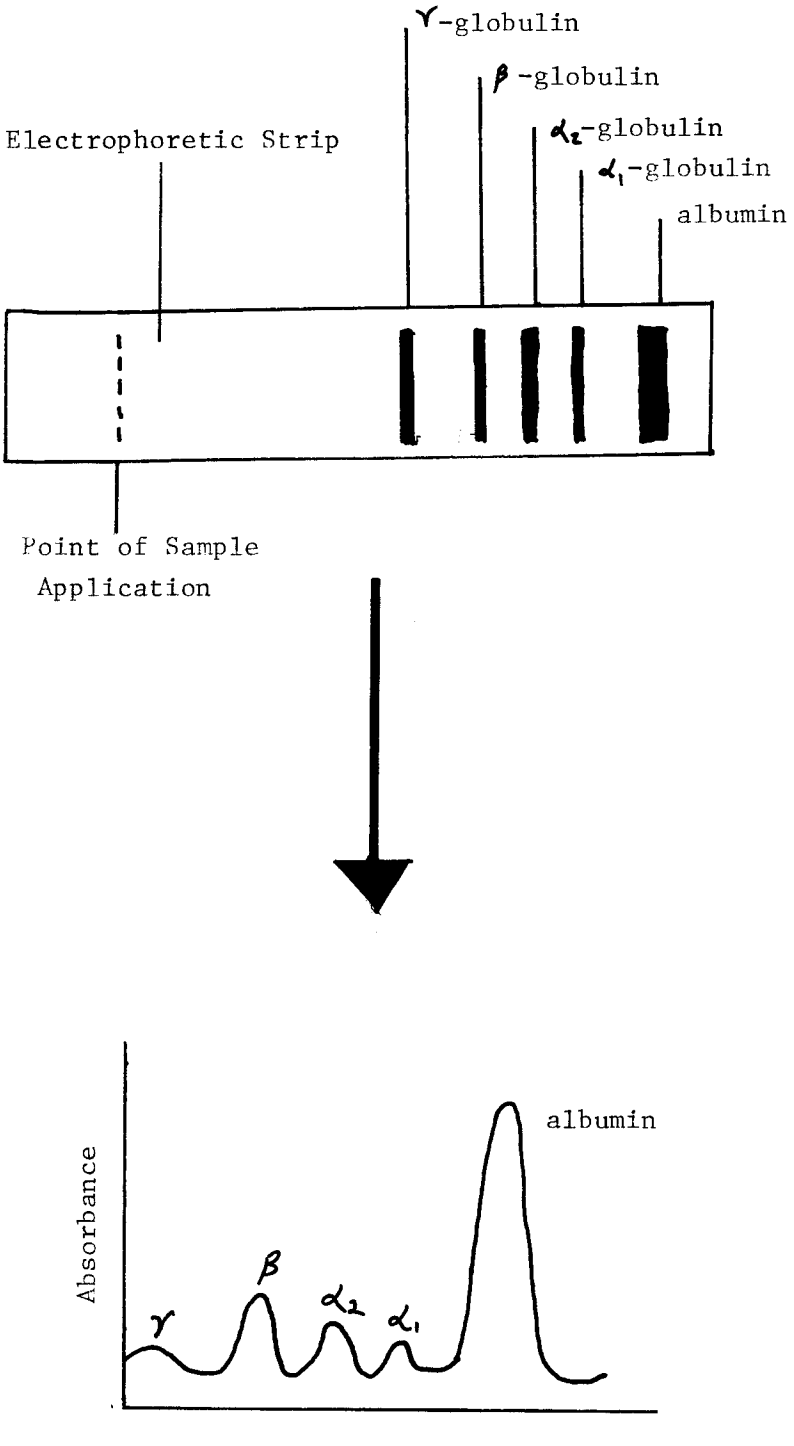
Voltage, the driving force behind electrophoresis, obeys Ohm's Law:

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

TABLE 2

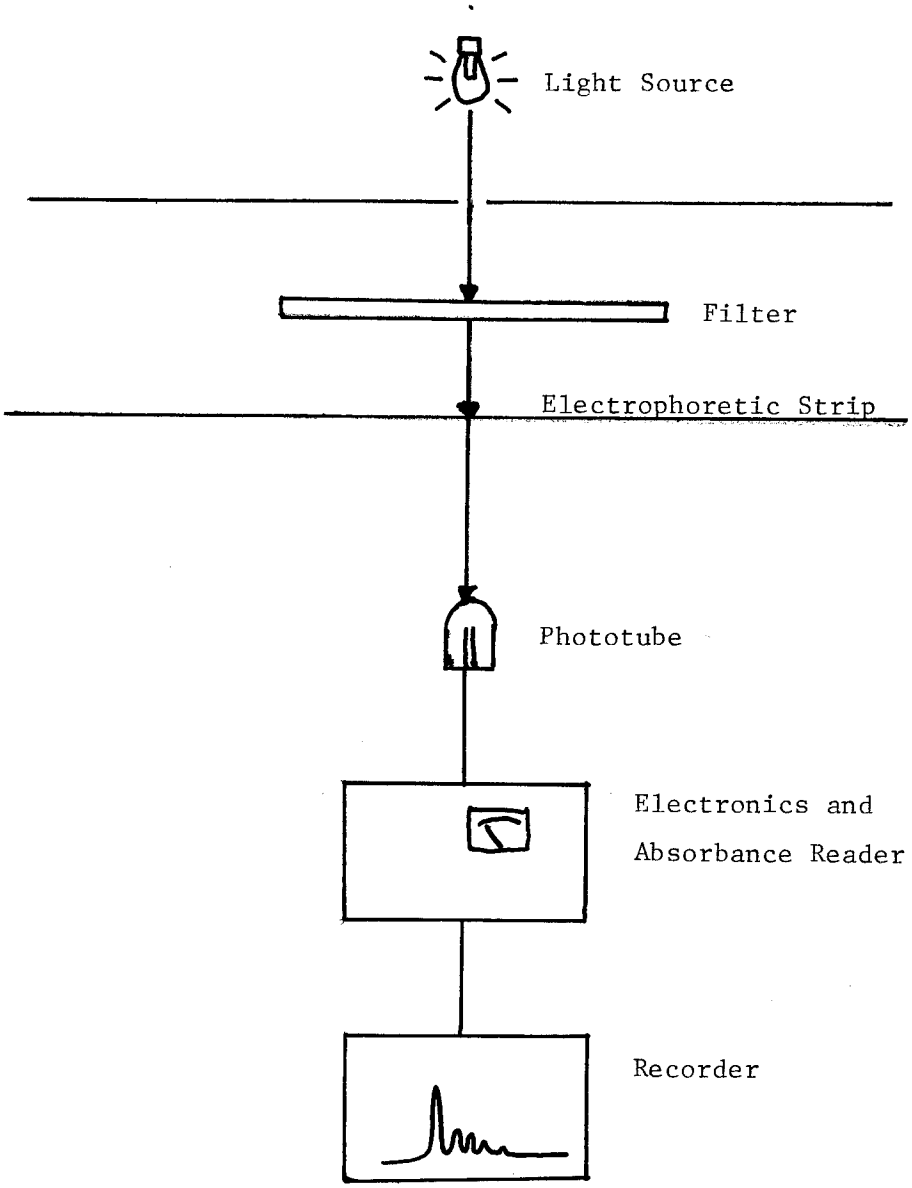
COMMONLY-USED STAINS FOR VARIOUS SUBSTANCES

Substance	Stain
Proteins	Ponceau S Bromophenol blue Light green SF Coomassie brilliant blue R250 Silver stain Stains-All Amindo black 10B
Lipoproteins	Sudan black B Oil red O Coomassie brillant blue R250
Glycoproteins	Periodic acid-Schiff Stains-All
Nucleic acids	Stains-All Silver stain Ethidium bromide
Enzymes:	
Dehydrogenases	NADH (fluorescence) Nitro blue tetrazolium chloride
Esterases & Cholinesterases	Beta-naphthyl esters and tetrazotized O-dianisidine
Phosphatases	1-Naphthylphosphate and fast blue B



Electrophoresis Results
of Serum Protein

Figure 4



Densitometer

Figure 5

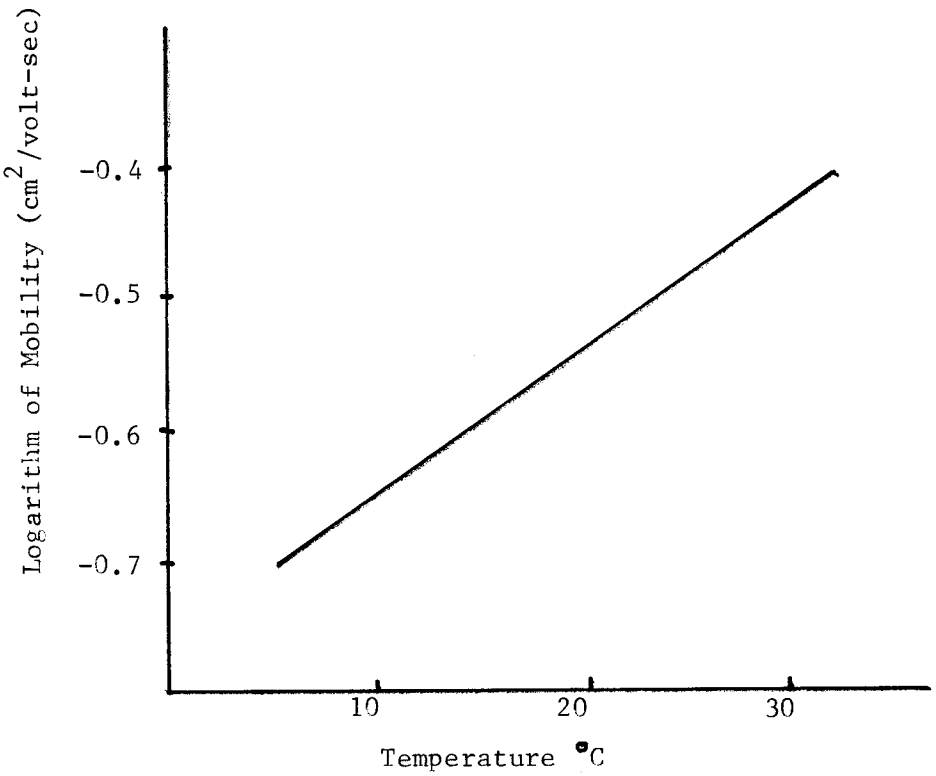
where the applied voltage, V, across the resistance of the medium, R, results in a current flow, I (5). As the voltage is increased, the migration rate of the ions increases which in turn causes the current to increase since it is the ions in the sample that carry the current. The increased current flow results in the production of heat:

$$\text{Heat} = V\ I\ t \qquad (8)$$

where V is the applied voltage, I is the current and t is the time (6). The effect of temperature on electrophoretic mobility can be seen in Figure 6 (4). As the temperature increases, resistance decreases and mobility and current increase. Unfortunately, higher temperatures denature proteins and cause evaporation of the buffer which leads to higher ionic strengths and consequently decreased mobility.

As seen in Figure 7, the device required to produce the necessary voltage for electrophoresis is totally analog in nature (7). The power supply can maintain its voltage or current directly. Power is read from a multiply circuit. The servo amplifier compares these readings to a voltage reference and sends an analog signal to control the voltage output of the power supply. By these means, the power supply can be used to maintain constant voltage, current or power.

In constant-voltage situations, current will increase and, due to increased thermal agitation of the dissolved ions, the migration rate of the sample will increase. A rise in temperature results in more rapid evaporation of



Mobility of Ions as
Temperature Increases

Figure 6

Analog Electrophoresis

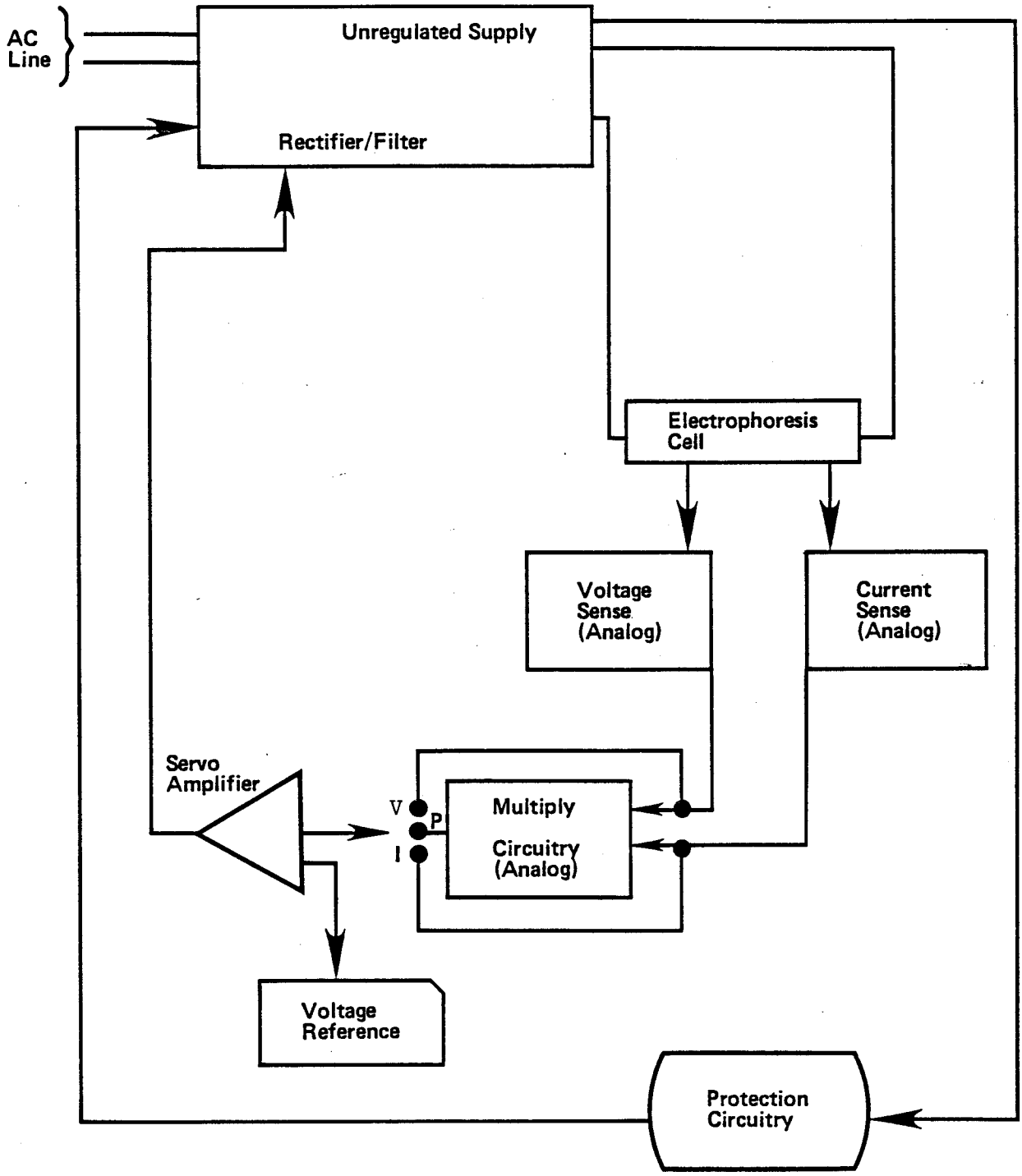


Figure 7

water from the buffer-moistened support medium and an increase in the ion concentration. The buffer compensates for this evaporation by drawing more buffer from both sides of the chamber. This factor tends to cause the sample to run towards the center of the solid matrix. These effects become negligible at lower voltages (6).

For constant current, the voltage decreases as the resistance drops. The temperature effect is lower than in constant voltage and this keeps the migration more constant for the whole experiment. Although these effects do not cause the protein to be denatured, the migration rate is slower and thus resolution is decreased (5).

It can be seen that in order to obtain optimum electrophoretic resolution, separation time must be minimized to reduce diffusion. This requires the highest possible electric field without allowing temperature to deteriorate the sample. The method of choice is constant-power electrophoresis.

Power is represented by the equation:

$$P = V\ I \qquad (9)$$

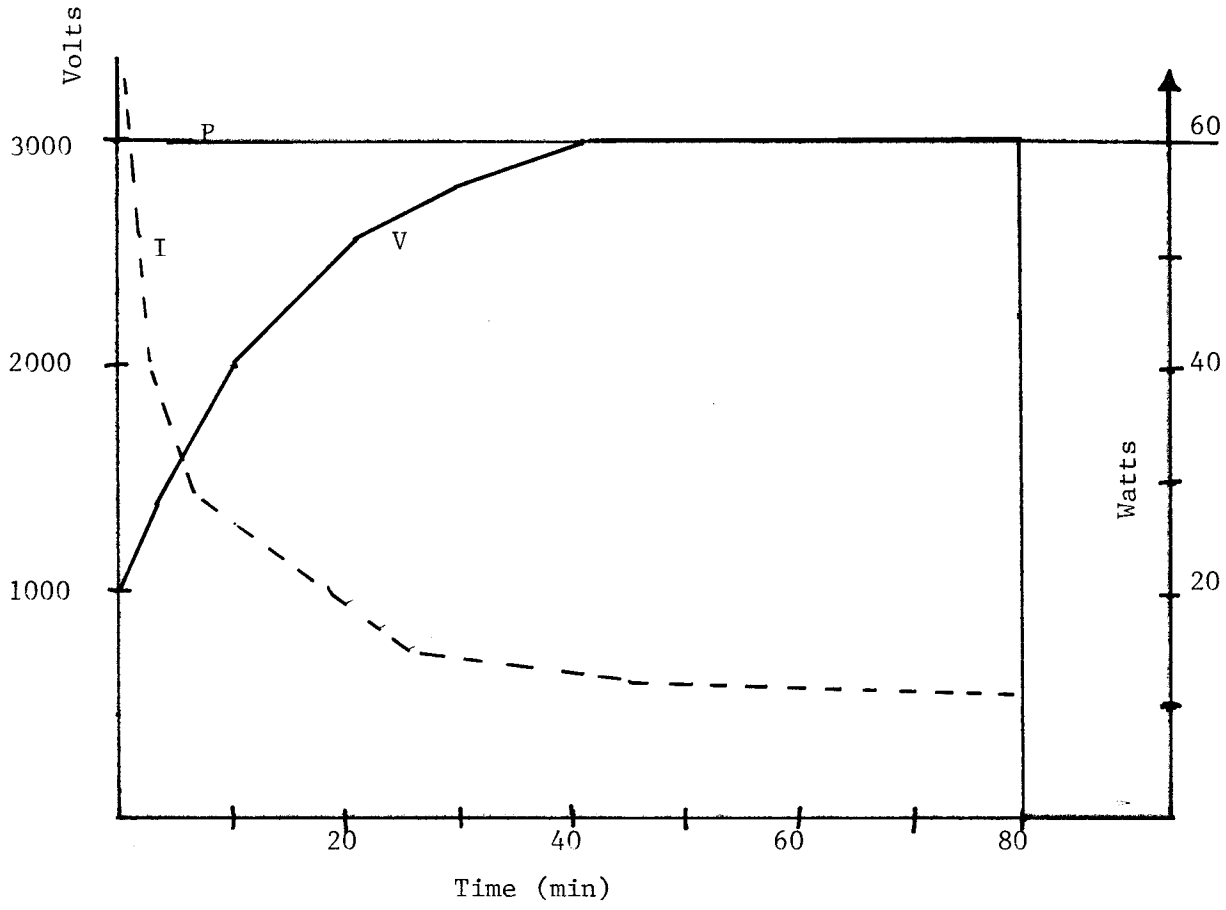
where the power, P, is in watts. Constant power operation delivers the optimun voltage throughout the experiment. As the resistance decreases, the current will increase and the voltage will decrease in order to maintain constant power. Current and voltage are fairly constant at the end of the run (8).

Another use for constant power is in isoelectric focusing which relies on establishing a pH gradient and focusing proteins to their isoelectric points, where migration stops. In this instance, resolution and mobility increase with field strength. The proteins focus faster and sharper at higher voltages (2). Resistance increases in this method so current must decrease as shown in Figure 8 (9). Table 3 shows the superiority of constant power over constant voltage and current (10).

Clinical Significance

Two of the most common electrophoretic analyses conducted in the clinical laboratory are the quantitation and identification of hemoglobin variants and of serum proteins. The procedures involve measuring the differences in electrophoretic patterns relative to a control pattern. From the obtained data, specific disease states are often diagnosed (2).

About 93 percent of plasma serum is water with the remainder consisting of proteins. The proteins serve a number of purposes such as: decomposition to produce amino acids for protein synthesis; transformation into carbohydrates and lipids; and maintenance of colloidal pressure and blood pH. Upon electrophoretic separation, five predominant fractions are observed. They are listed in Table 4 with their main constituents (6). Figure 9 shows a set of electrophoretic patterns depicting densitometer scans



Time Dependence of Current (I),
Voltage (V) and Power (P)
during Isoelectric Focusing

Figure 8

TABLE 3

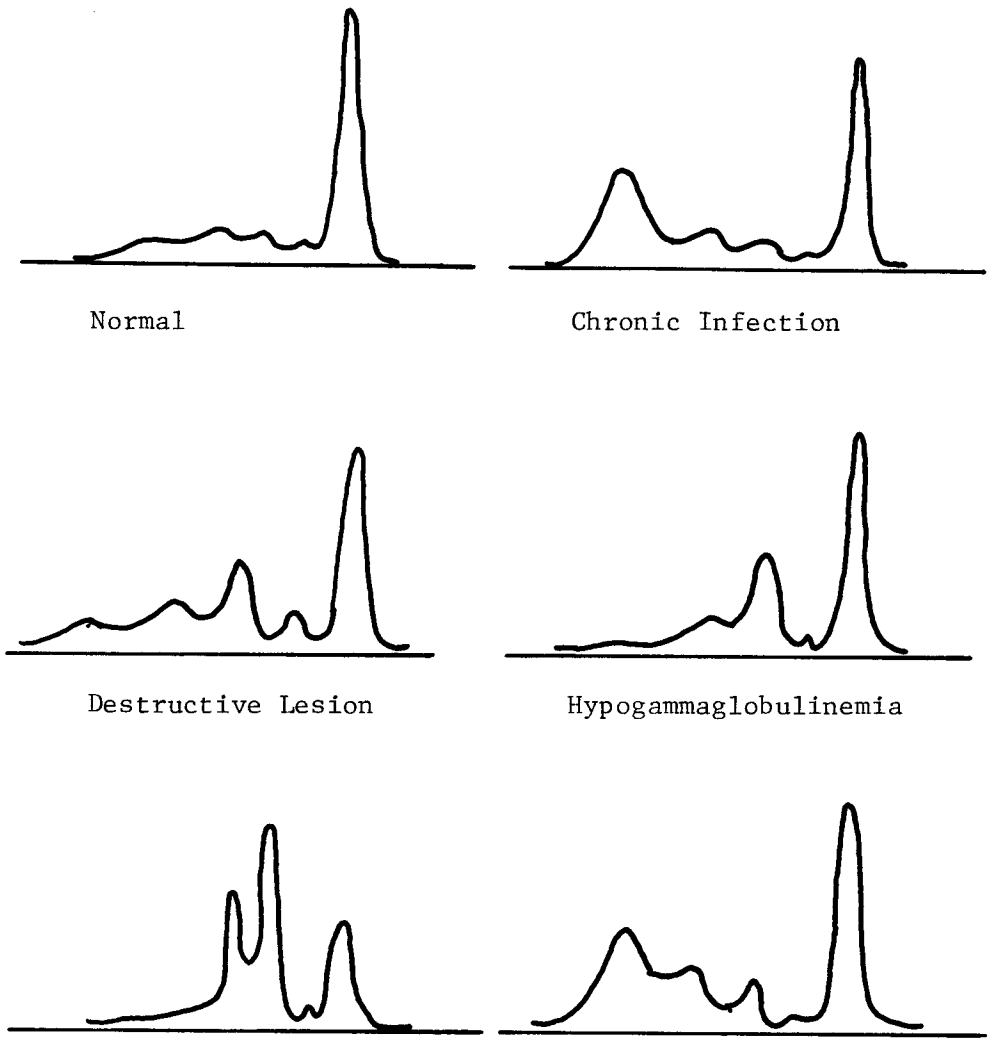
COMPARISON OF ELECTROPHORESIS UNDER
CONSTANT VOLTAGE, CURRENT AND POWER CONDITIONS

Test Data	Constant Current	Constant Voltage	Constant Power
Init. Voltage (volt)	170	159	150
Final Voltage (volt)	206	162	260
Init. Current (amps)	.079	.085	.090
Final Current (amps)	.079	.025	.053
Init. Resistance (Ω)	2150	1870	1670
Final Resistance (Ω)	3750	6480	4910
Init. Power (watts)	13.4	13.5	13.5
Final Power (watts)	23.4	4.1	13.8
Migration (cm)	10.3	4.5	7.3
Quality of Results	poor	poor	excellent

TABLE 4

ELECTROPHORETIC FRACTIONS
AND THEIR MAIN CONSTITUENTS

Electrophoretic Bands	Normal Range g/100 mL	Main Constituents
Albumin	3.5-5.0	Albumin
Alpha-one(α_1)	0.17-0.33	α_1-anti-trypsin
		α_1-lipoprotein
		Orosomucoid
Alpha-two(α_2)	0.42-0.90	α_2-macroglobulin
		Haptoglobin
		Pre-β-lipo-protein
Beta-one(β_1)	0.42-1.05	Transferrin
		Hemopexin
		β-lipoprotein
Gamma(γ)	0.71-1.65	Immunoglobulin G
		Immunoglobulin A
		Immunoglobulin M



Serum Protein Scans

Figure 9

of a normal and several abnormal serum proteins (6). A partial list of diseases associated with abnormal serum patterns is given in Table 5 (11).

Hemoglobin is a spheroidal molecule with a central cavity and four tetrahedrally-arranged loops of globin presenting to the exterior of the sphere an iron-porphyrin ring, the site for oxygen binding. The tetramer is composed of identical globin loops designated α and β. In normal adult human blood, these groups are referred to as A. Variations in the amino acid sequencing of the β loops, or chains, result in either other hemoglobins such as A_2 and F or abnormalities in the erythrocytes as well as disease states such as hemolytic anemia (6). Table 6 lists some clinically important hemoglobinopathies (6). Electrophoresis patterns for normal and several abnormal hemoglobin states are illustrated in Figure 10 (12).

Declaration of the Problem

Many variations of the electrophoretic technique have been made in order to increase its resolving power. Variables including the buffer, media, staining, temperature and analysis have been altered in the hope of obtaining better resolution. The only variable in which very little has been done is the power supplied to the electrophoretic cell. Constant voltage and current have been employed for some time, and constant power was developed in the early 1970's. There existed no other way

TABLE 5

ELECTROPHOROGRAMS ASSOCIATED WITH DISEASE

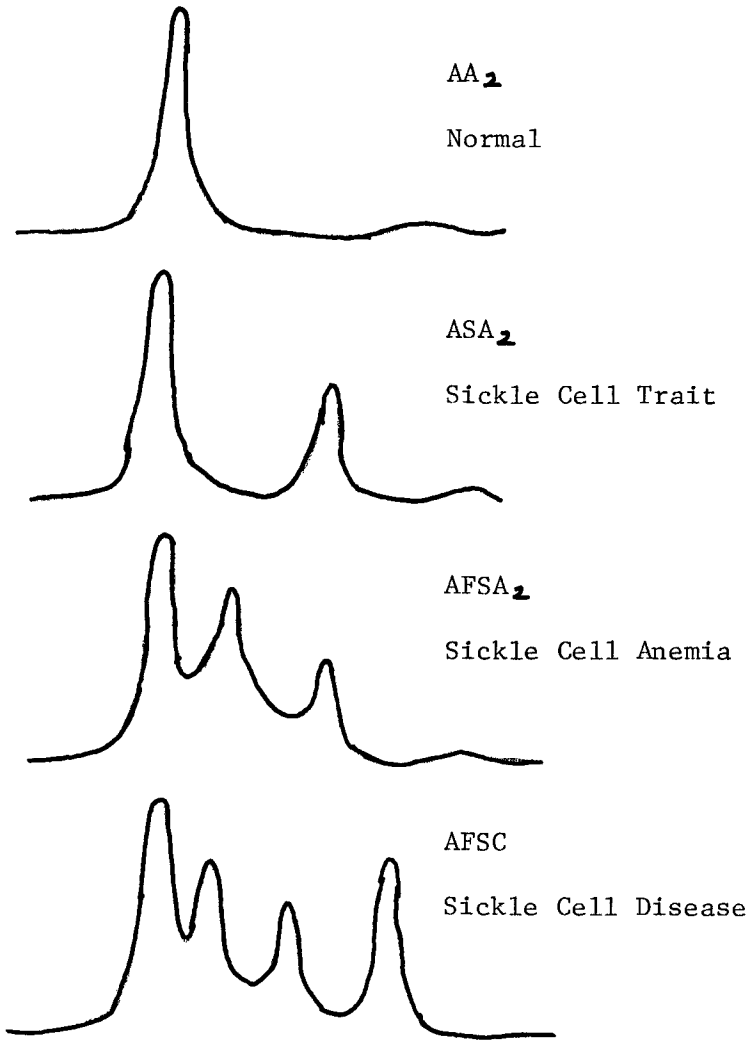
A	1	2	B	G	Disease State
–		+			Acute Infection
–		+		–	Asthma, other Allergies with poor response to therapy
–	+	+			Carcinomatosis
–				+	Chronic Infection
				+	Cryoglobulinemia
–		+	+		Diabetes Mellitus
–		+			Glomerulonephritis
–				+	Hepatic Cirrhosis
–	–	–	+	+	Hepatitis, Viral
–		+		+	Hodgkins Disease
				–	Hypogamma globulinemia
–				+	Leukemia, Myelogenous
–		+		+	Lupus Erythematosis
–				–	Lymphoma and Lymphocytic Leukemia
–			+	+	Macroglobulinemia
–				+	Myeloma
–				+	Myesthenia
–		+		+	Myxedema
–		+		–	Nephrosis
–		+			Rheumatic Fever
–		+		+	Rheumatoid Arthritis
–		+	+	+	Sarcoidosis
–	–	–	–	–	Scleroderma
–	+	+	–	–	Ulcerative Colitis

+ = increase from standard normal
– = decrease from standard normal

TABLE 6

CLINICALLY IMPORTANT HEMOGLOBINOPATHIES

Clinical Manifestation	Hemoglobin Designation	Comments
Hemolytic anemia	S	Sickling
	C	Target cells
	E	Target cells
	O (Arab)	Target cells
	Koln	Unstable hemoglobin
	Zurich	Unstable hemoglobin
	H	Unstable hemoglobin
Cyanosis	M(Iwate,Kankakee)	This group of rare abnormal hemoglobins is characterized by by abnormal adsorption spectra and by normal methemoglobin values when tested by the usual spectrophotometric measurement of methemoglobin
	M(Boston,Osaka)	
	M(Saskatoon,Chicago)	
	M(Milwaukee I)	
	M(Hyde Park)	
Erythrocytosis	Chesapeake	This group of abnormal hemoglobins is characterized by marked leftward displacement of the oxygen dissociation curve
	J(Capetown)	
	Malmo	
	Ypsilanti	
	Kempsey	
	Yakima	
	Rainier	
	Bethesda	



Hemoglobin Scans

Figure 10

to vary these until the advent of the microcomputer.

With the microcomputer and electronic integrated circuits such as analog-to-digital (A/D) and digital-to analog (D/A) converters, it is possible to control an analog power supply digitally. By interfacing and writing programs which would monitor and update the output of a power supply, better control can be placed upon voltage, current and power. Also, the power supply could be used to perform various pulse techniques. This type of programming may enable electrophoresis to be run at reduced temperatures. Further, with the interfacing of an IR temperature probe, electrophoresis could be conducted on a constant temperature basis. It is hoped that control of the power supply will lead to an increase in the resolving power of electrophoresis.

CHAPTER II

HISTORICAL REVIEW

In 1809, the Russian physicist, Alexander Reuss, was the first to describe the electrokinetic movement of colloidal particles. In his experiment, two glass tubes were placed vertically in a bed of wet clay. The tubes were then filled with water and a small amount of sand was placed at the bottom of each tube. The terminals of a battery were connected to the tubes and two distinct principles were observed by Reuss. First, he discovered that charged colloidal particles of clay migrated toward the positive electrode when electricity was applied. And second, he noticed that an increase in the volume of water occurred at the negative electrode. The latter principle is called electro-osmosis or electro-endosmosis while the first observation is now known today as electrophoresis (4).

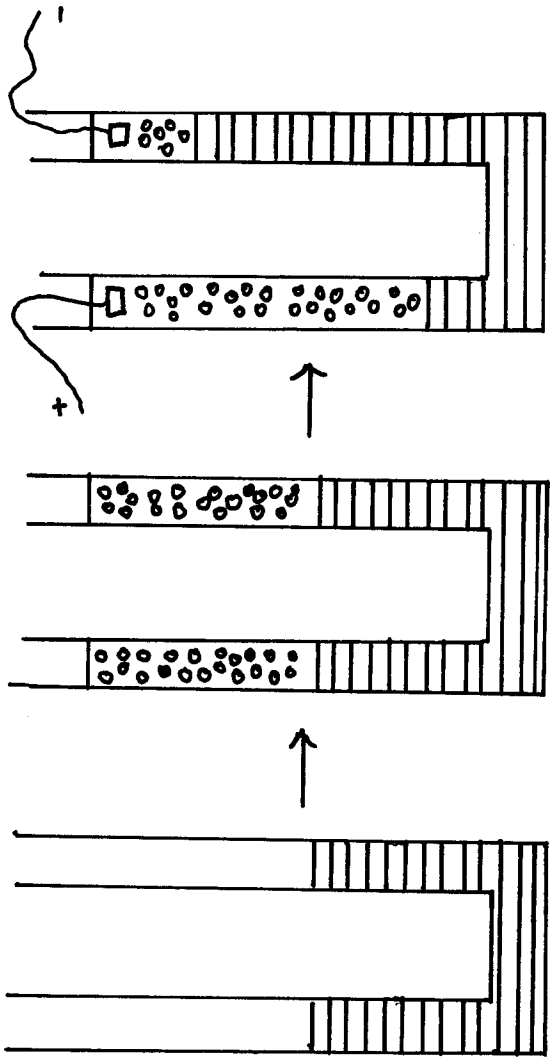
Later this discovery was extended and confirmed independently by Michael Faraday in England and E. H. DuBois-Reymond in Germany. Both men demonstrated that negatively-charged particles moved toward the anode and positively-charged particles moved toward the cathode in a solution or suspension. Also, it was determined that particles moved at different rates depending on the number of excess charges present. Thus electrophoresis was recognized as a means of separating particles in a mixture

according to their electrical properties (4).

Three separate techniques have evolved from these initial experiments. They are microscopic, moving boundary and zone electrophoresis. The oldest method is microscopic electrophoresis and requires an observer to measure particle migration through a solution in a glass tube with a microscope. Ideal objects such as blood cells, protozoa, bacteria and colloidal particles are large enough to be viewed through the microscope. Unfortunately, smaller particles could not be studied by this method and thus the more useful moving boundary procedure emerged in the early 1890's (13).

The basic difference between microscopic and moving boundary electrophoresis is the number of particles being observed. Moving boundary electrophoresis measures the movement of the boundary of a mass of charged particles instead of individual charged particles as in the microscopic technique. Identification of hundreds of proteins and other biologically active substances is obtained by observing the isoelectric points of these materials (13).

As shown in Figure 11, the material to be studied is placed in a glass U-tube (13). A buffer solution is placed carefully in both arms of the tube so that a sharp boundary is visible between the solutions. An electric current is passed through the solution and the materials' speed, motion and isoelectric point are observed.



Migration of a Protein

Figure 11

This apparatus was employed by Linder and Picton to study the migration of inorganic compounds and hemoglobin in solution. In addition, Michaelis used the U-shaped tube to determine the isoelectric points of several enzyme preparations. He was able to determine a pH value for each enzyme at which no net migration occured. Later, Michaelis used the term electrophoresis to describe this technique of charged particle interaction in an electric field. However, the man whose contributions to the field caused him to become known as the modern day father of electrophoresis was Tiselius (4).

In 1937, the Swedish chemist, Arne Wilhelm Kaurin Tiselius, was responsible for developing moving-boundary electrophoresis into an important diagnostic tool. He devised a new apparatus for electrophoretic analysis which alleviated several problems that interfered with resolution and analysis. The development of this new device was linked to Tiselius so much that it became known as "the Tiselius apparatus" (13).

With the older instruments, resolution of boundaries was not well defined due to convection currents within the liquid media. The problem was the result of heat generation due to the application of an electric field. Tiselius devised two methods to deal with this phenomenon. He altered the shape of the U-tube to increase its surface area and immersed the new tube in a cold water bath. These measures decreased the temperature, which decreased the

convection currents and thus increased the resolution of the boundaries (14).

Another addition which Tiselius incorporated into the new device was an optical system that made observations of the boundaries more discernible and precise. This optical system employed the schlieren method which utilizes the fact that the boundary between two transparent materials with different densities causes light rays to refract. The refraction from these two materials creates a shadow at the boundary and thus enables the researcher to view the boundary with greater ease (14).

A third innovation involved the trapping of specific fractions after separation of a mixture into its components. Tiselius designed the U-tube to have a series of sections which could be separated from one another. By shifting a section to one side, he could examine a specific fraction with little disturbance to the contents of the whole tube (13).

Later, he introduced a pistonlike plunger which acted as a counter pressure against the moving buffer solution. The piston was mechanically lowered into the arm in which the migration was ascending. The effect was similar to having a longer path of migration which would allow a more complete separation of the components (13).

Using this improved moving boundary instrument, Tiselius and his colleagues showed that the globulin resulting from the fractionation of human serum consisted of

four subfractions. Further, the properties and electrophoretic mobilities of these subfractions varied in such a manner that specific pathological conditions could be identified. Thus, the importance of electrophoresis as a clincial tool emerged (14).

The moving boundary work performed by Tiselius was rapidly followed by the development of a new technique which involved electrophoresis on a solid support medium unlike the former which occurs strictly in a liquid matrix. Many rigid and semirigid materials were found to remain stable while saturated with buffer and form efficient supporting structures for the separation of charged particles. These charged particles upon separation did not exhibit moving boundary characteristics but instead bunched together in individual areas or zones. Thus, this new technique which was developed independently by several researchers including Tiselius became known as zone electrophoresis (13).

In 1939, Konig performed what is considered the first electrophoretic separation using a solid support medium. He successfully isolated a yellow pigment from snake venom using filter paper as a medium. However, as early as 1886, Lodge used gelatin as a medium to measure the migration of inorganic ions. Agar jelly was employed by Field and Teague to study diphtheria toxin and antitoxin. Also, agar gel was used to carry out electrophoretic separations of metals by Kendall, Crittenden and White (4).

Zone electrophoresis differs from moving boundary electrophoresis in several important ways. For example, zone electrophoresis requires rather simple and inexpensive equipment. Another difference is that in zone electrophoresis the use of a rigid or semirigid support medium allows the components that were separated to be studied long after moving boundary results are discarded. Also, zone electrophoresis is more sensitive than moving boundary electrophoresis and requires smaller sample quantities for analysis (14).

Today, zone electrophoresis, which is the most highly used form of electrophoresis, has obtained a position of great importance in analytical biochemistry. Practical application of zone electrophoresis involves the use of various shapes and forms of media. For example, strips or sheets of paper of cellulose acetate and columns, plates, blocks, or slabs of starch, acrylamide, or agar gels are used in the electrophoretic process (1). With the application of these new media, procedures which used to take the better part of a day are performed in less than an hour.

CHAPTER III

METHODOLOGY

Microcomputers

Over the centuries, man has strived to devise a machine which could perform calculations. In the seventeenth century, Pascal was credited with the development of the first calculating machine even though the abacus had been in use for over two thousand years. Almost two centuries later, Charles Babbage drew up plans for a mechanical computing machine whose architecture is recognized as similar to that in use in today's computers. The mid-twentieth century saw the creation of the first-generation computer. It was bulky and expensive to build and operate. However, in the early 1970's, a revolution in electronics occurred. The evolution of large-scale integrated circuit manufacturing techniques brought rapid and continuing decrease in size, cost and power consumption of the digital computer (15).

The digital computer is known for its ability to follow a predetermined list of simple operations and solve complex problems at a rapid rate. The results are obtained in response to commands called instructions which follow an order of execution called a program. The instructions are encoded in binary words which are decoded and executed

before the computer retrieves the next instruction. The sequencing of instructions is performed by the central processing unit (CPU). When the CPU comprises a few integrated circuits, it is called a microprocessor. A computer which incorporates the CPU as its microprocessor is known as a microcomputer (16).

The CPU is the principal manager of the main communication channel and one of four essential components in any computer. The other components are the memory unit, the input-output control (I/O) and the arithmetic and logic unit (ALU). Communication with instruments, people or other computers is accomplished with the I/O control. Input information is sent to the memory unit for storage until the CPU directs further operations. The CPU may then direct this information to the ALU for processing and return this data to the memory. Ultimately, whenever the instructions are completed, the results are transferred to the I/O unit and made available to the instrument, user or other computer (15).

In the lab, the advent of the digital computer has improved the control of chemical instruments to points well beyond the limits of manual abilities. By interfacing the microcomputer to instruments, the chemist has been able to save time, reduce costs, achieve more accurate results and increase data acquisition. Thus, in a computer-managed laboratory, scientific observations in the nanosecond range and the control of events within microseconds are a reality

(17).

Microcomputer Interfacing

Whenever a scientist makes a measurement, he desires a digital number as the result. Analog signals were interpreted as a digital number by the position of a recorder pen or meter needle. The recent developments in electronics have changed these readings and now provide direct digital readouts of analog signals. There are several advantages to direct digital readings such as less sensitivity to noise, improvement of precision and accuracy, and direct computer processing of the information without reading error (17).

In order to perform these tasks, the microcomputer employs A/D and D/A convertors. The A/D circuit transforms an analog-domain signal such as current or voltage into a digital signal which is encoded as a number proportional to the analog magnitude. The D/A circuit converts a digital signal into an electrical analog quantity which is related to an encoded digital number. By programming the microcomputer, we can interpret analog inputs and provide a proper analog output (16).

Interfacing the Power Supply

In this work, an electrophoresis power supply has been modified to be controlled by analog signals from the D/A of a microcomputer. The D/A connects to a hand-made

interface box and then to a coupler on the power supply. The interface box has a simple hardware design which allows analog signals to be picked up by the A/D and sent out by the D/A. The connector is tied in with a 10-μF capacitor and 10-k ohm resistor in a parallel circuit which acts as a filter to convert pulsating DC voltages into constant DC voltages. The circuit continues from this filter to the operational amplifier which controls the voltage regulation of the power supply. Table 7 and Figure 12 show the millivolt input from the D/A and the resulting voltage output from the power supply.

In order to perform this task as well as monitor the output voltage and current of the power supply, the twelve bit A/D and D/A ADA-LAB(tm) interface card illustrated in Figure 13 is employed. The schematic representation of the card is shown in Figure 14 (18). ADA-LAB(tm) has four voltage ranges, however, none of them is capable of handling the large analog signals generated by the power supply. Therefore, the circuit in Figure 15, which is included as part of the interface box, is used to decrease the input signal. The overall idea is shown in Figure 16 which is the digital version of the device required to produce electrophoretic voltages (19).

Programming

The ADA-LAB(tm) interface card is controlled by a software operating system called QUICKI/O(tm) which uses

TABLE 7

POWER SUPPLY OUTPUT VIA D/A INPUT

Input (mV)	Output (V)	Input (mV)	Output (V)	Input (mV)	Output (V)
9	0	484	376	964	850
18	0	492	384	978	864
28	0	500	392	988	875
38	0	508	400	998	885
48	0	517	409	1008	894
57	0	527	418	1018	905
67	0	536	428	1028	914
77	0	547	438	1038	924
87	0	558	449	1048	934
97	0	569	460	1058	943
106	0	581	472	1068	953
116	0	588	479	1078	964
125	23	596	487	1088	974
135	32	605	495	1098	984
144	41	614	504	1108	994
154	51	623	513	1118	1004
164	61	633	523	1128	1014
174	71	644	533	1138	1024
184	80	654	544	1148	1034
194	91	666	555	1158	1044
203	100	679	568	1168	1054
212	108	686	575	1178	1063
221	117	693	582	1188	1074
231	127	702	591	1198	1084
240	136	710	599	1208	1094
250	145	719	608	1218	1104
259	155	730	618	1228	1114
269	165	741	629	1238	1124
280	175	752	640	1248	1133
291	186	764	652	1258	1143
299	194	777	665	1268	1153
308	203	784	672	1278	1164
317	212	791	679	1288	1174
326	221	800	688	1298	1184
335	230	809	695	1308	1194
345	240	818	705	1318	1204
355	249	828	715	1328	1214
366	260	839	726	1338	1224
376	270	851	737	1348	1234
387	281	863	750	1358	1244
395	289	877	763	1368	1253
404	297	884	769	1378	1263
413	306	891	777	1388	1273
422	314	898	785	1398	1284
431	324	907	794	1408	1294
441	333	917	803		
451	343	928	813		
461	354	939	824		

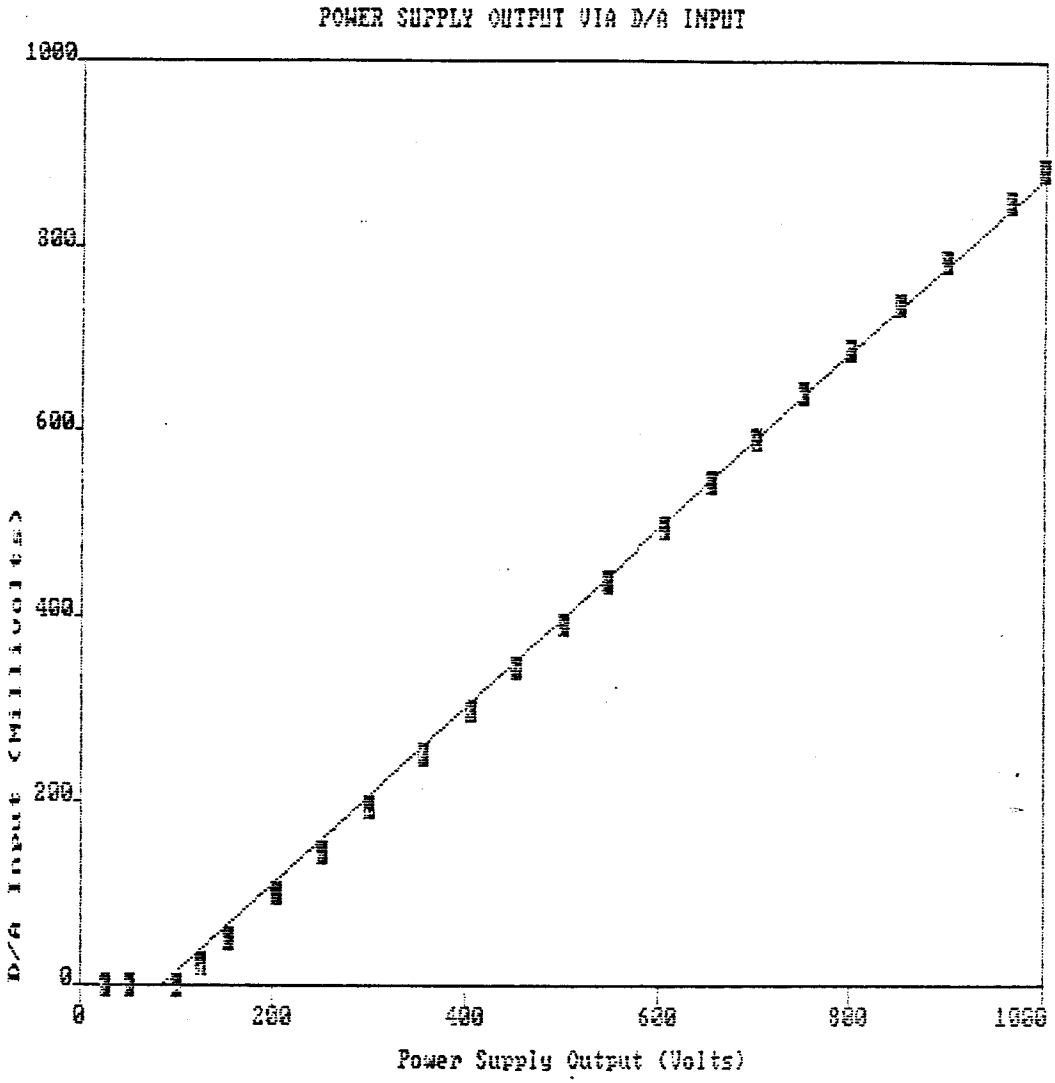
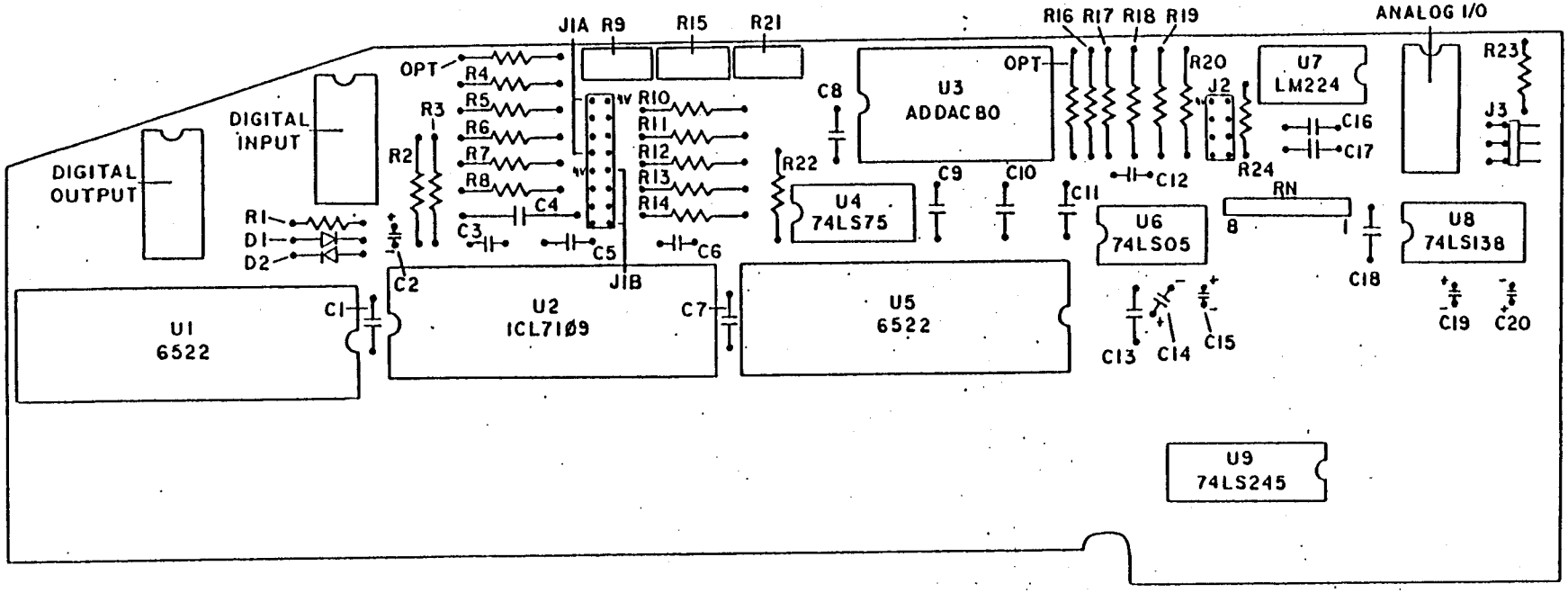

POWER SUPPLY OUTPUT VIA D/A INPUT
1000
800
600
400
200
0
D/A Input (Millivolts)
0
200
400
600
800
1000
Power Supply Output (Volts)

Figure 12



A/D-D/A Convertor Board

Figure 13

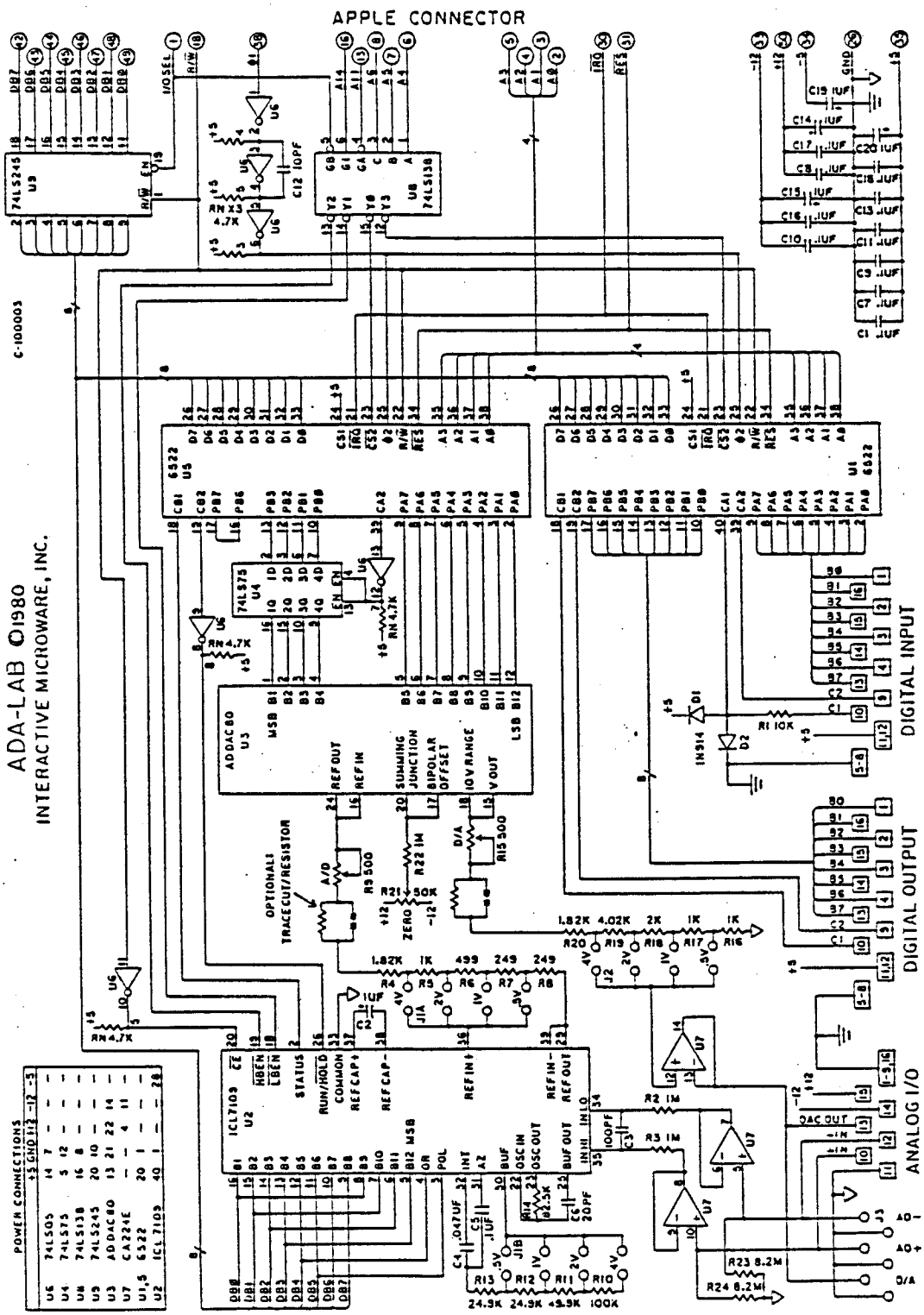
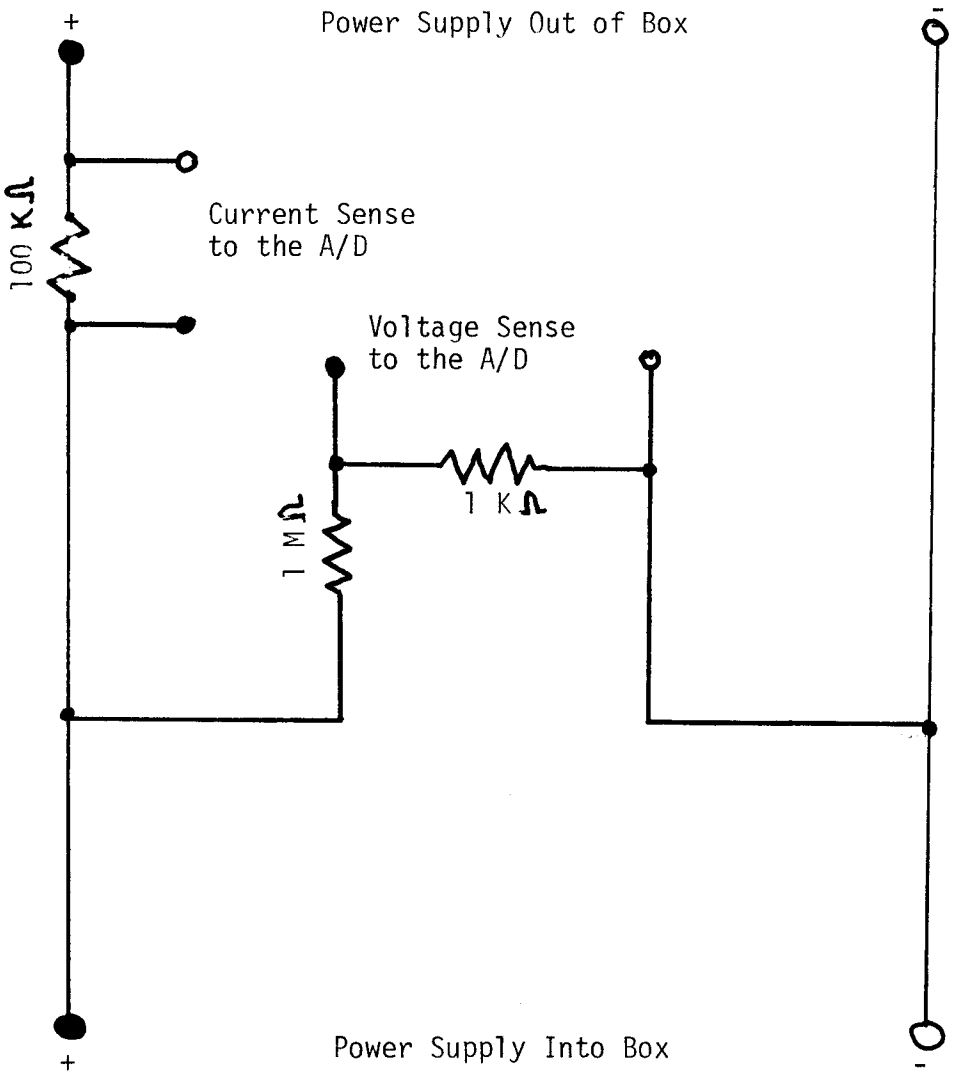

APPLE CONNECTOR
ADA-LAB ©1980
INTERACTIVE MICROWARE, INC.
DIGITAL INPUT
DIGITAL OUTPUT
ANALOG I/O
OPTIONAL! TRACECUT/RESISTOR
POWER CONNECTIONS

Figure 14



A/D Protection Circuit

Figure 15

Digital Electrophoresis

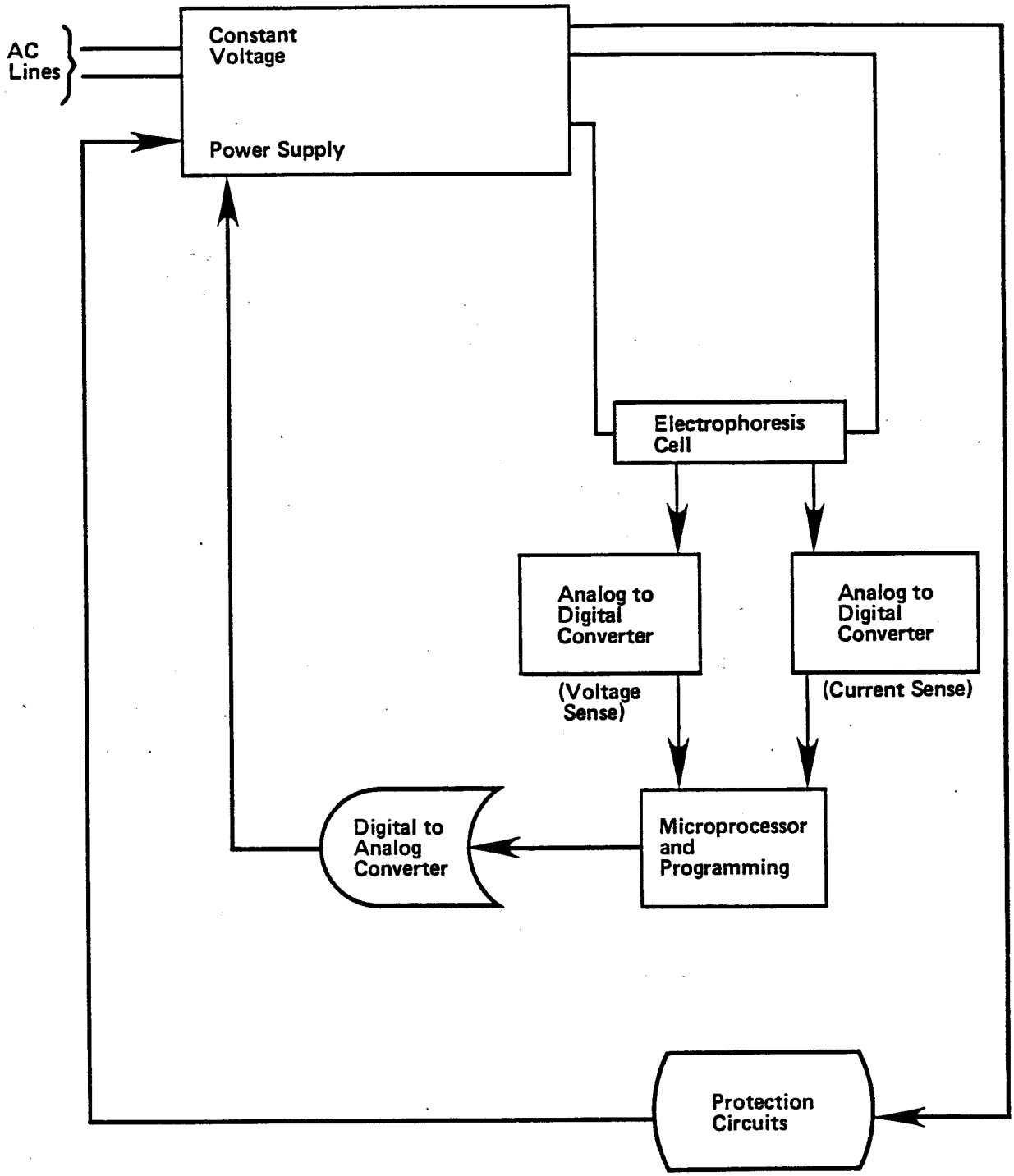


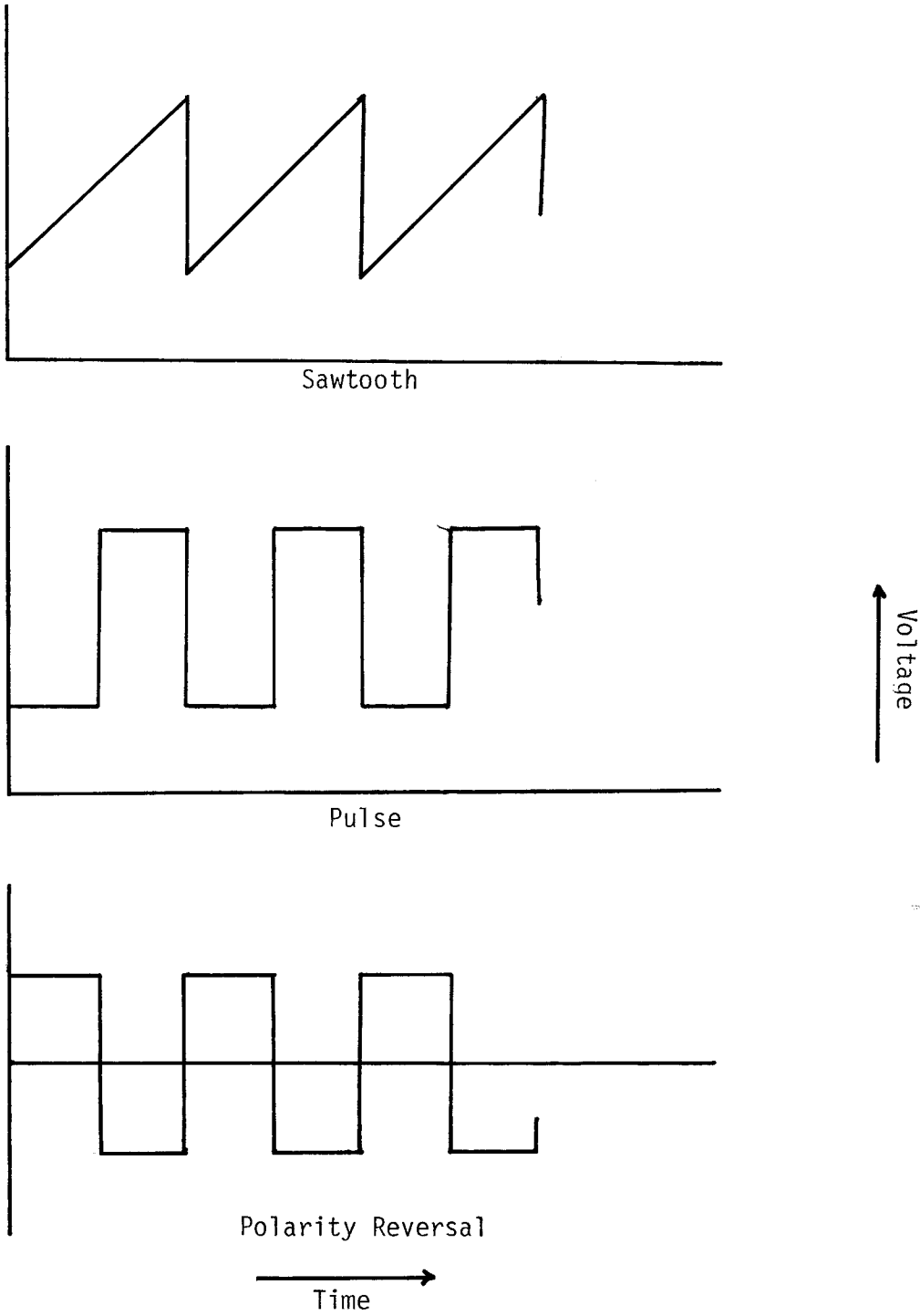
Figure 16

machine-language programs for data collection. By employing short and simple commands which may be included in a BASIC program, the microcomputer can access and execute these machine-language programs. Together with an APPLE(tm) computer and APPLESOFT(tm) BASIC, they form a development system for data acquisition and control (20).

It was necessary for this researcher to write BASIC programs to mimic the normal modes of operation of an electrophoresis power supply such as constant voltage, current and power. Appendix A includes these programs as well as certain waveform programs which may maximize efficiency while minimizing the amount of heat produced during the experiment. As stated previously, heat results from increases in current and will damage proteins. It was hoped that, by pulsing an electrophoresis power supply, better separations could be achieved by producing less heat (21). Some of the various waveforms included in the programs are illustrated in Figure 17 (19).

IR Probe

As can be inferred, our major concern in electrophoresis is heat. A convenient way to monitor heat is by the introduction of the non-invasive IR temperature probe in Figure 18. The IR temperature probe measures heat as temperature by detecting the amount of infrared radiation given off by an object. It then converts this infrared radiation to a millivolt output which can be read directly



Waveforms

Figure 17

IR Probe

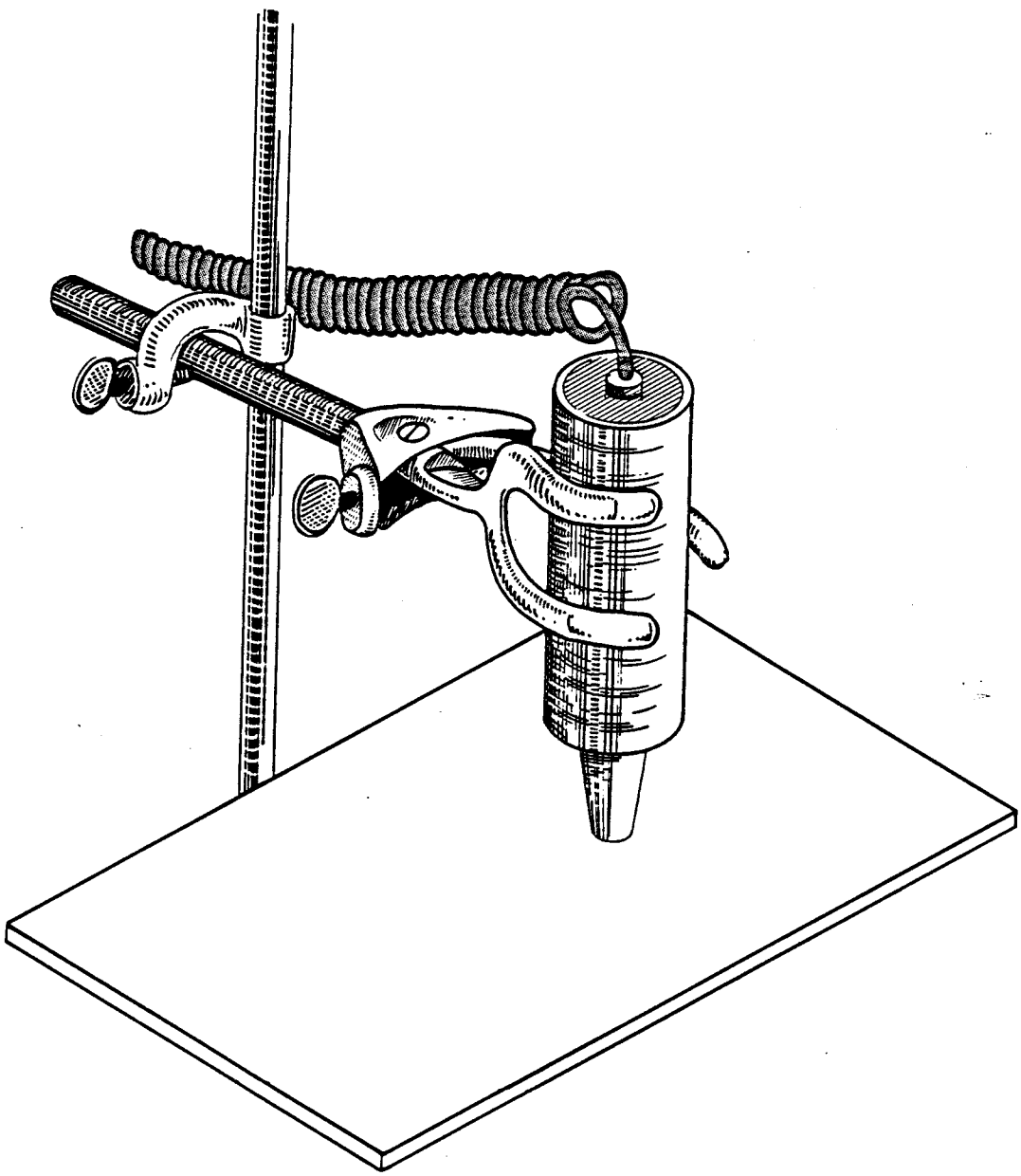


Figure 18

by the microprocessor as shown in Figure 19. Unfortunately, induced electrical interference was encountered when transmitting an analog signal between the probe and the ADA-LAB(tm) interface card. The amplifier in Figure 20 was necessary to minimize the noise. Now, with the IR probe interface, the constant temperature electrophoresis program in Appendix A is introduced. As with the waveforms, it was hoped that maximum efficiency could be achieved by controlling the amount of heat generated on the support media (19).

Temperature Monitored Electrophoresis

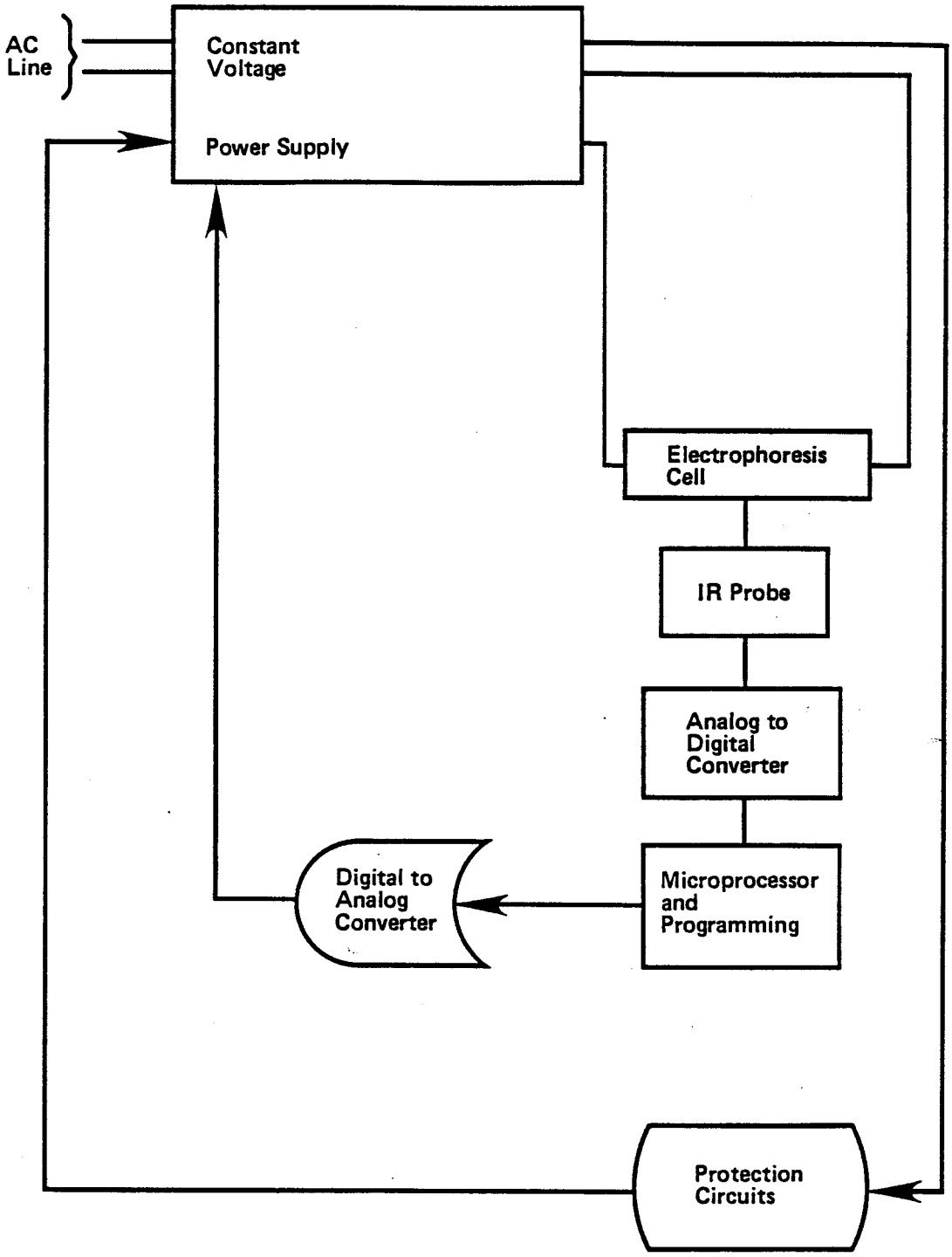
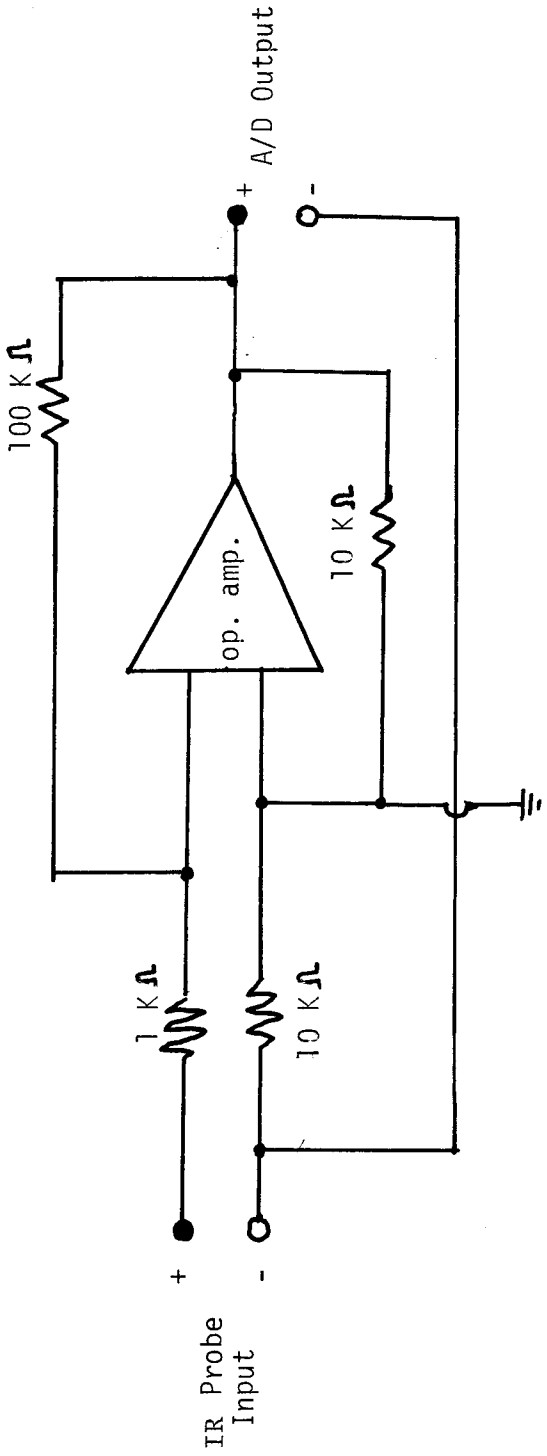


Figure 19



Amplifier for IR Probe

Figure 20

CHAPTER IV

MATERIALS AND APPARATUS

Materials

Table 8 provides a list of materials used to perform electrophoresis experiments. The hemoglobin and titan gel agarose electrophoresis systems are kits which include the stains, buffers, media and all the necessary equipment for electrophoresis except a power supply and standard laboratory chemicals.

Refrigerated control samples of human hemoglobin and serum were stored in tightly capped vials at 2 to 6 °C. The serum controls are stable for one to two days while the hemoglobin controls are stable for several months.

All reagents were prepared as instructed by the Helena Laboratory guide. Deionized water was used for all preparations.

Apparatus

Power Supply

The electrophoresis power supply is a BioRad Laboratories, model 1420B/150. It can maintain constant voltage, current or power and is capable of producing a potential of 1500 volts and passing a maximum current of 150 milliamps.

TABLE 8

MATERIALS

Material	Grade	Manufacturer
Hemoglobin Kit	-------	Helena Labs.
Titan Gel Agarose Electrophoresis System	------	Helena Labs.
ASA_2 Control	Human Hemoglobin	Helena Labs.
AFSC Control	Human Hemoglobin	Helena Labs.
$AFSA_2$ Control	Human Hemoglobin	Helena Labs.
Serum Control	Human Serum	Clinical sample
Acetic Acid	Reagent, ACS	Fisher Scientific
Glacial Acetic Acid	Reagent, ACS	Fisher Scientific
Methanol	Reagent	Eastman

Computer

A 128/K APPLE(tm) IIe, model A9M0108, complete with dual disk drive, monitor and printer was used. These particular computers come with seven interface slots for additional features. In this study, the computer used a printer card, an 80-column card, a disk drive card and an ADA-LAB(tm) card in four of the seven slots.

Interface Card

Interfacing is accomplished with an ADALAB(tm) interface card, model ICI 1980. This card includes a 12-bit A/D voltage input, a 12-bit D/A voltage output, eight digital sense inputs, eight digital sense outputs, a 32-bit real time clock, two 16-bit timers and QUICKI/O(tm) data acquisition software.

Electrophoresis Chamber

The electrophoresis chamber is manufactured by Helena Laboratories, catalog number 1283. It was modified for the introduction of the IR temperature probe. A one-quarter inch diameter hole was drilled such that the probe could be placed directly above the center of the cellulose acetate or agarose gel plate.

Monitors and Detectors

The detector used to measure the absorbance of the electrophoresed proteins in this study is the Helena

Laboratories Flur-Vis Quick Scan Densitometer. To insure the proper potentials and currents of the electrophoresis power supply, several Keithley 169 digital multimeters were employed. Temperature was monitored by the Omega infrared to millivolt convertor, model OS-500C.

CHAPTER V

PROCEDURE AND RESULTS

Preparation of Solutions

Most of the reagents came as part of Helena electrophoresis kits except for those that are commonly found in the laboratory. All solutions can be prepared in advance and are stable for two months at 15 to 30 °C.

Buffers

All of the buffers are provided in the kits and come as pre-packaged solids. The buffer used for hemoglobin electrophoresis is tris-EDTA-boric acid. This buffer is dissolved in 980 mL of deionized water to provide a specific ionic strength and give a pH range of 8.2 to 8.6. For serum protein electrophoresis, a package of barbital-sodium barbital with thimerosal is dissolved in 1500 mL of deionized water with a pH range of 8.4 to 8.8.

Hemolysate Reagent

A 0.005-M EDTA solution in deionized water is used to prepare a hemolysate for hemoglobin electrophoresis.

Fixative/Destain Solution

This solution is used in serum protein electrophoresis. It is a mixture of 1000 mL of methanol, 1000 mL of purified water and 200 mL of glacial acetic acid.

Stains

Dry powder stains are provided in the electrophoresis kits. For hemoglobin analysis, Ponceau S is dissolved in 1000 mL of 5.0 percent acetic acid such that the stain is 0.25 percent (w/v) in the acetic acid. Amido black, a serum protein stain, is dissolved in 1000 mL of fixative/destain solution such that the stain is 0.25 percent (w/v) in this solution.

Clearing Solution

A clearing solution is necessary to achieve a transparent background on cellulose acetate plates. It is a mixture of thirty parts glacial acetic acid, seventy parts methanol and four parts polyethylene glycol.

Miscellaneous

Acetic acid is mixed as a 5 percent (v/v) solution in deionized water as well as being used in reagent strength. Methanol and glacial acetic acid are needed in reagent concentrations only.

Instrument Hookup and Procedure

Before performing an electrophoresis experiment, the following connections are necessary in order for the equipment to function as a unit. The three leads from each of the two ADA-LAB(tm) boards must be plugged into the sixteen-pin connectors on the back of the interface box. The leads and the back of the interface box are labeled to make this easier. Next, a wire must plug into the D/A output on the front of the interface box to the connector on the back of the power supply. The ground switch near the connector must be turned off. Also, from the front of the power supply, negative and positive leads connect to the power supply input on the front of the interface box. The electrophoresis chamber links to the power supply output directly below the input terminal. If the IR temperature probe is needed, an A/D input is provided to the right of the input terminal.

After these connections are made, the diskette containing the electrophoresis programs is placed into drive number 1 of the microcomputer. By turning on the power, the diskette is booted. Since only capital letters are recognized by the microcomputer, the CAP LOCK key must be in the proper position. The command, CATALOG, is typed in order to read the directory. The directory is a list of all the electrophoresis programs available on the disk. At this point, either the cellulose acetate- or agarose-gel procedure should be followed.

Cellulose Acetate Electrophoresis

The following procedure is used to perform hemoglobin electrophoresis on cellulose acetate. Figure 21 is a pictorial representation of these directions.

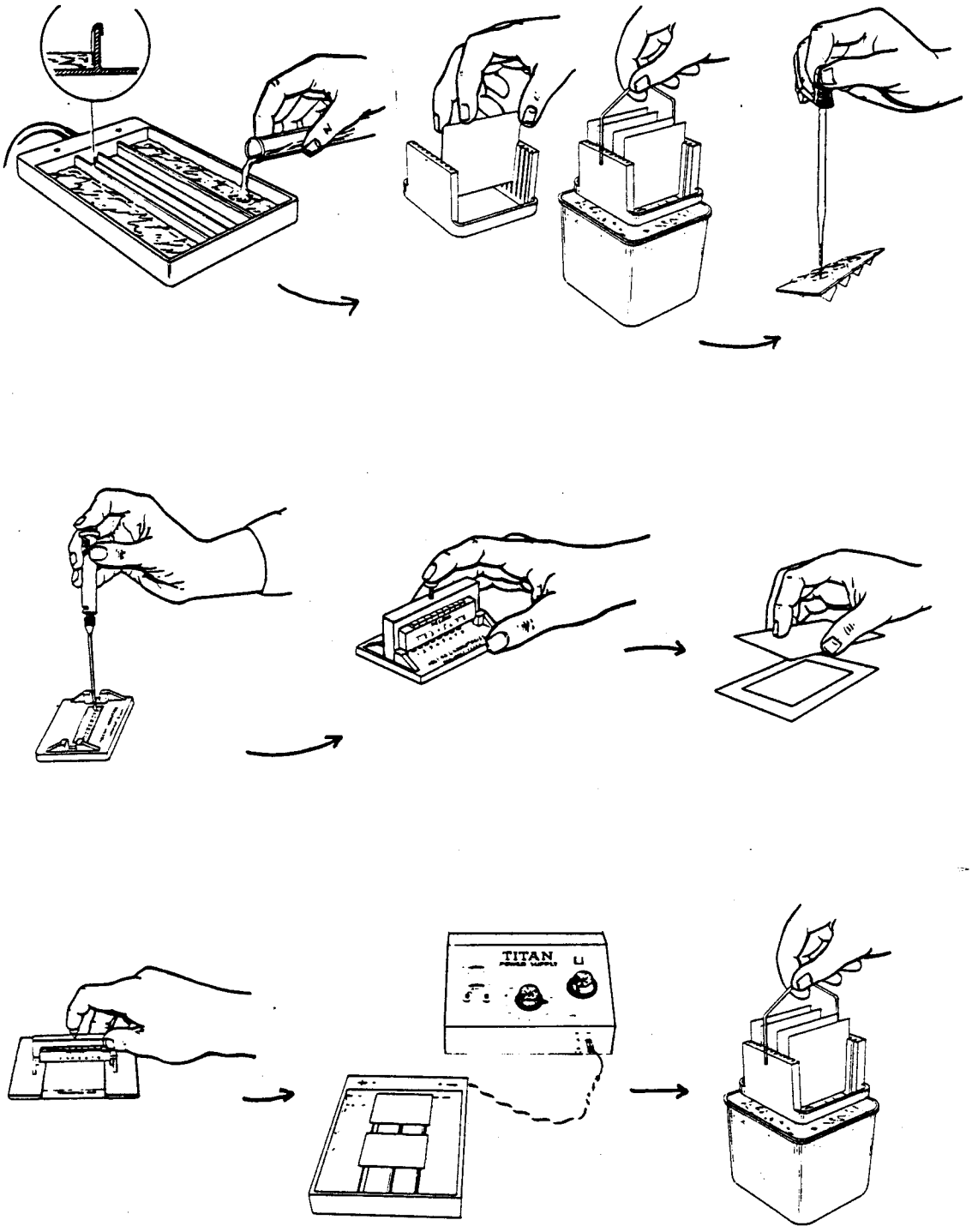
Pour equal amounts of buffer into the outer compartments of an electrophoresis chamber. Wet two wicks in the buffer and drape one over each support bridge so that they make buffer contact. The chamber is ready for electrophoresis but should be kept covered while not in use.

Soak cellulose acetate plates approximately five minutes before use by slowly and uniformly lowering a rack of the plates into the buffer. In the meantime, mix by swirling one part of the hemoglobin sample to six parts of the hemolysate reagent. After one minute, dispense 3 µL of this mixture into each of the wells in a sample holder and cover with a glass slide to prevent evaporation.

Load the applicator as shown, then blot in order to achieve a more uniform application upon the second loading. Once loaded, the application must be made within 15 s.

A previously-soaked cellulose acetate plate should be blotted, then placed in the aligning base with the acetate side up. Quickly transfer the applicator to the aligning base approximately 25 mm above the cathode end. Press down and hold for five seconds.

Now, place the strip, acetate side down, into the chamber with the sample site nearest the cathode side. Once the cell is ready, load the microcomputer with the program



Cellulose Acetate Electrophoresis

Figure 21

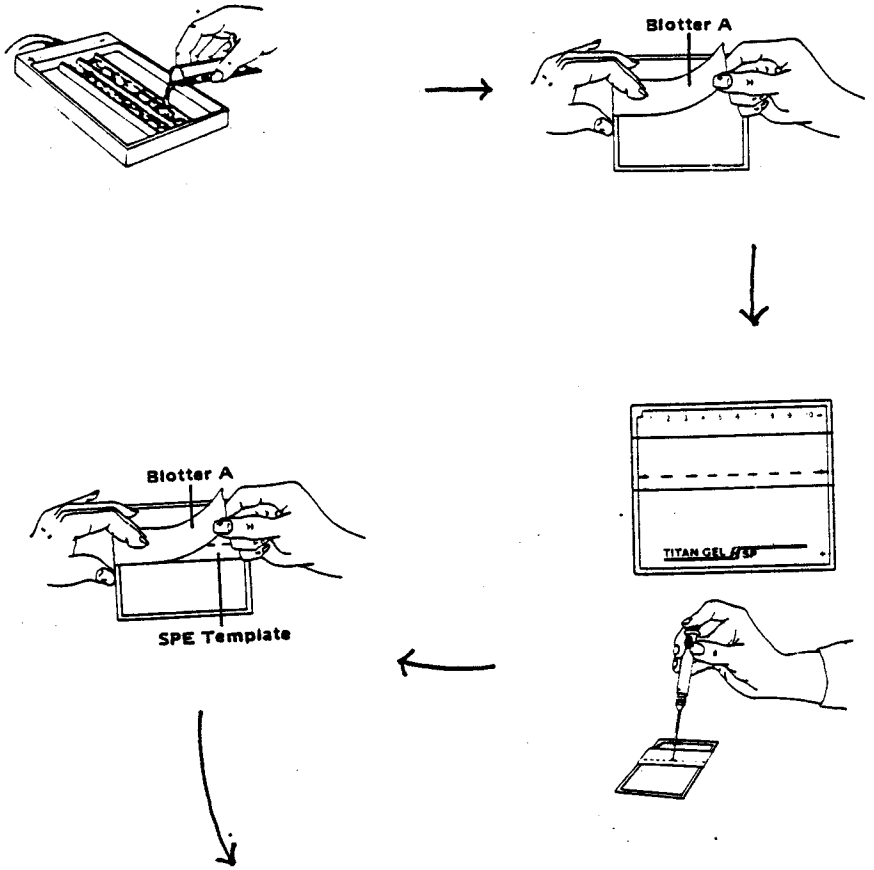
of choice and input the desired voltage, current, wattage or temperature and the amount of time for the run. Turn the power supply on but make sure that the power control knob is turned counterclockwise to zero. The microcomputer will automatically run the experiment and shut down at the end of the indicated time. If the constant-temperature program is to be used, the IR probe must be placed 5 to 15 mm above the center of the cellulose acetate plate through the specially drilled hole in the cover.

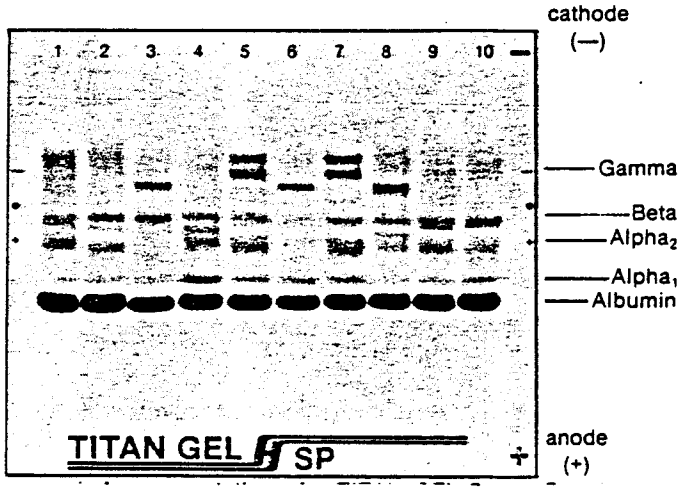
Once electrophoresis is completed, the plate is stained in Ponceau S for five minutes. Three successive washings in 5 percent acetic acid are performed until the stain is completly removed from the white background. At this point, only the hemoglobin fractions retain the color of the stain.

The plate is dehydrated in methanol twice for two minutes each and then soaked in a clearing solution for five minutes. Air dry for one minute and oven dry for several minutes at 60 ^{o}C. The white background is no longer visible and only the clear mylar background remains. The absorption of the hemoglobin fractions is determined by densitometer scans at 525 nm.

Agarose Gel Electrophoresis

The following procedure is used to perform serum protein electrophoresis on agarose gel. Figure 22 is a pictorial representation of these directions.





Agarose Gel Electrophoresis

Figure 22

Pour equal amounts of buffer into the inner compartments of an electrophoresis chamber. Cover the chamber until it is ready to be used.

Remove an agarose gel plate from the protective packaging and gently blot the plate at the area of application using fingertip pressure. Next, place the template on the gel as shown. Apply fingertip pressure to assure that there are no air bubbles under the template. Place 3 μL of the sample which is one part serum to three parts buffer onto the template slits. Allow four minutes for the samples to diffuse into the agarose then blot the template and remove it after waiting thirty seconds.

Place the plate, agarose side down, into the inner sections of the chamber making sure that the edges of the agarose are in contact with the buffer and the application point is on the cathodic side. The cover is placed on the chamber such that it does not touch the plate.

Load the microcomputer with the program of choice and input the desired parameters as in the previous procedure. Turn on the power supply as before. Also, several pulse programs are available for both the gel and the acetate procedures. They require input for pulse height, base height, time of pulse, time between pulses and the amount of time for the experiment to last. Once again, the microcomputer completely controls the electrophoresis experiment. If temperature is to be monitored, the IR probe is placed as indicated earlier.

Upon completion of the run, remove the plate from the chamber and place it in methanol for five minutes. Remove the plate from the methanol and place it on a blotter. Set it in an oven at 60 °C until completely dry. Then immerse the plate into the amido black stain for ten minutes. Two successive washings in destain solution are performed until the plate's background is clear. At this point, only the protein fractions retain the color of the stain. Again, dry the plate in the oven at 60 °C. Scan the plate using the densitometer at 595 nm.

Temperature Study

Various constant voltage, current and wattage studies were performed using a microcomputer-controlled power supply. Temperature was monitored during each experiment by the IR temperature probe while electrophoresis was conducted for hemoglobin on cellulose acetate and serum protein on agarose gel plates. Also, the temperature of hemoglobin on cellulose acetate was recorded for different buffer concentrations. Tables 9 through 15 report these temperatures while Figures 23 through 29 are graphical representations.

Hemoglobin Study

Several hemoglobin variant controls, namely ASA_2, $AFSA_2$ and AFSC, were monitored for resolution under different constant voltage, current and wattage conditions.

TABLE 9

IR TEMPERATURES
FOR VARIOUS BUFFER CONCENTRATIONS

Time (min)	@ 700 mL (°C)	@ 800 mL (°C)	@ 900 mL (°C)	@ 1000 mL (°C)
0	18.0	19.2	17.0	17.0
1	26.4	25.6	24.2	24.6
2	28.0	27.4	26.4	26.4
3	29.0	28.6	27.6	27.1
4	29.5	29.2	28.5	27.5
5	29.9	29.8	29.0	27.9
6	30.5	30.3	29.3	28.2
7	31.2	30.5	29.6	28.5
8	31.5	30.8	29.9	28.7
9	31.9	31.0	30.2	28.9
10	32.1	31.2	30.5	29.1
11	32.5	31.3	30.7	29.2
12	32.7	31.5	30.9	29.3
13	32.8	31.7	31.0	29.4
14	33.0	31.9	31.1	29.4
15	33.1	32.1	31.3	29.5
16	33.2	32.1	31.3	29.6
17	33.4	32.3	31.4	29.7
18	33.5	32.3	31.6	29.8
19	33.6	32.4	31.7	30.0
20	33.8	32.4	31.8	30.5
Averages	30.9	30.2	29.3	28.1

NOTE: Concentrations are for Supre Heme buffer in deionized water. All runs were conducted on cellulose acetate at constant voltage (350 V).

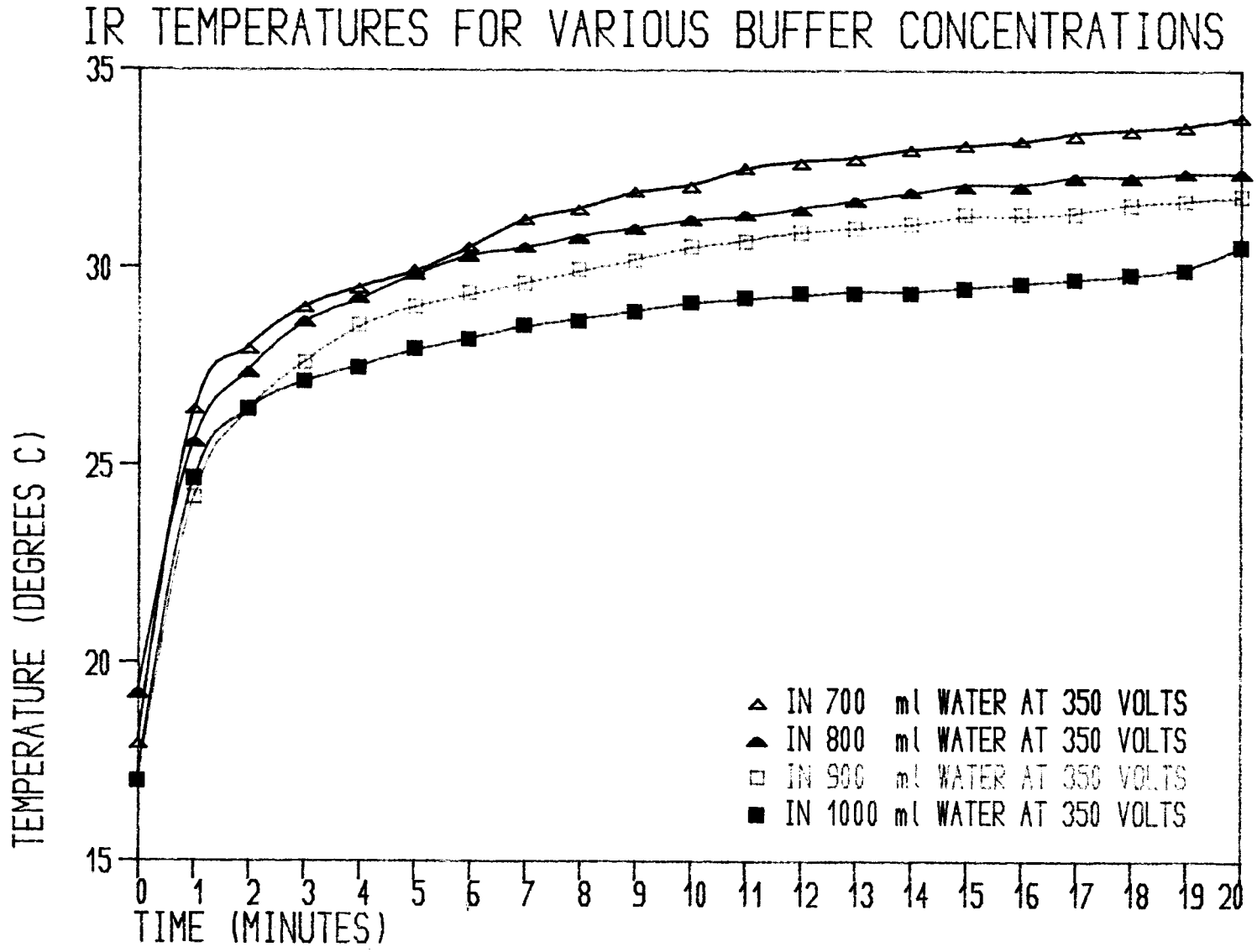

IR TEMPERATURES FOR VARIOUS BUFFER CONCENTRATIONS
TEMPERATURE (DEGREES C)
35
30
25
20
15
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
TIME (MINUTES)
IN 700 ml WATER AT 350 VOLTS
IN 800 ml WATER AT 350 VOLTS
IN 900 ml WATER AT 350 VOLTS
IN 1000 ml WATER AT 350 VOLTS

Figure 23

TABLE 10

CELLULOSE ACETATE IR TEMPERATURES
CONSTANT VOLTAGE

Time (min)	@ 300 V (°C)	@ 350 V (°C)	@ 450 V (°C)	@ 550 V (°C)
0	16.9	17.0	17.0	19.0
1	22.2	24.6	28.0	37.2
2	23.9	26.4	30.7	42.0
3	24.8	27.1	32.7	43.3
4	25.5	27.5	34.7	44.0
5	26.2	27.9	35.2	46.4
6	26.6	28.2	35.9	47.5
7	26.8	28.5	36.1	47.4
8	27.0	28.7	36.4	47.0
9	27.2	28.9	36.7	46.3
10	27.4	29.1	36.8	45.7
11	27.5	29.2	36.9	45.1
12	27.6	29.3	36.9	44.6
13	27.7	29.4	36.9	44.2
14	27.8	29.4	37.0	43.7
15	27.9	29.5	37.1	43.6
Averages	25.8	27.5	35.8	42.9

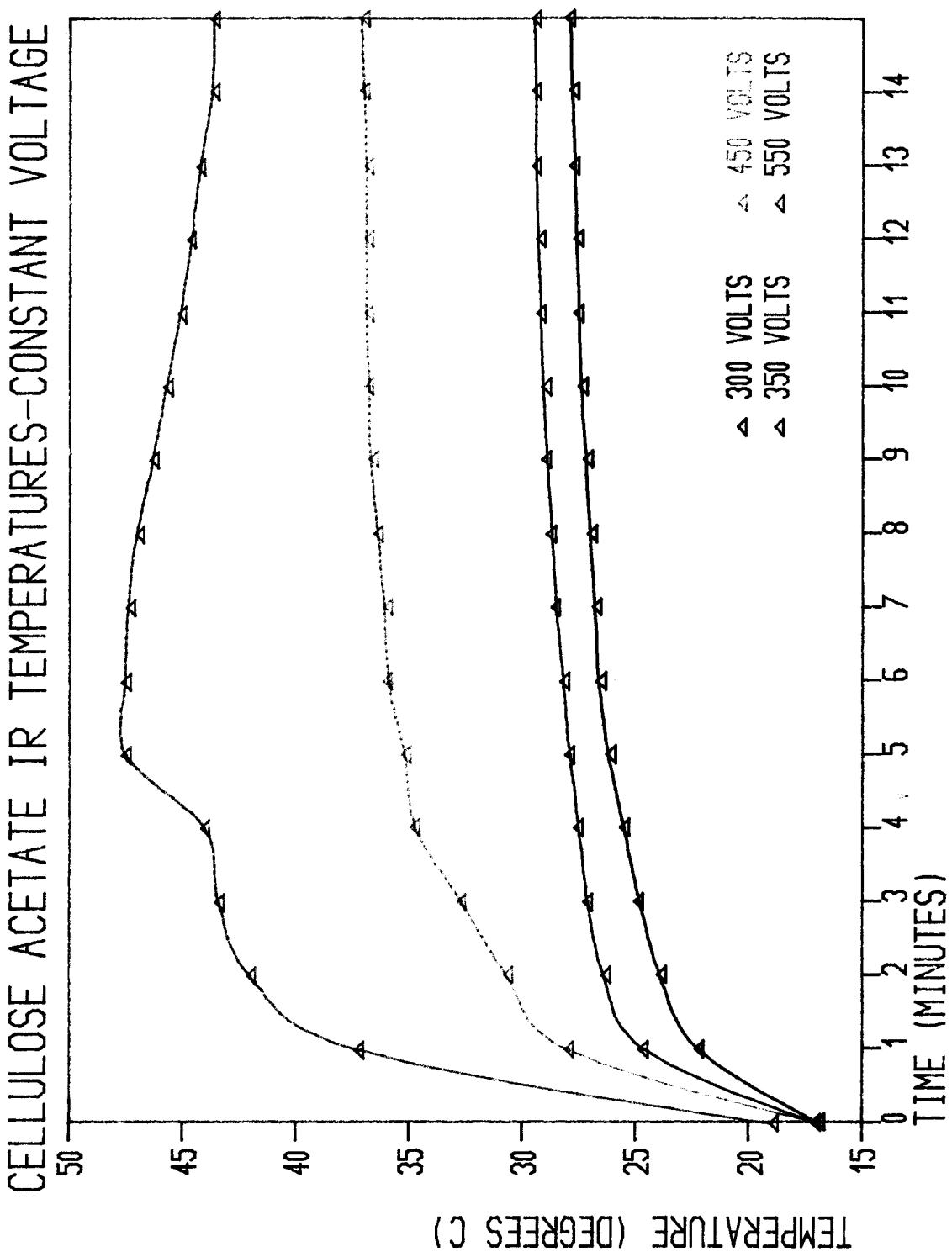

CELLULOSE ACETATE IR TEMPERATURES-CONSTANT VOLTAGE
TEMPERATURE (DEGREES C)
TIME (MINUTES)
300 VOLTS
350 VOLTS
450 VOLTS
550 VOLTS
50
45
40
35
30
25
20
15
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 24

TABLE 11

CELLULOSE ACETATE IR TEMPERATURES
CONSTANT CURRENT

Time (min)	@ 3 mA (°C)	@ 5 mA (°C)	@ 7 mA (°C)	@ 9 mA (°C)
0	20.0	19.0	23.4	23.7
1	27.7	33.1	40.9	42.6
2	28.1	34.5	42.3	46.5
3	28.1	35.7	42.1	47.6
4	28.1	36.0	42.5	48.7
5	28.4	36.6	43.2	48.1
6	28.6	37.2	42.1	47.3
7	28.6	37.5	42.8	46.5
8	28.6	37.3	42.4	45.8
9	28.8	37.2	42.1	45.2
10	29.0	37.0	41.8	44.6
11	29.3	36.9	41.5	44.2
12	29.4	36.7	41.2	43.8
13	29.3	36.6	40.9	43.6
14	29.3	36.5	40.6	43.5
15	29.3	36.4	40.5	43.2
Averages	28.2	35.3	40.6	44.1

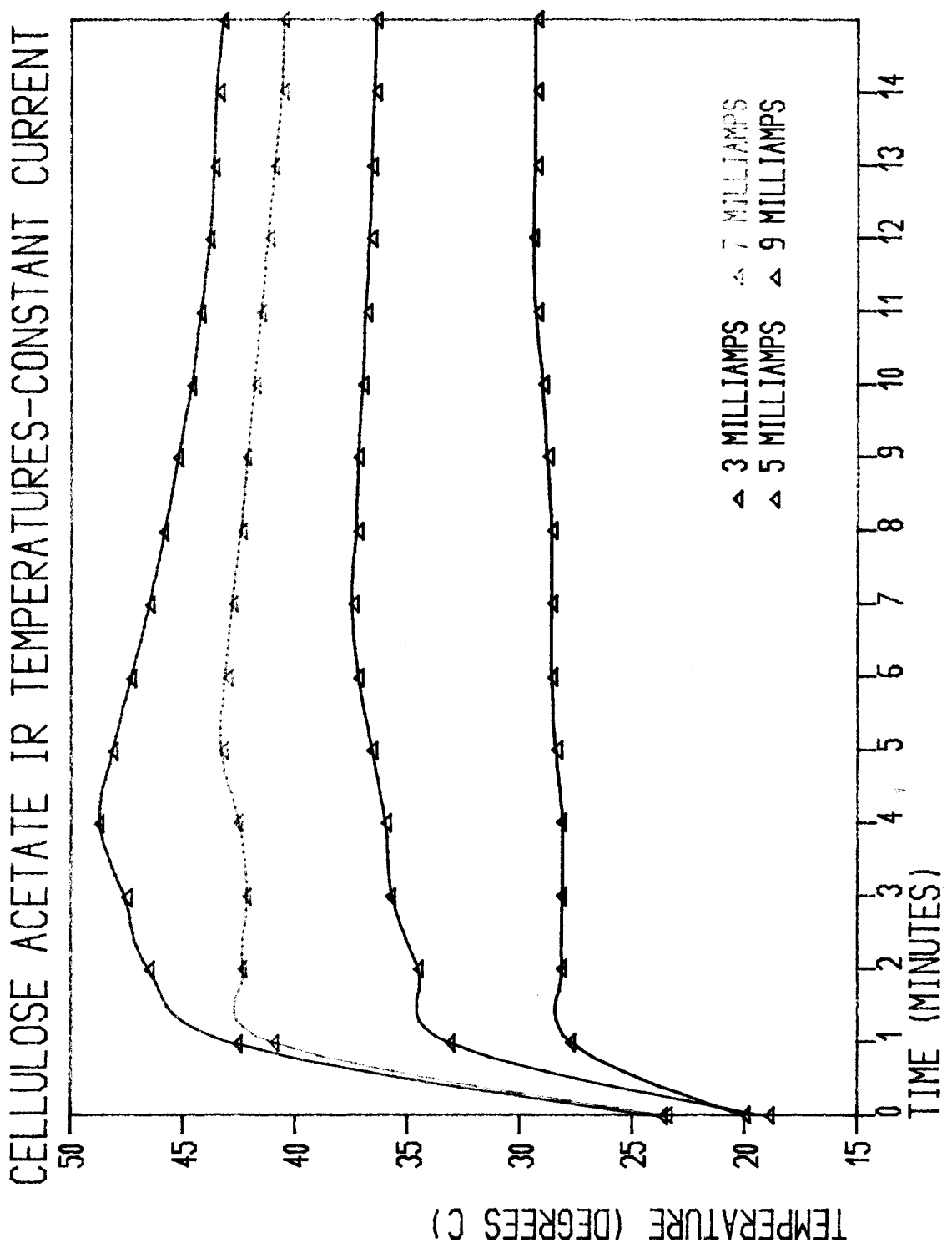

CELLULOSE ACETATE IR TEMPERATURES-CONSTANT CURRENT
TEMPERATURE (DEGREES C)
TIME (MINUTES)
3 MILLIAMPS
5 MILLIAMPS
7 MILLIAMPS
9 MILLIAMPS
50
45
40
35
30
25
20
15
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 25

TABLE 12

CELLULOSE ACETATE IR TEMPERATURES
CONSTANT POWER

Time (min)	@ 1 W (°C)	@ 3 W (°C)	@ 5 W (°C)	@ 7 W (°C)
0	17.0	18.0	20.0	18.0
1	25.2	31.0	36.1	40.5
2	26.3	33.4	38.0	42.2
3	27.2	34.2	39.9	42.9
4	27.9	34.6	40.3	43.3
5	28.2	34.6	40.4	43.0
6	28.6	34.7	40.3	43.3
7	28.8	34.7	40.1	43.9
8	28.7	34.6	40.0	44.0
9	28.7	34.5	39.9	44.0
10	28.5	34.4	39.6	43.9
11	28.5	34.3	39.5	43.7
12	28.4	34.2	39.3	43.5
13	28.3	34.1	39.0	43.4
14	28.3	34.0	38.8	43.4
15	28.2	33.9	38.8	43.1
Averages	27.3	33.1	38.0	41.5

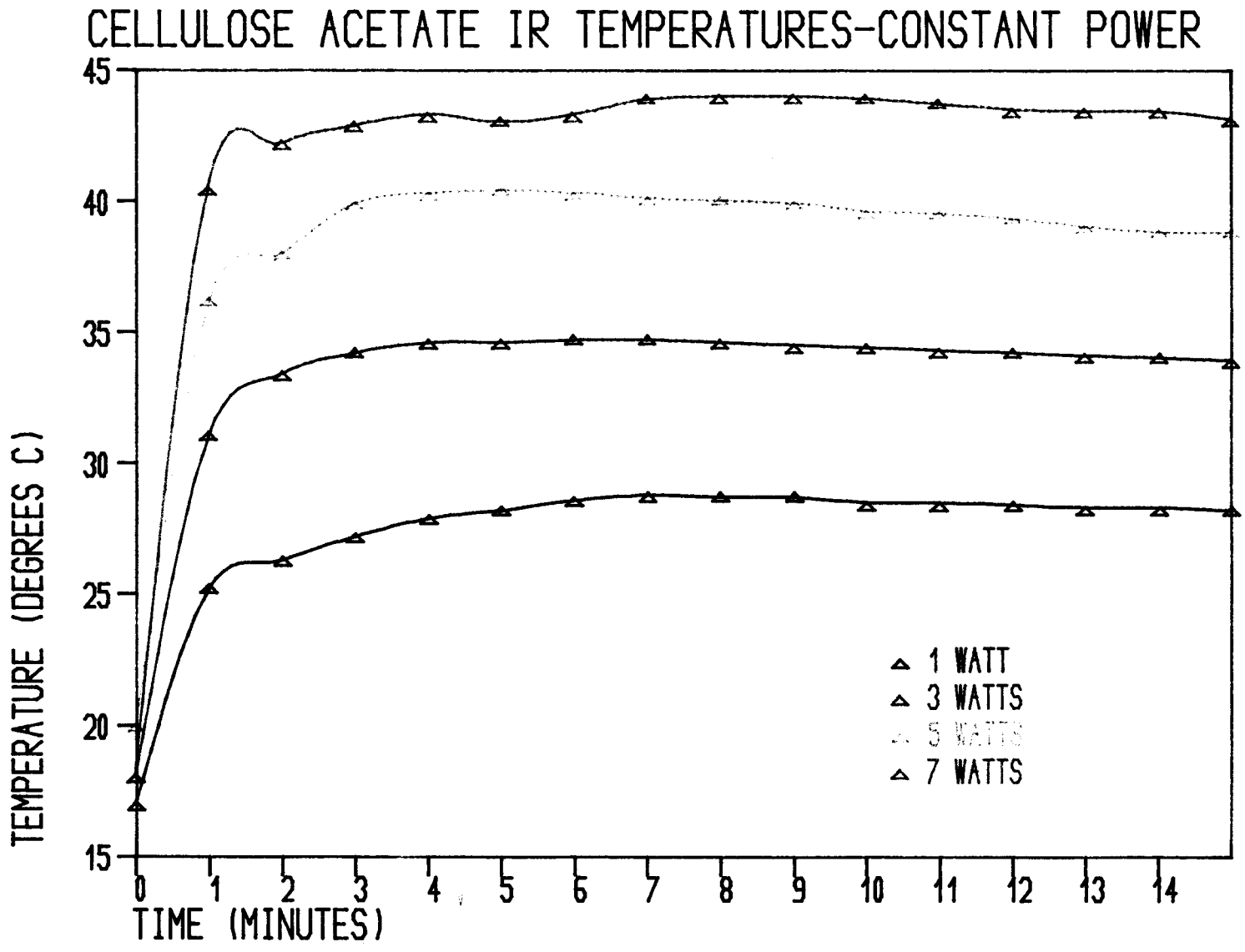

CELLULOSE ACETATE IR TEMPERATURES-CONSTANT POWER
TEMPERATURE (DEGREES C)
TIME (MINUTES)
45
40
35
30
25
20
15
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
1 WATT
3 WATTS
5 WATTS
7 WATTS

Figure 26

TABLE 13

AGAROSE GEL IR TEMPERATURES
CONSTANT VOLTAGE

Time (min)	@ 120 V (°C)	@ 220 V (°C)	@ 320 V (°C)	@ 420 V (°C)
0	21.4	21.3	21.7	21.5
1	23.5	29.0	40.9	53.0
2	25.3	34.3	55.3	64.6
3	26.5	36.3	56.6	69.8
4	27.2	37.5	57.4	71.5
5	27.7	38.2	58.4	72.0
6	28.3	38.6	58.9	72.5
7	28.4	39.0	59.1	72.4
8	28.6	39.2	59.3	72.6
9	28.7	39.5	59.6	72.9
10	28.8	39.6	60.2	73.3
11	28.9	39.6	60.6	73.5
12	29.0	39.8	60.8	73.6
13	29.0	39.9	60.9	73.7
14	29.4	40.0	61.0	73.8
15	29.3	40.1	61.1	73.9
Averages	27.5	38.4	55.7	67.8

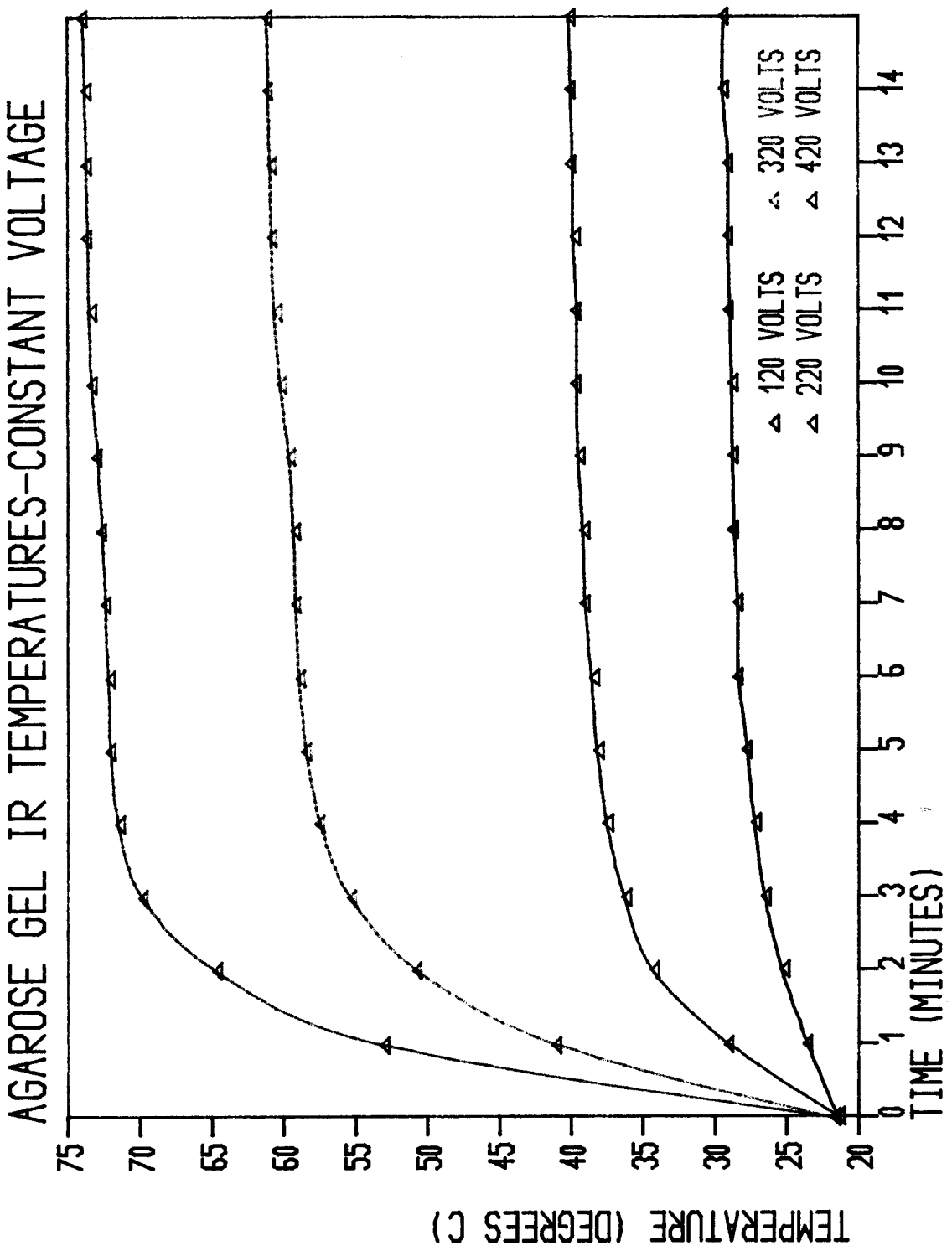

AGAROSE GEL IR TEMPERATURES-CONSTANT VOLTAGE
TEMPERATURE (DEGREES C)
TIME (MINUTES)
120 VOLTS
220 VOLTS
320 VOLTS
420 VOLTS
75
70
65
60
55
50
45
40
35
30
25
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 27

TABLE 14

AGAROSE GEL IR TEMPERATURES
CONSTANT CURRENT

Time (min)	@ 10 mA (°C)	@ 20 mA (°C)	@ 30 mA (°C)
0	21.2	22.2	21.4
1	26.5	36.4	44.3
2	28.6	39.0	48.4
3	29.5	39.9	51.0
4	30.2	40.2	52.5
5	30.7	40.8	53.2
6	30.9	41.6	53.6
7	31.4	43.4	54.0
8	31.7	44.4	54.8
9	32.2	44.9	56.6
10	32.5	45.2	58.7
11	32.8	45.7	--
12	33.0	46.3	--
13	33.1	46.8	--
14	33.2	47.1	--
15	33.3	47.4	--
Averages	30.7	42.0	50.0

Note: The gel plate was destroyed during the 30 mA run at 11 minutes. The average temperature is given for that amount of time and may not be comparable to the other runs.

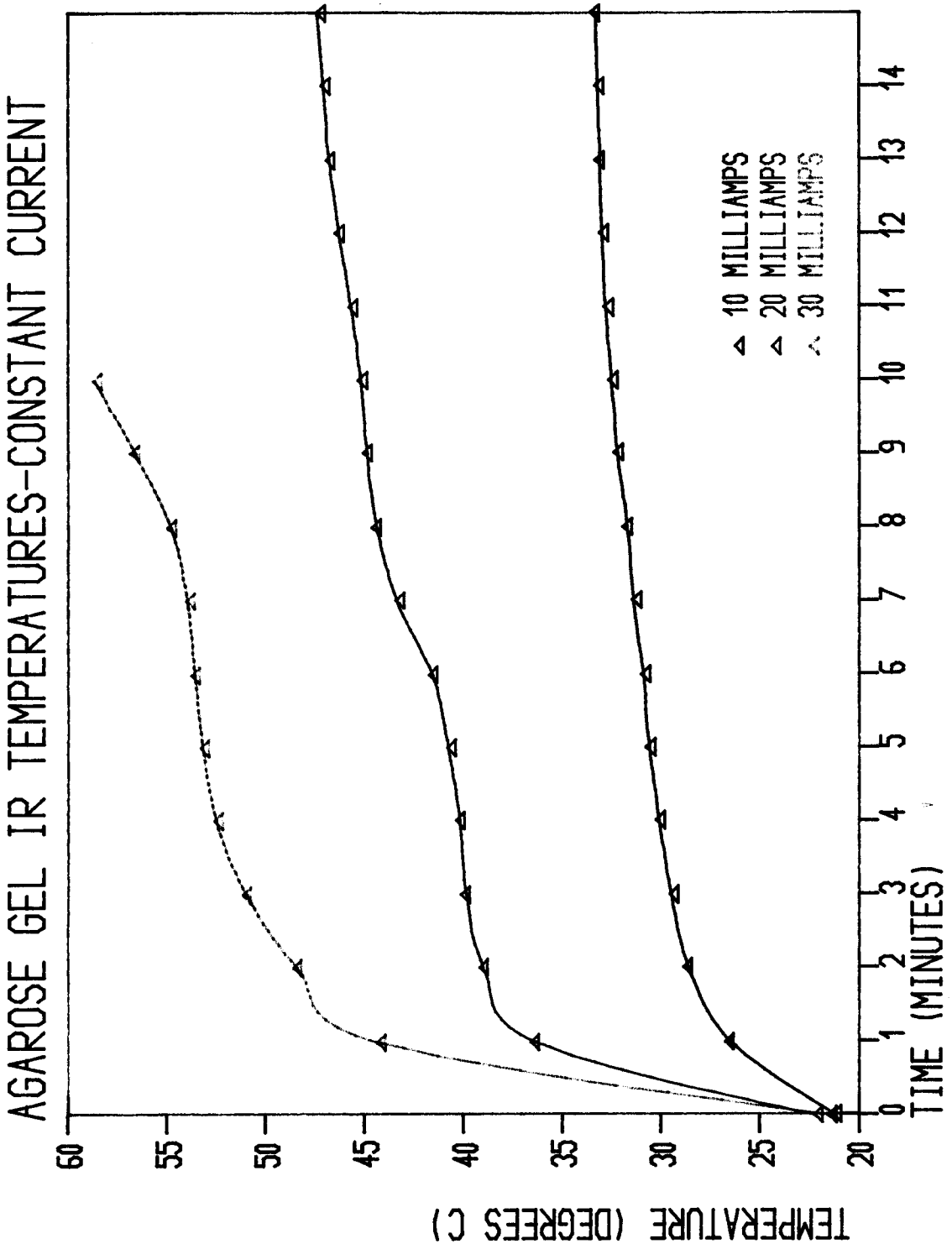

AGAROSE GEL IR TEMPERATURES-CONSTANT CURRENT
TEMPERATURE (DEGREES C)
TIME (MINUTES)
10 MILLIAMPS
20 MILLIAMPS
30 MILLIAMPS
60
55
50
45
40
35
30
25
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

Figure 28

TABLE 15

AGAROSE GEL IR TEMPERATURES
CONSTANT POWER

Time (min)	@ 1 W (°C)	@ 5 W (°C)	@ 10 W (°C)
0	21.8	21.2	22.9
1	24.0	30.1	41.4
2	25.8	35.4	51.1
3	27.0	37.4	55.8
4	27.7	38.6	57.9
5	28.2	39.3	58.9
6	28.8	39.7	59.4
7	28.9	40.1	59.6
8	29.1	40.3	59.8
9	29.2	40.6	60.1
10	29.3	40.7	60.7
11	29.4	40.8	60.8
12	29.5	40.9	60.9
13	29.5	41.1	60.9
14	29.4	41.0	61.0
15	29.4	41.0	61.1
Averages	27.9	38.0	55.8

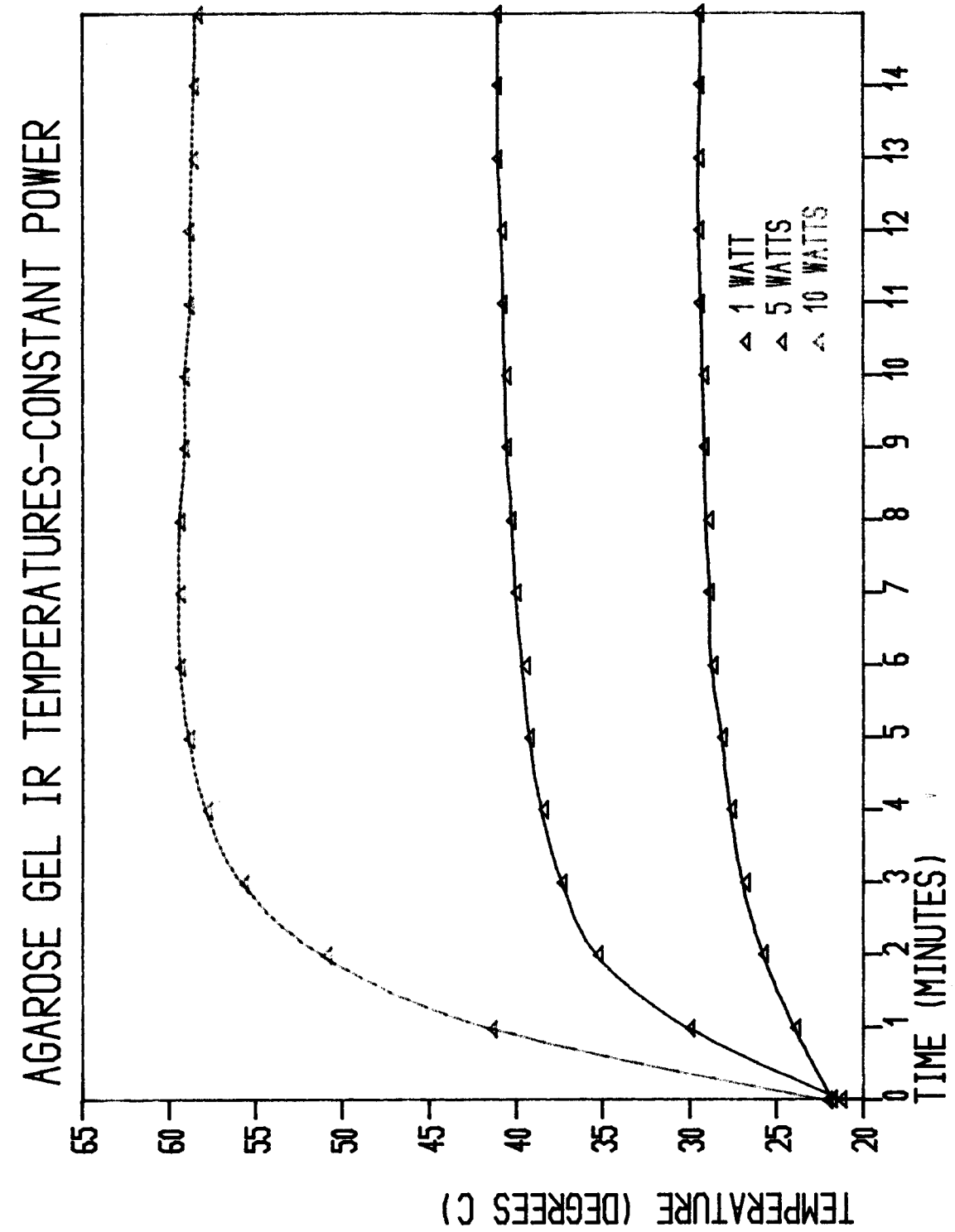

AGAROSE GEL IR TEMPERATURES-CONSTANT POWER
TEMPERATURE (DEGREES C)
TIME (MINUTES)
1 WATT
5 WATTS
10 WATTS
65
60
55
50
45
40
35
30
25
20
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14

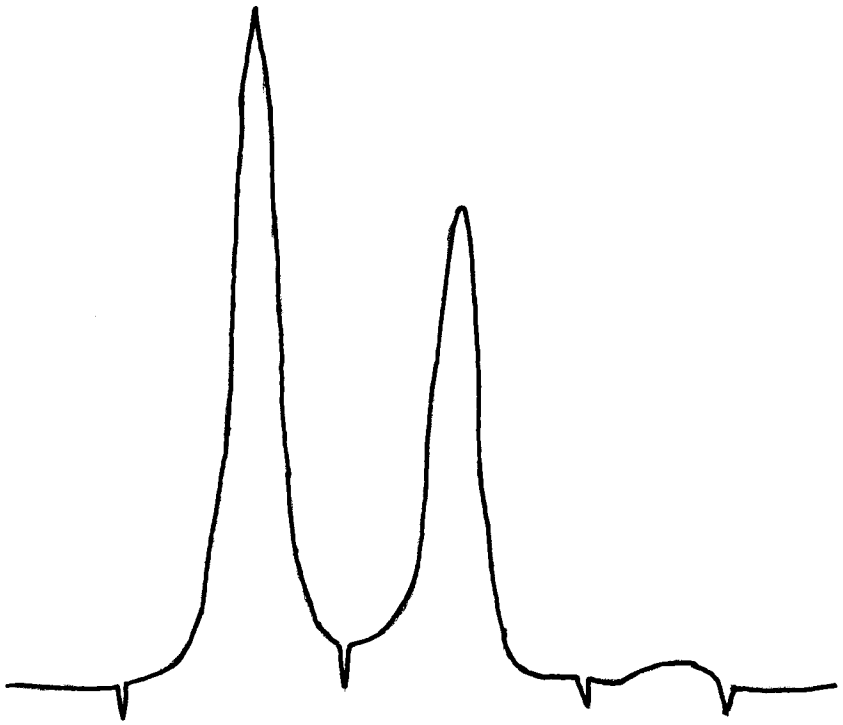
Figure 29

In this study, both the microcomputer and analog-controlled electrophoresis techniques were utilized. Figures 30 through 32 were provided by Helena Laboratories. Figures 33 through 35 illustrate densitometer scans from cellulose acetate plates for the best separation achieved by the microcomputer. The best analog electrophoresis separations occurred under the same conditions and were identical to those generated by the microcomputer. Also, the same hemoglobin controls were used to study the constant-temperature electrophoresis system. Figure 36 depicts the choice separation on a cellulose acetate plate at 32 °C.

Serum Protein Study

As in the hemoglobin study, normal human serum resolution was examined using analog and microcomputer-generated constant voltage, current and wattage methods. Once again, the best analog and microcomputer separations for these three methods occurred under the same conditions. Figure 37 was provided by Helena Laboratories. The densitometer scans in Figures 38 through 40 were obtained using the microcomputer.

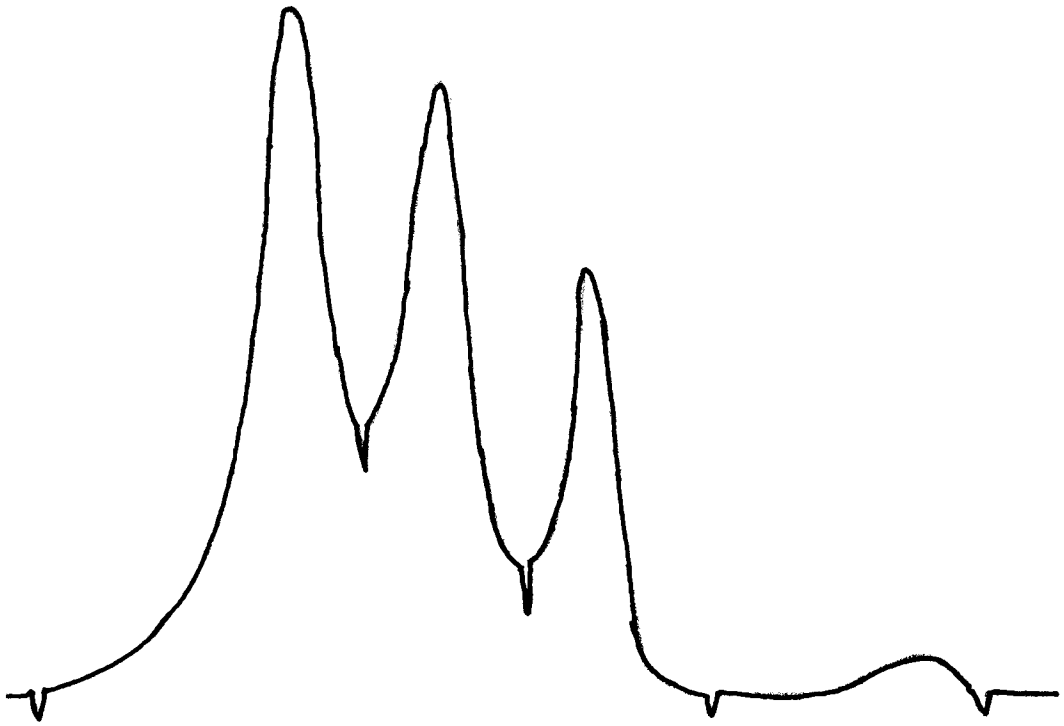
Resolution of human serum also was studied by employing several pulse programs written for the microcomputer-controlled power supply. Variations on the input parameters were attempted in order to achieve maximum separation. The densitometer scans in Figures 41 through 45 illustrate these results. The parameters, average



Hemoglobin	%	Range %
A	66.1	61.0-71.2
S	31.2	26.4-36.0
A_2	2.8	1.4-4.2

ASA_2 Hemo Control

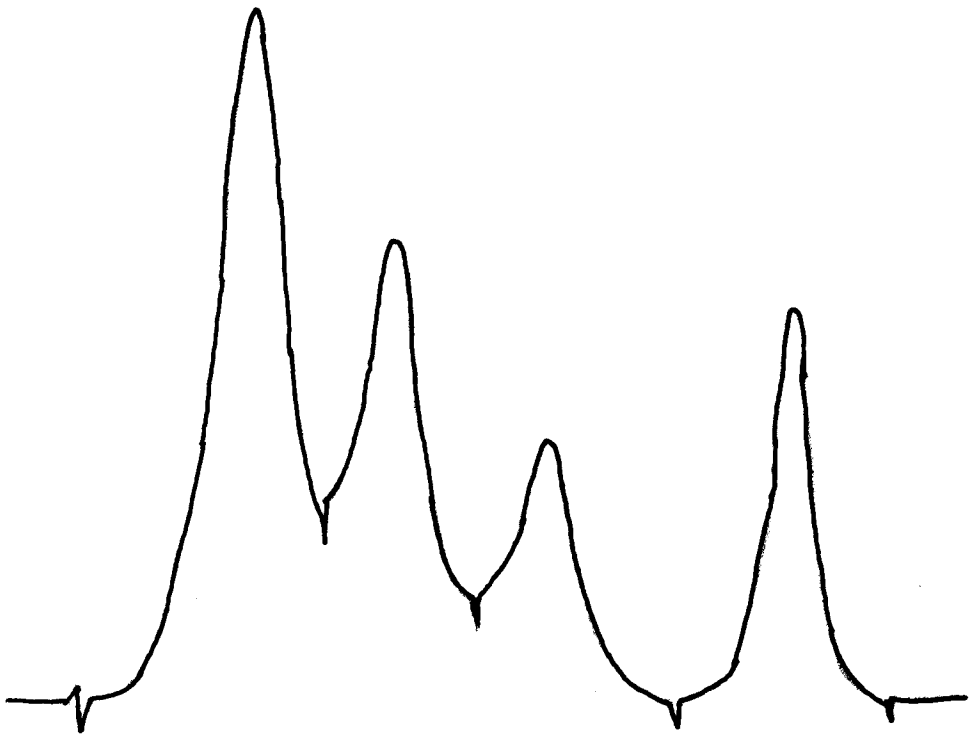
Figure 30



Hemoglobin	%	Range %
A	49.0	42.0-56.0
F	29.6	25.7-33.5
S	19.8	15.8-23.8
A_2	1.6	0.7-2.5

$AFSA_2$ Hemo Control

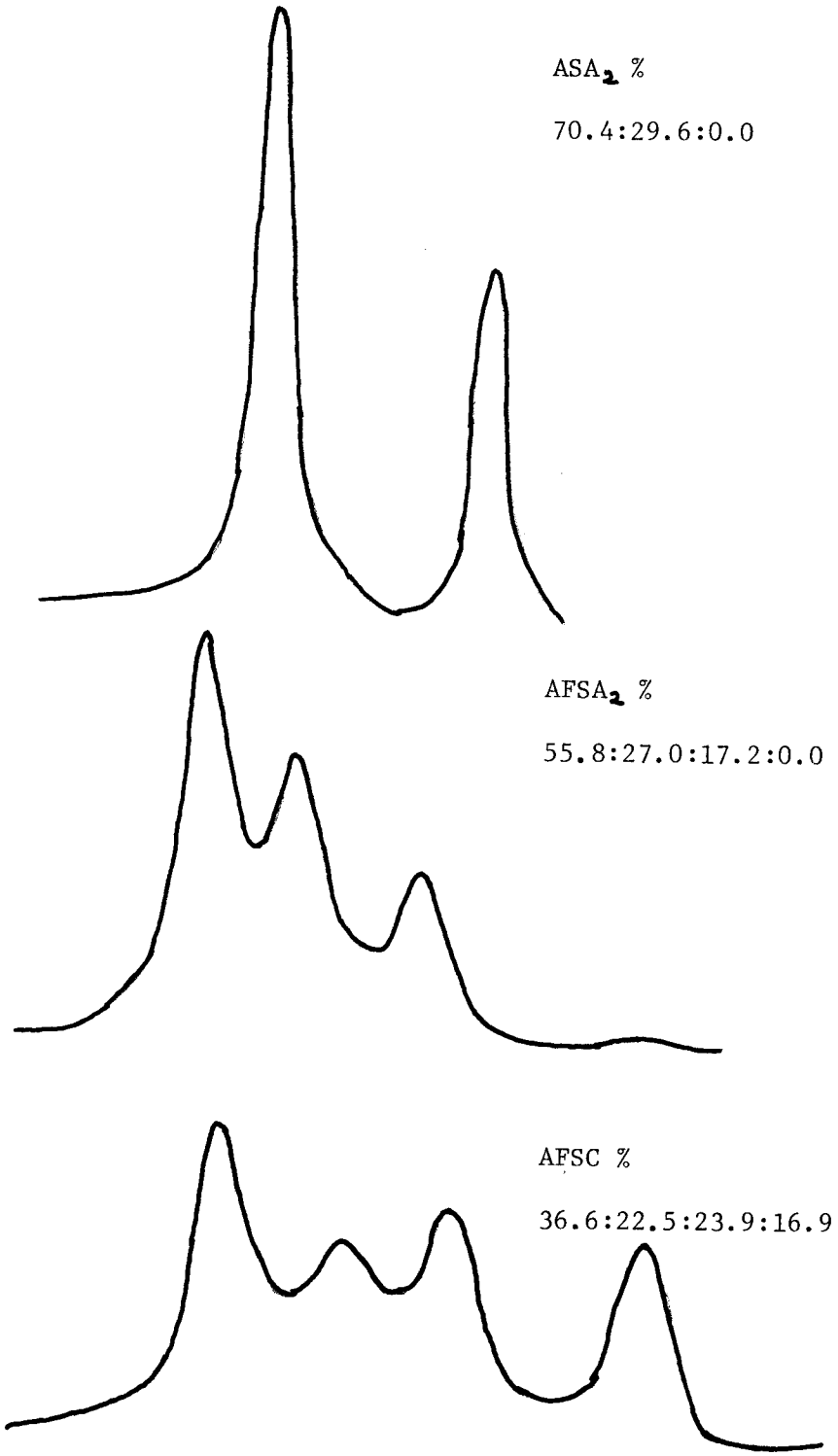
Figure 31



Hemoglobin	%	Range %
A	40.1	35.3-44.8
F	20.2	17.5-22.9
S	20.1	18.0-22.2
C	19.7	17.2-22.5

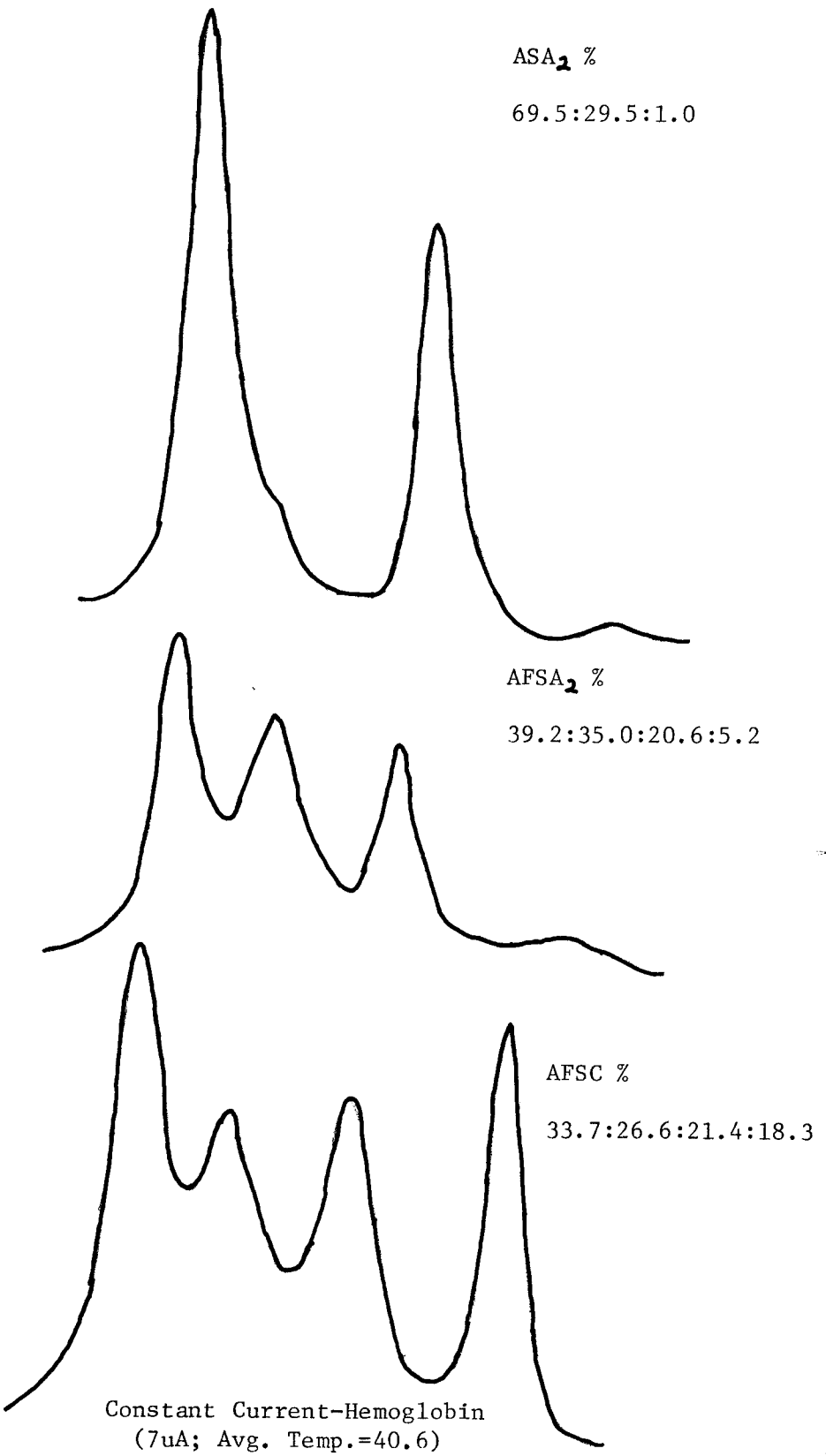
AFSC Hemo Control

Figure 32



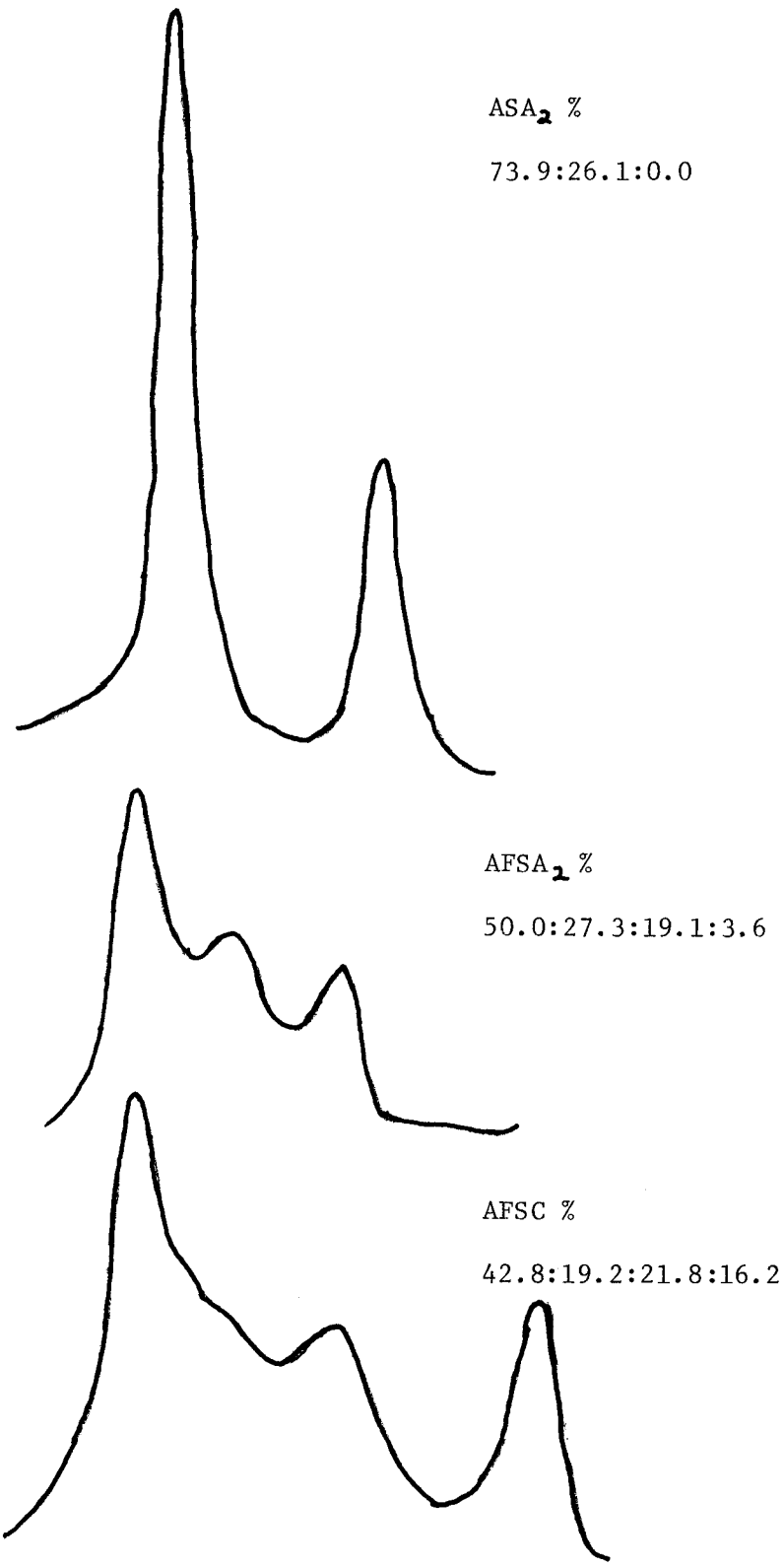
Constant Voltage-Hemoglobin
(350V; Avg. Temp.=27.5)

Figure 33



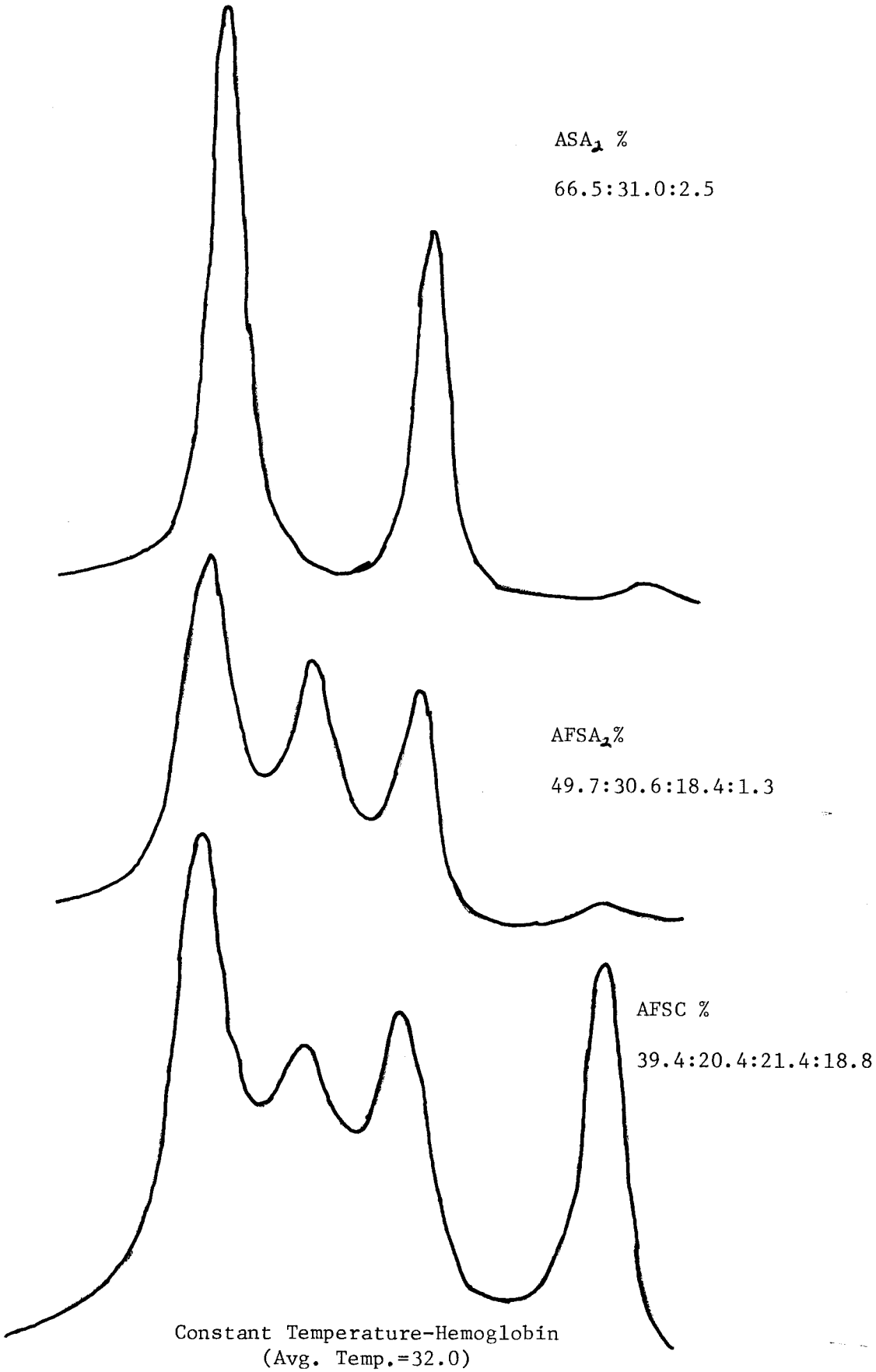
Constant Current-Hemoglobin
(7uA; Avg. Temp.=40.6)

Figure 34



Constant Wattage-Hemoglobin
(3W; Avg. Temp.=33.1)

Figure 35



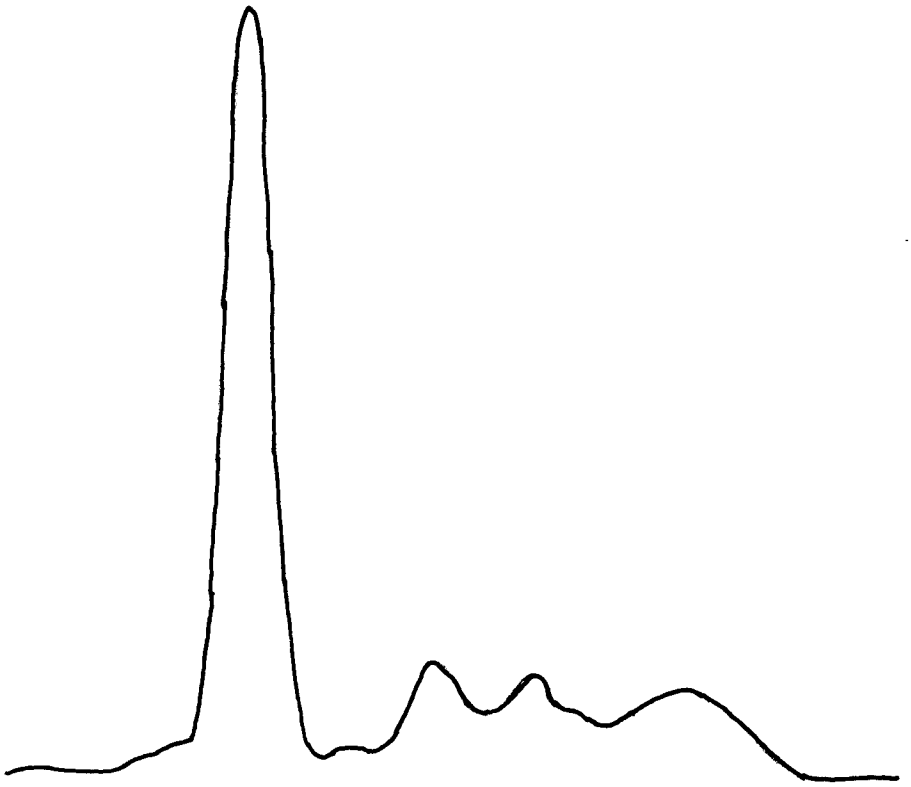
Constant Temperature-Hemoglobin
(Avg. Temp.=32.0)

Figure 36

Serum Protein	%	Range %
Albumin	57.1	51.9-62.3
Alpha one	3.4	2.2-4.6
Alpha two	11.2	9.2-13.3
Beta	13.7	11.5-15.9
Gamma	14.6	12.0-17.2

Normal Serum Control

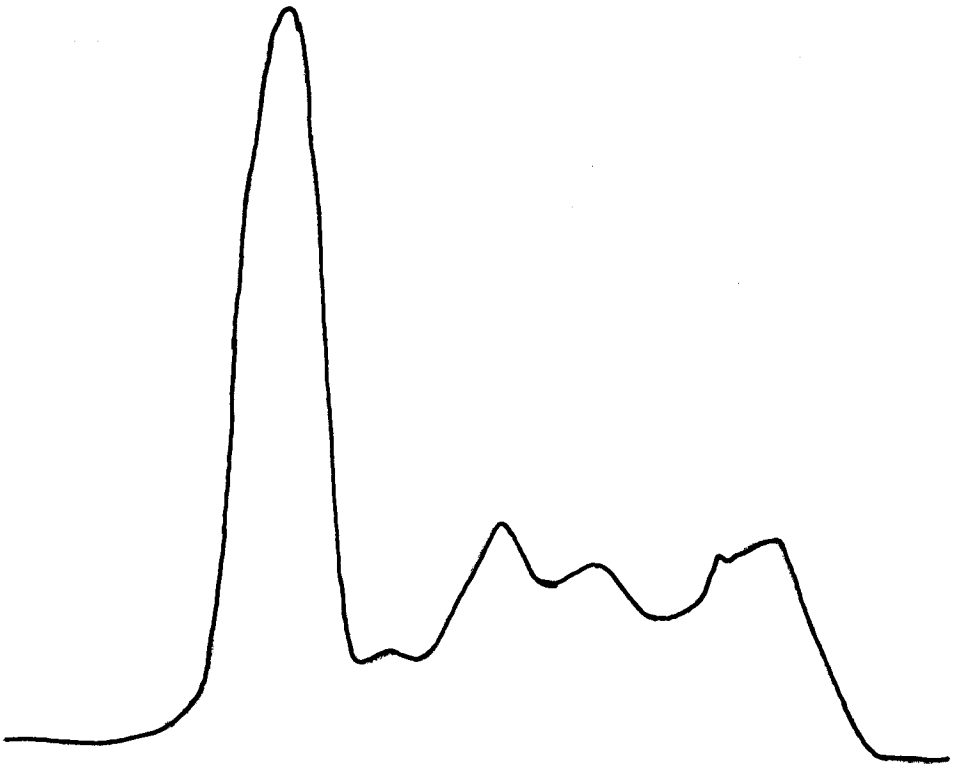
Figure 37



Serum Protein	%
Albumin	68.3
Alpha one	0.8
Alpha two	10.7
Beta	8.5
Gamma	11.7

Constant Voltage-Serum
(120V; Avg. Temp.=27.5)

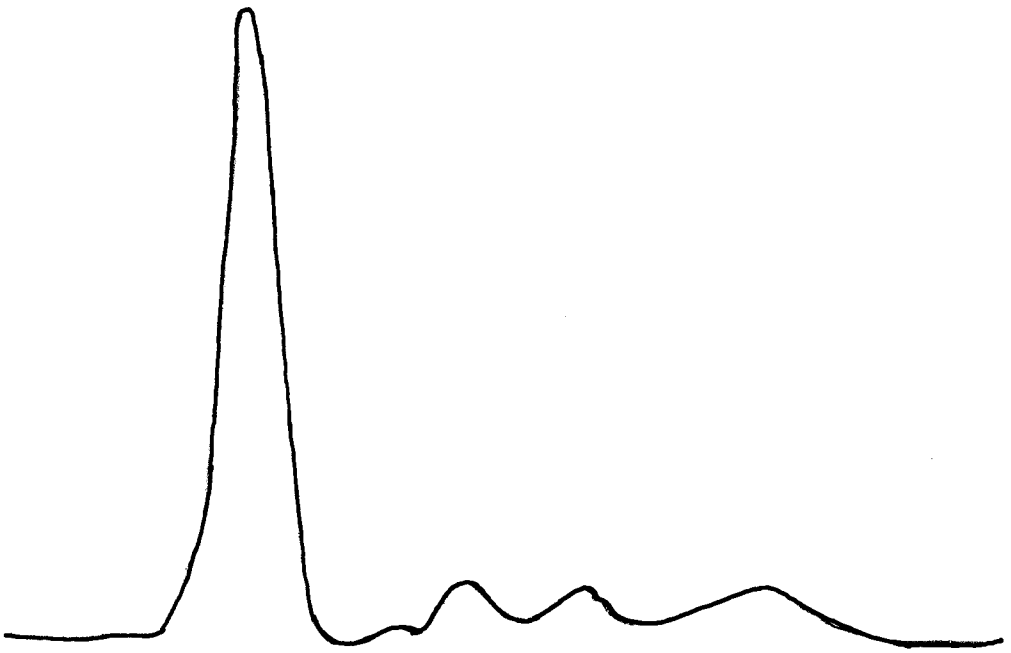
Figure 38



Serum Protein	%
Albumin	49.6
Alpha one	3.0
Alpha two	15.4
Beta	12.3
Gamma	19.7

Constant Current-Serum
(3uA; Avg. Temp.=28.7)

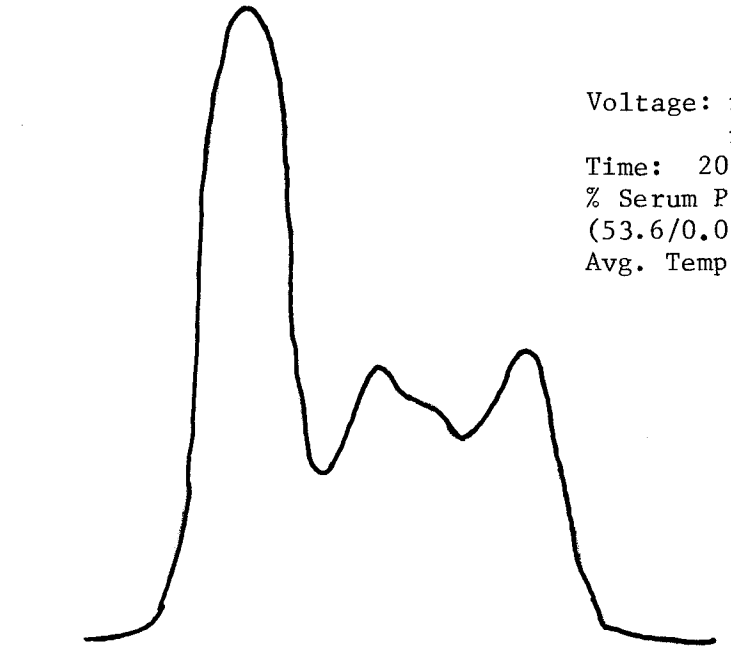
Figure 39



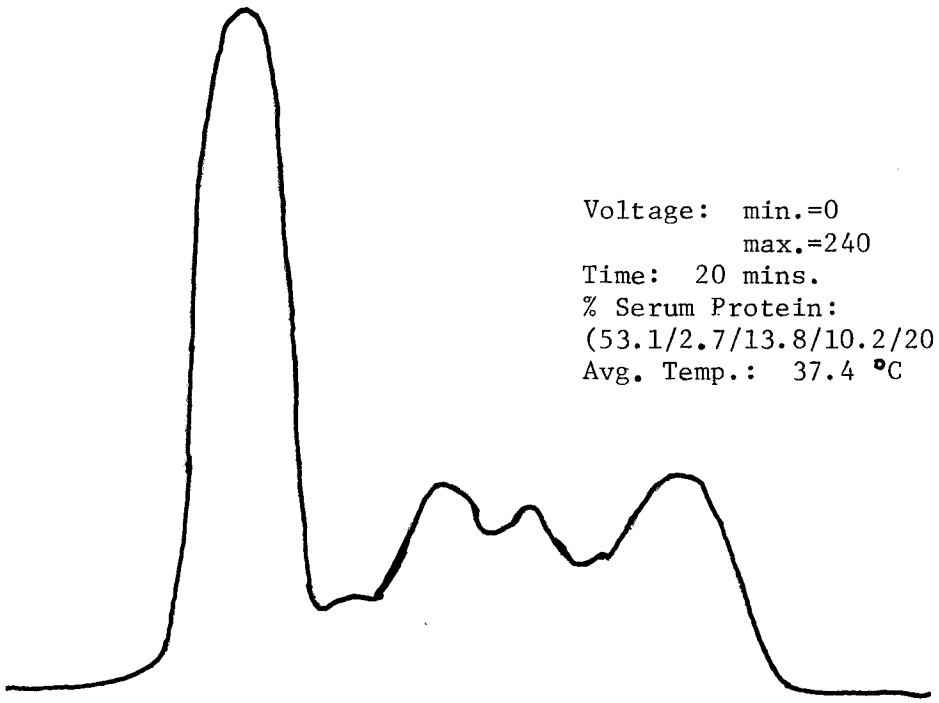
Serum Protein	%
Albumin	86.5
Alpha one	0.5
Alpha two	4.2
Beta	3.6
Gamma	5.2

Constant Wattage-Serum
(3W; Avg. Temp.=28.3)

Figure 40



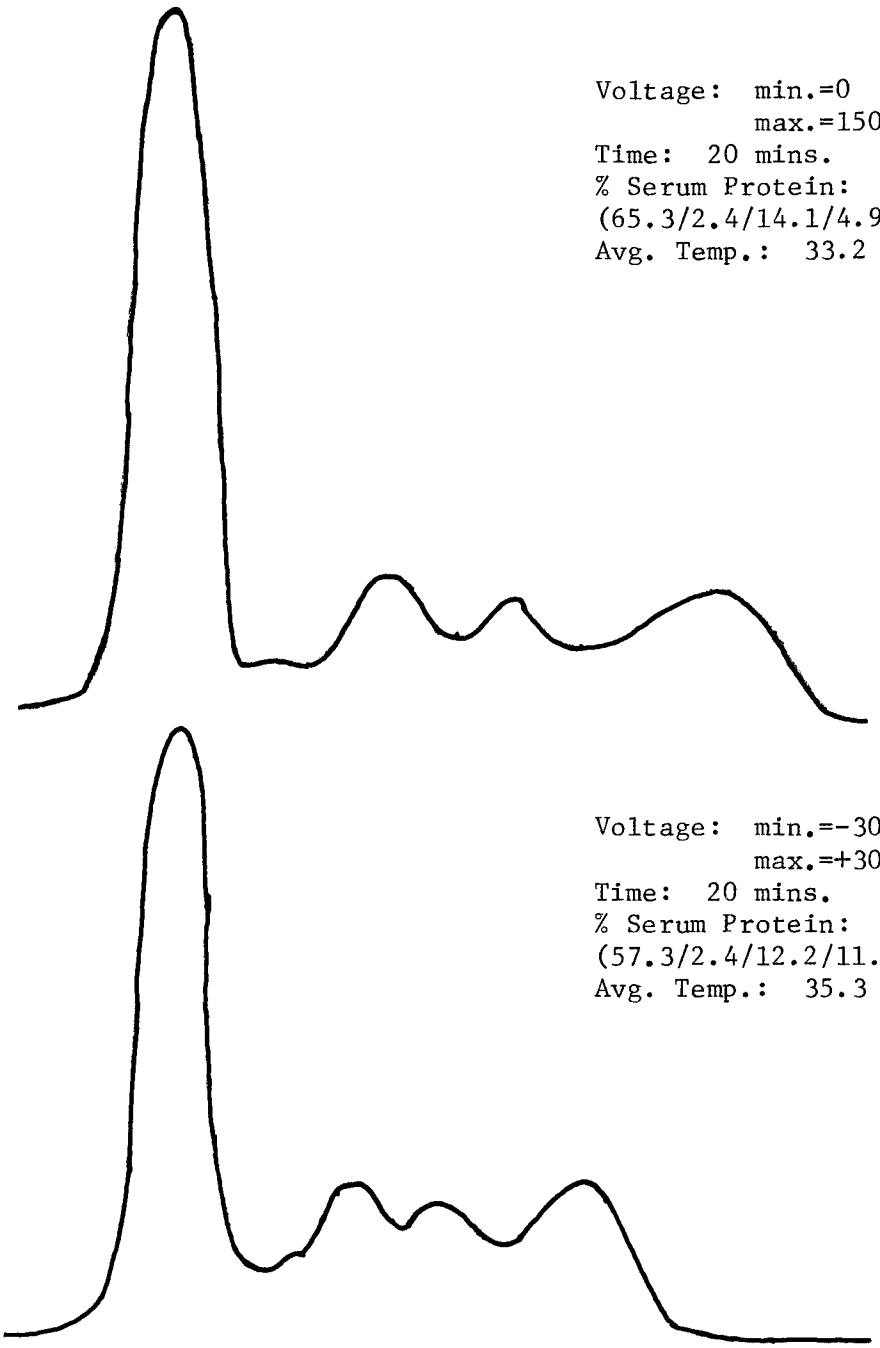
Voltage: min.=0
max.=230
Time: 20 mins.
% Serum Protein:
(53.6/0.0/15.9/9.9/20.6)
Avg. Temp.: 34.1 °C



Voltage: min.=0
max.=240
Time: 20 mins.
% Serum Protein:
(53.1/2.7/13.8/10.2/20.2)
Avg. Temp.: 37.4 °C

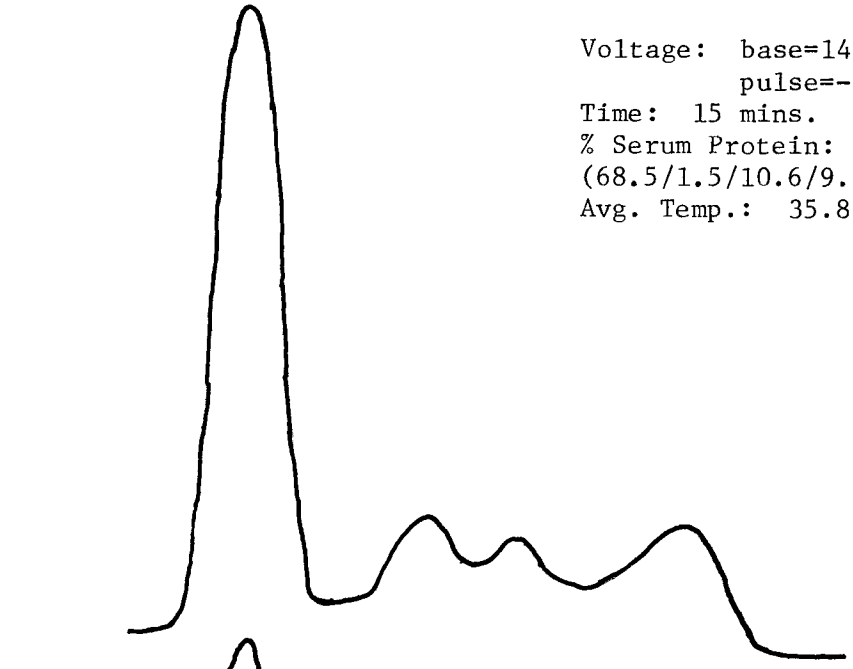
Sawtooth Pulses for Serum

Figure 41

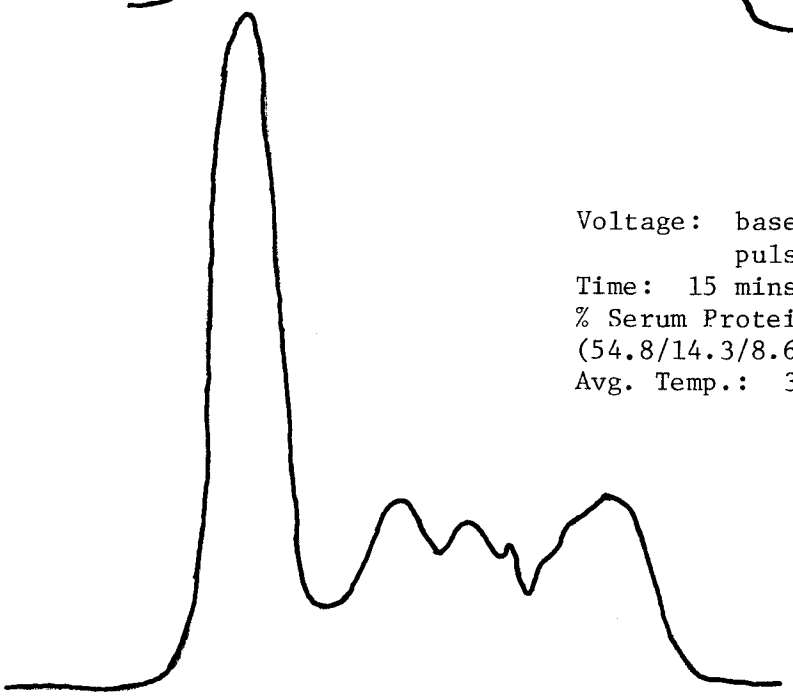


Square Wave and Polarity Reverse
Pulses for Serum

Figure 42



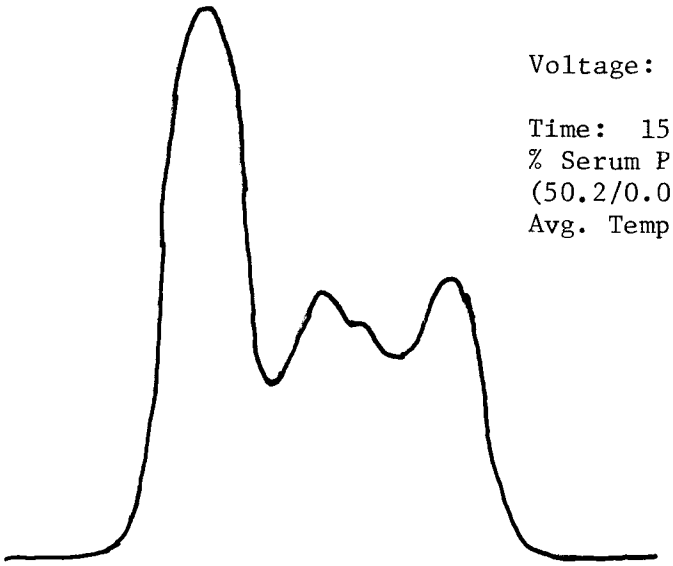
Voltage: base=145
pulse=-20 @1 sec.
Time: 15 mins.
% Serum Protein:
(68.5/1.5/10.6/9.9/9.5)
Avg. Temp.: 35.8 °C



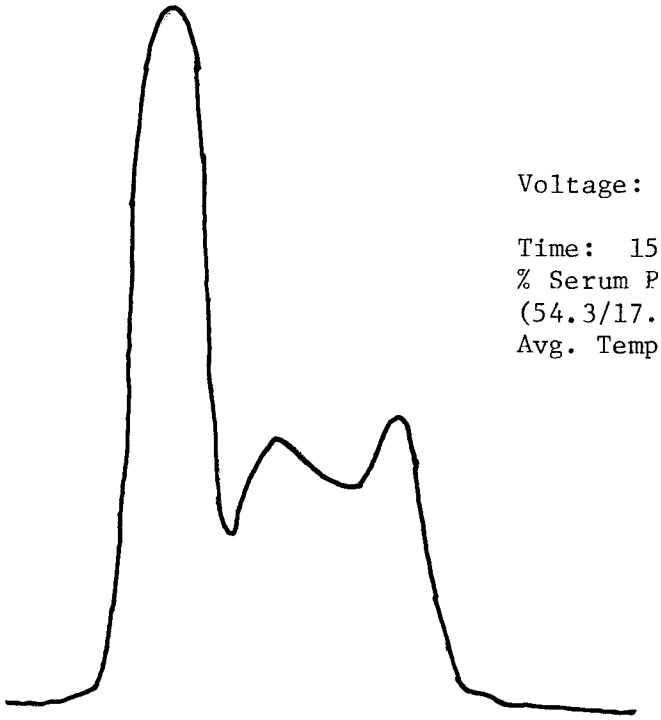
Voltage: base=150
pulse=+50,-50 @1 sec.
Time: 15 mins.
% Serum Protein:
(54.8/14.3/8.6/2.6/19.7)
Avg. Temp.: 36.1 °C

High Voltage Square Wave
Pulses for Serum

Figure 43



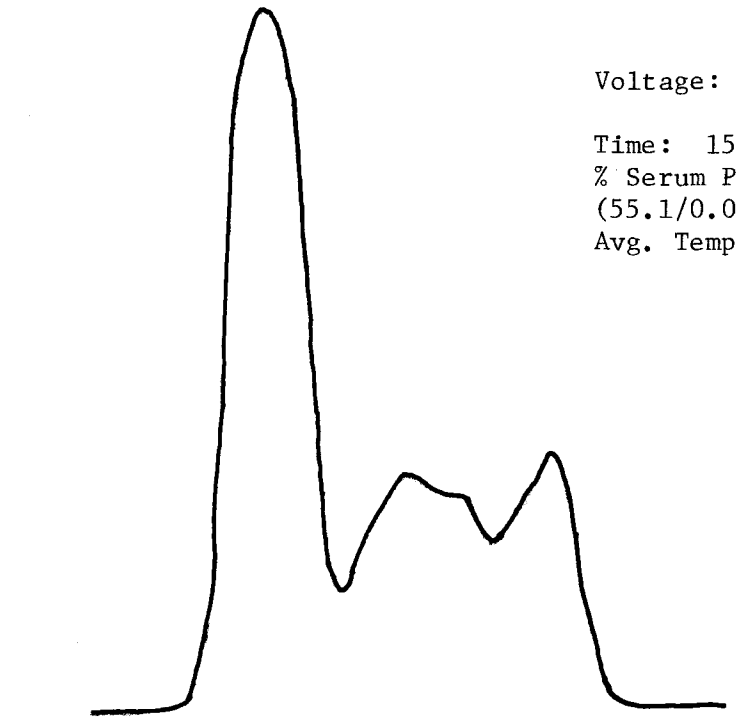
Voltage: base=100
pulse=+20 @1 sec.
Time: 15 mins.
% Serum Protein:
(50.2/0.0/16.7/10.4/22.7)
Avg. Temp.: 30.1 °C



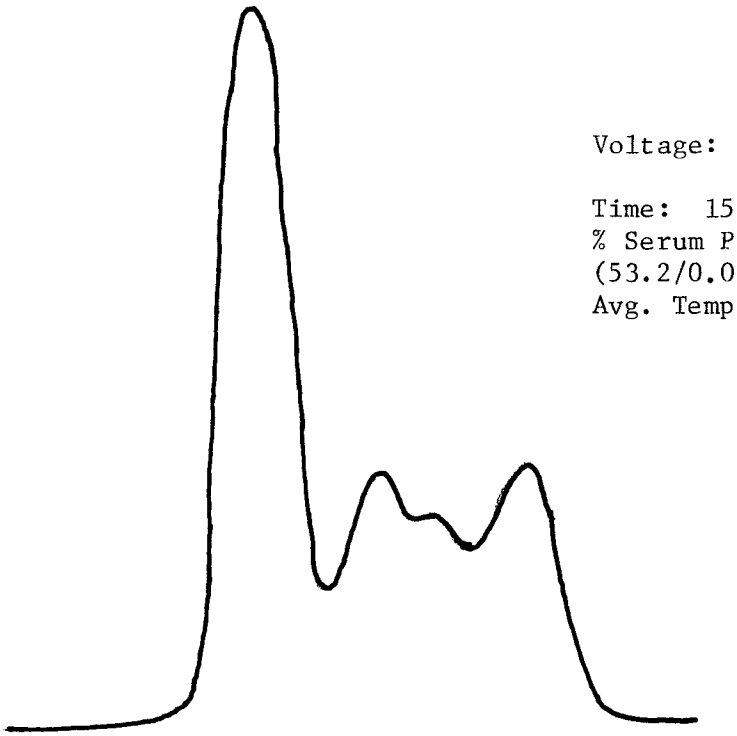
Voltage: base=100
pulse=-20 @1 sec.
Time: 15 mins.
% Serum Protein:
(54.3/17.4/8.7/19.6)
Avg. Temp.: 30.0 °C

Low Voltage Square Wave
Pulses for Serum

Figure 44



Voltage: base=100
pulse=-20,+20 @1 sec.
Time: 15 mins.
% Serum Protein:
(55.1/0.0/17.0/10.1/17.8)
Avg. Temp.: 29.4 °C



Voltage: base=100
pulse=-20,+20 @1 sec.
Time: 15 mins.
% Serum Protein:
(53.2/0.0/15.0/11.4/20.4)
Avg. Temp.: 30.4 °C

More Low Voltage Square Wave
Pulses for Serum

Figure 45

temperature of the run and percent separation were included with these figures.

CHAPTER VI

DISCUSSION AND CONCLUSIONS

Discussion of Results

In this study, a microcomputer-controlled electrophoresis power supply was examined as a possible means of increasing the resolution of human hemoglobin and serum protein. It was hoped that a computer program could be devised which would control the power supply to yield such a result.

Several programs were developed using the APPLE(tm) computer and ADA-LAB(tm) interface board to control and monitor the power supply. Although only one program was successful in increasing resolution, the other programs could not be considered as being unsuccessful. For example, the microcomputer-controlled power supply gave results as good as the analog version for constant voltage, current and wattage. Optimum resolution was achieved for both techniques using the same voltage, current and wattage conditions. Variations on these conditions yielded only poorer resolution for both techniques. Also, it made no difference in these results whether the medium was cellulose acetate or agarose gel or whether the sample was human hemoglobin or serum.

Several pulse programs were employed in an attempt to increase the resolution of human serum on agarose gel. These techniques were successful in controlling temperature buildup on the plate but failed to give the anticipated enhanced resolution. The pulse programs could only equal the resolution found by the best constant voltage experiment.

The only program providing increased resolution was one used to study hemoglobin separations on cellulose acetate. The program, constant-temperature electrophoresis, led to an increase in resolution by 10 percent. This resulted from monitoring the voltage signal from the IR temperature probe. With this information, the microcomputer could adjust the voltage from the power supply in order to maintain a selected temperature to within 1 °C. The use of a temperature of 32 °C was found to achieve optimum resolution.

The IR temperature probe was used to provided insight into other areas as well. By observing temperature profiles, the probe was used to confirm some known facts while demonstrating two previously-unnoticed trends. The confirmed facts involve temperature increases for voltage, current or wattage increases as well as for increases in the ionic strength of the buffer. One previously-unnoticed trend showed small temperature decreases at the end of runs which employed higher than normal voltages, currents or wattages. Another previously-unobserved trend indicated

that the best resolution for constant voltage and wattage occurred in the range of 29 to 34 °C. However, the use of constant current did not follow this trend.

Conclusions

The intent of this study was to increase the resolution obtained in electrophoresis by controlling the power supply with a microcomputer. This was accomplished in the constant temperature system. However, questions remain as to its effect on resolution for other biological materials on various media. Also, the hardware and software of this system need to be further refined if it is to become a practical tool for the laboratory.

Microcomputer control of constant voltage, current or wattage electrophoresis experiments was not found to increase resolution. It gave equally good results when compared to the analog methods. The greatest gain resulting from its use would be to free an analyst from constantly monitoring the power supply. For example, electrophoresis experiments which require more than one hour to separate biological components would benefit the most.

As with the above technique, pulse programs were not able to increase resolution. The best separations were only equal to the best constant voltage experiment. However, this study did not apply all of the possible input data which the computer could have used for these programs. Therefore, the potential for better resolution may still

exist.

The temperature data were not really unexpected. The three facts obtained from use of the IR temperature probe were found to be quite logical. For example in Chapter 1 was discussed temperature increases for increases in voltage, current, wattage and the buffers' ionic strength. The second fact in which slight temperature decreases occurred during a run at higher than normal voltages, currents or wattages is the result of media degradation which was observed upon examination of the plates. Finally, the best resolution occurred for a temperature range in which biological material is most active.

In the future, use of the microcomputer should make it possible to control two other forms of electrophoresis, conductivity and isoelectric focusing. The microcomputer would be programmed to control the power supply much as it does now. However, separations would be performed until a specified conductance or pH gradient was established.

Preliminary studies were conducted to determine the feasibility of interfacing an electrochemical detection cell into a gel system. The microcomputer would be programmed to turn the power supply off and on as it takes readings from the cell. These readings would record current decreases as separated fractions pass through the cell. The microcomputer would then be able to generate a facsimile of a densitometer scan for these fractions. This would enable

an analyst to by-pass the messy and time consuming process of staining. Also, the gel would become reusable since the migration of the fractions would create a continuous flow. A proper chemical label which would attach to each fraction and be reduced by the electrochemical cell appears to be the only major obstacle. This study is to be left to future researchers.

APPENDIX A

Electrophoresis Programs

CONSTANT VOLTAGE PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
2000  REM  CONSTANT VOLTAGE SUBROUTINE
2001  PRINT
2002  PRINT "THE CORRECTION FACTOR (CRR) MAY NEED TO BE ADJUSTED ON A DAI
     LY BASIS.  IT'S VALUE IS DEFINED ON LINE 2139."
2004  PRINT
2005  PRINT "THE POWER SUPPLY SHOULD BE INSTALLED IN THE FOUR LOWER LEFT
     HAND POSTS"
2009  PRINT
2010  PRINT "THE VOLTAGE MONITOR SHOULD BE BOARD ONE AND THE CURRENT MONI
     TOR SHOULD BE BOARD ZERO."
2019  PRINT
2020  PRINT "MAKE SURE THE A/D VOLTAGE SETTINGS AND THE D/A VOLTAGE SETTI
     NGS ARE +/-1."
2029  PRINT
2030  PRINT "THE D/A OUTPUT MUST BE THE SAME CHANNEL AS THE LAST ACCESSED
      A/D. FOR EXAMPLE, IN THE CONSTANT VOLTAGE CASE THE A/D AND THE D/A
     MUST BE BOARD ONE."
2031  PRINT
2032  PRINT "SET POWER SUPPLY MODE FOR CONSTANT VOLTAGE"
2049  PRINT
2050  INPUT "WHAT VOLTAGE WOULD YOU LIKE TO  APPLY?";APLVLT
2059  PRINT
2060  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
2069  PRINT
2070  PRINT "THE VALUES YOU HAVE SELECTED ARE:"
2090  PRINT "ELECTROPHORESIS TIME="TIMELEC" MINUTES"
2100  PRINT "THE APPLIED VOLTAGE="APLVLT" VOLTS"
2104  PRINT
2105  INPUT "IS THIS CORRECT? (Y OR N)";YN$
2106  IF YN$ = "Y" GOTO 2110
2107  IF YN$ = "N" GOTO 2050
2110 D% = TIMELEC * 600: CALL 880
2120  REM   LINE 2130 WILL READ A/D BOARD ONE TO GET THE PRESENT VOLTAGE
     VALUE
2130  & AI1: & AI1
2135  REM  VLT WILL BE THE VOLTAGE FROM THE (VOLTAGE)A/D TIMES A CORRECTI
     ON FACTOR(CRR)
2139 CRR = 2.3
```

```
2140 VLT =  INT (D% / CRR)
2152 V = APLVLT
2196  GOSUB 10000
2197 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2210  REM  VLT WILL BE THE PRESENT VOLTAGE INPUTTED INTO THE A/D
2215  PRINT VLT
2220  CALL 908
2222  IF D% <  = 0 GOTO 15000
2225  IF VLT < APLVLT - 10 GOTO 2300
2230  IF VLT < APLVLT - 5 GOTO 2325
2240  IF VLT < APLVLT - 1 GOTO 2350
2245  IF VLT < APLVLT GOTO 2450
2250  IF VLT = APLVLT GOTO 2375
2255  IF VLT > APLVLT + 5 GOTO 2425
2260  IF VLT > APLVLT + 1 GOTO 2525
2270  IF VLT > APLVLT GOTO 2400
2300 V = V + 10
2305  GOSUB 10000
2307 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2310  & AI1: & AI1
2314 VLT =  INT (D% / CRR)
2315  PRINT "THIS IS THE +10 VOLT CORRECTION  "VLT" INPUT VOLTS"
2317  PRINT "OUTPUTTING "V" VOLTS"
2321  GOTO 2220
2325 V = V + 5
2327  GOSUB 10000
2330 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2335  & AI1: & AI1
2340 VLT =  INT (D% / CRR)
2341  PRINT "THIS IS THE +5 VOLT CORRECTION  "VLT" INPUT VOLTS"
2342  PRINT "OUTPUTTING "V" VOLTS"
2345  GOTO 2220
2350 V = V + 1
2353  GOSUB 10000
2355 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2360  & AI1: & AI1
2365 VLT =  INT (D% / CRR)
2366  PRINT "THIS IS THE +1 VOLT CORRECTION  "VLT" INPUT VOLTS"
2367  PRINT "OUTPUTTING "V" VOLTS"
2371  GOTO 2220
2375 V = V
2378  GOSUB 10000
2380 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2385  & AI1: & AI1
2390 VLT =  INT (D% / CRR)
2391  PRINT "THIS IS THE VOLTAGE IT SHOULD BE  "VLT" INPUT VOLTS"
2392  PRINT "OUTPUTTING "V" VOLTS"
2395  GOTO 2220
2400 V = V - .5
2403  GOSUB 10000
2405 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2410  & AI1: & AI1
2415 VLT =  INT (D% / CRR)
2416  PRINT "THIS IS THE -0.5 VOLT CORRECTION  "VLT" INPUT VOLTS"
```

```
2417  PRINT "OUTPUTTING "V" VOLTS"
2420  GOTO 2220
2425 V = V - 5
2427  GOSUB 10000
2430 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2435  & AI1: & AI1
2440 VLT =  INT (D% / CRR)
2441  PRINT "THIS IS THE -5 VOLT CORRECTION  "VLT" INPUT VOLTS"
2442  PRINT "OUTPUTTING "V" VOLTS"
2445  GOTO 2220
2450 V = V + .5
2455  GOSUB 10000
2460 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2465  & AI1: & AI1
2470 VLT =  INT (D% / CRR)
2475  PRINT "THIS IS THE +0.5 VOLT CORRECTION  "VLT" INPUT VOLTS"
2476  PRINT "OUTPUTTING "V" VOLTS"
2485  GOTO 2220
2525 V = V - 1
2530  GOSUB 10000
2535 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2540  & AI1: & AI1
2545 VLT =  INT (D% / CRR)
2550  PRINT "THIS IS THE -1 VOLT CORRECTION  "VLT" INPUT VOLTS"
2551  PRINT "OUTPUTTING "V" VOLTS"
2555  GOTO 2220
10000  REM  THIS IS AVOLTAGE ADJUSTMENT SUBROUTINE
10010  IF 0 < V AND V < 101 THEN FUG = 1866
10020  IF 101 < V AND V < 201 THEN FUG = 1666
10030  IF 201 < V AND V < 301 THEN FUG = 1466
10040  IF 301 < V AND V < 401 THEN FUG = 1266
10050  IF 401 < V AND V < 501 THEN FUG = 1066
10060  IF 501 < V AND V < 601 THEN FUG = 866
10070  IF 601 < V AND V < 701 THEN FUG = 666
10080  IF 701 < V AND V < 801 THEN FUG = 466
10090  IF 801 < V AND V < 901 THEN FUG = 266
10100  IF 901 < V AND V < 1001 THEN FUG = 66
10110  IF V > 1001 THEN FUG = 10
10120  RETURN
15000 D% = 0: & A01
15001  PRINT
15002  INPUT "WOULD YOU LIKE TO RUN ANOTHER CONSTANT VOLTAGE ELECTROPHORE
     SIS? (Y OR N)";YN$
15003  IF YN$ = "Y" GOTO 2050
15010  END
```

CONSTANT CURRENT PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
2000  REM   CONSTANT CURRENT SUBROUTINE
2001  PRINT
2002  PRINT "THE CORRECTION FACTOR (CRR) MAY NEED TO BE ADJUSTED ON A DAI
     LY BASIS.  IT IS LOCATED ON LINE 2139 OF THIS PROGRAM."
2004  PRINT
2005  PRINT "THE POWER SUPPLY SHOULD BE INSTALLED IN THE FOUR LOWER LEFT
     HAND POSTS"
2009  PRINT
2010  PRINT "THE VOLTAGE MONITOR SHOULD BE BOARD ONE AND THE CURRENT MONI
     TOR SHOULD BE BOARD ZERO."
2019  PRINT
2020  PRINT "MAKE SURE THE A/D VOLTAGE SETTINGS AND THE D/A VOLTAGE SETTI
     NGS ARE +/-1."
2029  PRINT
2030  PRINT "THE D/A OUTPUT MUST BE THE SAME CHANNEL AS THE LAST ACCESSED
      A/D. FOR EXAMPLE, IN THE CONSTANT CURRENT CASE THE A/D AND THE D/A
     MUST BE BOARD ZERO."
2031  PRINT
2032  PRINT "SET POWER SUPPLY MODE FOR CONSTANT CURRENT"
2049  PRINT
2050  INPUT "WHAT CURRENT WOULD YOU LIKE TO HOLD CONSTANT?";CSTCUR
2059  PRINT
2060  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
2069  PRINT
2070  PRINT "THE VALUES YOU HAVE SELECTED ARE:"
2090  PRINT "ELECTROPHORESIS TIME="TIMELEC" MINUTES"
2100  PRINT "THE DESIRED CURRENT="CSTCUR" MILLIAMPS"
2104  PRINT
2105  INPUT "IS THIS CORRECT? (Y OR N)";YN$
2106  IF YN$ = "Y" GOTO 2110
2107  IF YN$ = "N" GOTO 2050
2110 D% = TIMELEC * 600: CALL 880
2120  REM  LINE 2130 WILL READ A/D BOARD ZERO TO GET THE PRESENT CURRENT
     VALUE
2130  & AI0: & AI0
2135  REM  REDCUR WILL BE THE CURRENT FROM THE (CURRENT)A/D DIVIDED BY A
     CORRECTION FACTOR(CRR)
2139 CRR = 179
2140 REDCUR = (D% / CRR)
2152 V = 30
2196  GOSUB 10000
2197 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2200  FOR X = 1 TO 10000: NEXT
2210  REM   REDCUR WILL BE THE PRESENT CURRENT INPUTTED INTO THE A/D
2215  PRINT REDCUR
2220  CALL 908
```

```
2222  IF D% <  = 0 GOTO 15000
2225  IF REDCUR < CSTCUR - 1 GOTO 2300
2230  IF REDCUR < CSTCUR - .5 GOTO 2325
2240  IF REDCUR < CSTCUR - .1 GOTO 2350
2245  IF REDCUR < CSTCUR GOTO 2450
2250  IF REDCUR = CSTCUR GOTO 2375
2255  IF REDCUR > CSTCUR + .5 GOTO 2425
2260  IF REDCUR > CSTCUR + .1 GOTO 2525
2270  IF REDCUR > CSTCUR GOTO 2400
2300 V = V + 10
2305  GOSUB 10000
2307 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2310  & AI0: & AI0
2314 REDCUR = (D% / CRR)
2315  PRINT "THIS IS THE +1 MILLIAMP CORRECTION.  READING "REDCUR" MILLIA
     MPS"
2317  PRINT
2321  GOTO 2220
2325 V = V + 5
2327  GOSUB 10000
2330 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2335  & AI0: & AI0
2340 REDCUR = (D% / CRR)
2341  PRINT "THIS IS THE +.5 MILLIAMP CORRECTION.  READING "REDCUR" MILLI
     AMPS"
2342  PRINT
2345  GOTO 2220
2350 V = V + 1
2353  GOSUB 10000
2355 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2360  & AI0: & AI0
2365 REDCUR = (D% / CRR)
2366  PRINT "THIS IS THE +.1 MILLIAMP CORRECTION.  READING "REDCUR" MILLI
     AMPS"
2367  PRINT
2371  GOTO 2220
2375 V = V
2378  GOSUB 10000
2380 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2385  & AI0: & AI0
2390 REDCUR = (D% / CRR)
2391  PRINT "THIS IS THE VOLTAGE IT SHOULD BE.  READING "REDCUR" MILLIAMP
     S"
2392  PRINT
2395  GOTO 2220
2400 V = V - .5
2403  GOSUB 10000
2405 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2410  & AI0: & AI0
2415 REDCUR = (D% / CRR)
2416  PRINT "THIS IS THE NEG. SMALL MILLIAMP CORRECTION.  READING "REDCUR
     " MILLIAMPS"
2417  PRINT
2420  GOTO 2220
```

```
2425 V = V - 5
2427  GOSUB 10000
2430 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2435  & AI0: & AI0
2440 REDCUR = (D% / CRR)
2441  PRINT "THIS IS THE -.5 MILLIAMP CORRECTION.  READING "REDCUR" MILLI
     AMPS"
2442  PRINT
2445  GOTO 2220
2450 V = V + .5
2455  GOSUB 10000
2460 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2465  & AI0: & AI0
2470 REDCUR = (D% / CRR)
2475  PRINT "THIS IS THE POS. SMALL MILLIAMP CORRECTION.  READING "REDCUR
     " MILLIAMPS"
2476  PRINT
2485  GOTO 2220
2525 V = V - 1
2530  GOSUB 10000
2535 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2540  & AI0: & AI0
2545 REDCUR = (D% / CRR)
2550  PRINT "THIS IS THE -.1 MILLIAMP CORRECTION.  READING "REDCUR" MILLI
     AMPS"
2551  PRINT
2555  GOTO 2220
10000  REM  THIS IS AVOLTAGE ADJUSTMENT SUBROUTINE
10010  IF 0 < V AND V < 101 THEN FUG = 1866
10020  IF 101 < V AND V < 201 THEN FUG = 1666
10030  IF 201 < V AND V < 301 THEN FUG = 1466
10040  IF 301 < V AND V < 401 THEN FUG = 1266
10050  IF 401 < V AND V < 501 THEN FUG = 1066
10060  IF 501 < V AND V < 601 THEN FUG = 866
10070  IF 601 < V AND V < 701 THEN FUG = 666
10080  IF 701 < V AND V < 801 THEN FUG = 466
10090  IF 801 < V AND V < 901 THEN FUG = 266
10100  IF 901 < V AND V < 1001 THEN FUG = 66
10110  IF V > 1001 THEN FUG = 10
10120  RETURN
15000 D% = 0: & A00
15001  INPUT "WOULD YOU LIKE TO RUN ANOTHER CONSTANT CURRENT ELECTROPHORE
     SIS? (Y OR N)";YN$
15002  IF YN$ = "Y" GOTO 2049
15010  END
```

CONSTANT POWER PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,0
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
2000  REM   CONSTANT WATTAGE SUBROUTINE
2001  PRINT
2002  PRINT "THE CORRECTION FACTORS FOR CURRENT AND VOLTAGE MAY NEED TO B
     E ADJUSTED ON A DAILY BASIS.  THESE VALUES ARE LOCATED ON LINES 2123
      AND 2139, RESPECTIVELY."
2004  PRINT
2005  PRINT "THE POWER SUPPLY AND CHAMBER SHOULD BE CONNECTED TO THE FOUR
      LOWER LEFT HAND POSTS"
2009  PRINT
2010  PRINT "THE VOLTAGE MONITOR SHOULD BE BOARD ONE AND THE CURRENT MONI
    - TOR SHOULD BE BOARD ZERO."
2019  PRINT
2020  PRINT "VOLTAGE SETTINGS:  A/D BOARD ONE +/-4; ALL OTHERS +/-1"
2029  PRINT
2030  PRINT "THE D/A OUTPUT MUST BE THE SAME CHANNEL AS THE LAST ACCESSED
      A/D. FOR EXAMPLE, IN THE CONSTANT WATTAGE CASE THE A/D AND THE D/A
     MUST BE BOARD ONE FOR CONTROLLING AND MONITOURING THE VOLTAGE.
2031  PRINT
2032  PRINT "HOWEVER THE A/D INPUT OF CURRENT FOR BOARD ZERO MUST BE READ
      PRIOR TO ANY VOLTAGE ACTION OCCURING ON BOARD ONE."
2033  PRINT
2034  PRINT "SET POWER SUPPLY MODE FOR CONSTANT VOLTAGE"
2049  PRINT
2050  INPUT "WHAT WATTAGE WOULD YOU LIKE TO APPLY?";APLPWR
2051  PRINT
2052  INPUT "WHAT MAXIMUM CURRENT WILL YOU ALLOW?";MAXCUR
2059  PRINT
2060  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
2069  PRINT
2070  PRINT "THE VALUES YOU HAVE SELECTED ARE:"
2090  PRINT "ELECTROPHORESIS TIME="TIMELEC" MINUTES"
2100  PRINT "THE APPLIED WATTAGE="APLPWR" WATTS"
2101  PRINT "THE MAXIMUM CURRENT ALLOWED="MAXCUR" MILLIAMPS"
2104  PRINT
2105  INPUT "IS THIS CORRECT? (Y OR N)";YN$
2106  IF YN$ = "Y" GOTO 2110
2107  IF YN$ = "N" GOTO 2050
2110 D% = TIMELEC * 600: CALL 880
2120  REM   LINE 2121 WILL READ THE A/D BOARD ZERO TO GET THE PRESENT CUR
     RENT VALUE
2121  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2122  REM  REDCUR WILL BE THE CURRENT FROM THE (CURRENT) A/D DIVIDED BY A
      CORRECTION FACTOR (FAC)
2123 FAC = 45
2125 REDCUR = D% / FAC
2129  REM   LINE 2130 WILL READ THE A/D BOARD ONE TO GET THE PRESENT VOLT
```

```
    AGE VALUE
2130  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2135  REM   VLT WILL BE THE VOLTAGE FROM THE (VOLTAGE) A/D DIVIDED BY A C
    ORRECTION FACTOR (CRR)
2139 CRR = 2.3
2140 VLT = D% / CRR
2142 PWR = (VLT * REDCUR) / 1000
2152 V = 50
2196  GOSUB 10000
2197 D% = ((2048 / 893) * V) + (FUG / 10): & AO1
2210  REM   PWR WILL BE THE PRESENT WATTAGE MANIPULATED FROM THE INPUT OF
     THE VOLTAGE AND CURRENT A/D'S
2215  PRINT PWR
2220  CALL 908
2222  IF D% <  = 0 GOTO 15000
2223  IF REDCUR >  = MAXCUR GOTO 5000
2225  IF PWR < APLPWR - 1 GOTO 2300
2230  IF PWR < APLPWR - .5 GOTO 2325
2240  IF PWR < APLPWR - .1 GOTO 2350
2245  IF PWR < APLPWR GOTO 2450
2250  IF PWR = APLPWR GOTO 2375
2255  IF PWR > APLPWR + .5 GOTO 2425
2260  IF PWR > APLPWR + .1 GOTO 2525
2270  IF PWR > APLPWR GOTO 2400
2300 V = V + 10
2305  GOSUB 10000
2307 D% = ((2048 / 893) * V) + (FUG / 10): & AO1
2308  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2309 REDCUR = D% / FAC
2310  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2311 VLT = D% / CRR
2313 PWR = (VLT * REDCUR) / 1000
2315  PRINT "THIS IS THE +1 WATT CORRECTION.  READING "PWR" WATTS"
2321  GOTO 2220
2325 V = V + 5
2327  GOSUB 10000
2330 D% = ((2048 / 893) * V) + (FUG / 10): & AO1
2331  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2332 REDCUR = D% / FAC
2335  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2336 VLT = D% / CRR
2340 PWR = (VLT * REDCUR) / 1000
2341  PRINT "THIS IS THE +.5 WATT CORRECTION.  READING "PWR" WATTS"
2345  GOTO 2220
2350 V = V + 1
2353  GOSUB 10000
2355 D% = ((2048 / 893) * V) + (FUG / 10): & AO1
2356  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2357 REDCUR = D% / FAC
2360  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2361 VLT = D% / CRR
2365 PWR = (VLT * REDCUR) / 1000
2366  PRINT "THIS IS THE +.1 WATT CORRECTION.  READING "PWR" WATTS"
2371  GOTO 2220
```

```
2375 V = V
2378  GOSUB 10000
2380 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2381  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2382 REDCUR = D% / FAC
2385  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2386 VLT = D% / CRR
2390 PWR = (VLT * REDCUR) / 1000
2391  PRINT "THIS IS THE WATTAGE IT SHOULD BE.  READING "PWR" WATTS"
2395  GOTO 2220
2400 V = V - .5
2403  GOSUB 10000
2405 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2406  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2407 REDCUR = D% / FAC
2410  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2411 VLT = D% / CRR
2415 PWR = (VLT * REDCUR) / 1000
2416  PRINT "THIS IS THE NEG. SMALL WATT CORRECTION.  READING "PWR" WATTS
    "
2420  GOTO 2220
2425 V = V - 5
2427  GOSUB 10000
2430 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2431  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2432 REDCUR = D% / FAC
2435  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2436 VLT = D% / CRR
2440 PWR = (VLT * REDCUR) / 1000
2441  PRINT "THIS IS THE -.5 WATT CORRECTION.  READING "PWR" WATTS"
2445  GOTO 2220
2450 V = V + .5
2455  GOSUB 10000
2460 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2461  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2462 REDCUR = D% / FAC
2465  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2466 VLT = D% / CRR
2470 PWR = (VLT * REDCUR) / 1000
2475  PRINT "THIS IS THE POS. SMALL WATT CORRECTION.  READING "PWR" WATTS
    "
2485  GOTO 2220
2525 V = V - 1
2530  GOSUB 10000
2535 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2536  & AI0: FOR I = 1 TO 50: NEXT : & AI0
2537 REDCUR = D% / FAC
2540  & AI1: FOR I = 1 TO 50: NEXT : & AI1
2541 2545 PWR = (VLT * REDCUR) / 1000
2550  PRINT "THIS IS THE -.1 WATT CORRECTION.  READING "PWR" WATTS"
2555  GOTO 2220
5000  GOTO 15000
10000  REM  THIS IS AVOLTAGE ADJUSTMENT SUBROUTINE
10010  IF 0 < V AND V < 101 THEN FUG = 1866
```

```
10020  IF 101 < V AND V < 201 THEN FUG = 1666
10030  IF 201 < V AND V < 301 THEN FUG = 1466
10040  IF 301 < V AND V < 401 THEN FUG = 1266
10050  IF 401 < V AND V < 501 THEN FUG = 1066
10060  IF 501 < V AND V < 601 THEN FUG = 866
10070  IF 601 < V AND V < 701 THEN FUG = 666
10080  IF 701 < V AND V < 801 THEN FUG = 466
10090  IF 801 < V AND V < 901 THEN FUG = 266
10100  IF 901 < V AND V < 1001 THEN FUG = 66
10110  IF V > 1001 THEN FUG = 10
10120  RETURN
15000 D% = 0: & A01
15001  PRINT
15002  INPUT "WOULD YOU LIKE TO RUN ANOTHER CONSTANT WATTAGE ELECTROPHORE
     SIS? (Y OR N)";YN$
15003  IF YN$ = "Y" GOTO 2050
15010  END
```

CONSTANT TEMPERATURE PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
2000  REM  CONSTANT TEMPERATURE SUBROUTINE
2001  PRINT
2002  PRINT "THE CORRECTION FACTOR (FAC) MAY NEED TO BE ADJUSTED ON A DAI
     LY BASIS.  IT'S VALUE IS DEFINED ON LINE 2150 OF THIS PROGRAM."
2004  PRINT
2005  PRINT "MAKE A DIRECT HOOK-UP BETWEEN THE POWER SUPPLY AND THE ELECT
     ROPHORESIS CHAMBER"
2006  PRINT
2008  PRINT "THE IR PROBE SHOULD BE INSTALLED IN THE A/D OF BOARD ZERO"
2009  PRINT
2010  PRINT "USE THE D/A OF BOARD ZERO TO CONTROL THE POWER SUPPLY VOLTAG
     E"
2011  PRINT
2015  PRINT "VOLTAGE SETTINGS:  A/D BOARD ZERO +/-0.5; ALL OTHERS +/-1"
2016  PRINT
2017  PRINT "SET POWER SUPPLY MODE TO CONSTANT VOLTAGE"
2029  PRINT
2030  INPUT "WHAT TEMPERATURE DO YOU DESIRE? (RECOMMENDED MAX = 50 DEGREE
     S CELSIUS) ";TEMP
2049  PRINT
2050  INPUT "WHAT MAXIMUM VOLTAGE WILL BE ALLOWED?";MAXVOLT
2051  PRINT
2055  INPUT "WHAT INITIAL VOLTAGE WOULD YOU LIKE?";V
2059  PRINT
2060  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
2067  PRINT
2070  PRINT "THE VALUES YOU HAVE SELECTED ARE:"
2071  PRINT
2080  PRINT "TEMPERATURE= "TEMP" DEGREES CELSIUS"
2090  PRINT "ELECTROPHORESIS TIME= "TIMELEC" MINUTES"
2100  PRINT "MAXIMUM VOLTAGE= "MAXVOLT" VOLTS"
2102  PRINT "INITIAL VOLTAGE= "V" VOLTS"
2104  PRINT
2105  INPUT "IS THIS CORRECT? (Y OR N)";YN$
2106  IF YN$ = "Y" GOTO 2110
2107  IF YN$ = "N" GOTO 2030
2110 D% = TIMELEC * 600: CALL 880
2130  & AI0: & AI0
2150 FAC = 1
2155 IRTEMP = FAC * D%
2160  PRINT "THE PRESENT TEMPERATURE= "IRTEMP" DEGREES CELSIUS"
2170  IF IRTEMP > TEMP GOTO 2190
2180  IF IRTEMP <  = TEMP GOTO 2194
2190  PRINT "THE PRESENT TEMPERATURE IS ALREADY GREATER THAN VALUE SET": GOTO
     2030
2194 MAXVOLT = MAXVOLT - 11
```

```
2195 V = V - 11
2196  GOSUB 10000
2197 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2198  FOR X = 1 TO 10000: NEXT
2200  CALL 908
2210  IF D% <  = 0 GOTO 15000
2215  PRINT V
2220  IF IRTEMP < TEMP - 4 GOTO 2300
2230  IF IRTEMP < TEMP - 2 GOTO 2325
2240  IF IRTEMP < TEMP - 1 GOTO 2350
2245  IF IRTEMP < TEMP GOTO 2450
2250  IF IRTEMP = TEMP GOTO 2375
2255  IF IRTEMP > TEMP + 2 GOTO 2425
2257  IF IRTEMP > TEMP + 1 GOTO 2525
2260  IF IRTEMP > TEMP GOTO 2400
2300 V = V + 50
2303  GOSUB 10000
2305 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2310  & AI0: & AI0
2315 IRTEMP = FAC * D%
2316  PRINT "THIS IS THE +50 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2317  FOR X = 1 TO 30000: NEXT
2320  IF V >  = MAXVOLT THEN  GOTO 2322
2321  GOTO 2200
2322 V = V - 50
2325 V = V + 10
2327  GOSUB 10000
2330 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2335  & AI0: & AI0
2340 IRTEMP = FAC * D%
2341  PRINT "THIS IS THE +25 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2345  IF V >  = MAXVOLT THEN  GOTO 2347
2346  GOTO 2200
2347 V = V - 10
2350 V = V + 5
2353  GOSUB 10000
2355 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2360  & AI0: & AI0
2365 IRTEMP = FAC * D%
2366  PRINT "THIS IS THE +5 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2370  IF V >  = MAXVOLT THEN  GOTO 2373
2371  GOTO 2200
2373 V = V - 5
2375 V = V
2378  GOSUB 10000
2380 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2385  & AI0: & AI0
2390 IRTEMP = FAC * D%
2391  PRINT "THIS IS WHERE IT SHOULD BE "IRTEMP
2395  IF V >  = MAXVOLT THEN  GOTO 2397
2396  GOTO 2200
2397 V = V
2400 V = V - 1
2403  GOSUB 10000
```

```
2405 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2410  & AI0: & AI0
2415 IRTEMP = FAC * D%
2416  PRINT "THIS IS THE -1 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2420  GOTO 2200
2425 V = V - 25
2426  FOR X = 1 TO 5000: NEXT
2427  GOSUB 10000
2430 D% = ((2048 / 893) * V) + (FUG / 10): & A01
2435  & AI0: & AI0
2440 IRTEMP = FAC * D%
2442  PRINT "THIS IS THE -25 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2445  GOTO 2200
2450 V = V + 1
2455  GOSUB 10000
2460 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2465  & AI0: & AI0
2470 IRTEMP = FAC * D%
2475  PRINT "THIS IS THE +1 VOLT CORRECTION"IRTEMP
2480  IF V >  = MAXVOLT THEN  GOTO 2490
2485  GOTO 2200
2490 V = V - 1
2500  GOTO 2200
2525 V = V - 5
2526  GOSUB 10000
2527 D% = ((2048 / 893) * V) + (FUG / 10): & A00
2530  & AI0: & AI0
2540 IRTEMP = FAC * D%
2543  PRINT "THIS IS THE -5 VOLT CORRECTION "IRTEMP" DEGREES CELSIUS"
2545  GOTO 2200
10000  REM  THIS IS A VOLTAGE ADJUSTMENT SUBROUTINE
10010  IF 0 < V AND V < 101 THEN FUG = 1866
10020  IF 101 < V AND V < 201 THEN FUG = 1666
10030  IF 201 < V AND V < 301 THEN FUG = 1466
10040  IF 301 < V AND V < 401 THEN FUG = 1266
10050  IF 401 < V AND V < 501 THEN FUG = 1066
10060  IF 501 < V AND V < 601 THEN FUG = 866
10070  IF 601 < V AND V < 701 THEN FUG = 666
10080  IF 701 < V AND V < 801 THEN FUG = 466
10090  IF 801 < V AND V < 901 THEN FUG = 266
10100  IF 901 < V AND V < 1001 THEN FUG = 66
10110  IF V > 1001 THEN FUG = 10
10120  RETURN
15000 D% = 0: & A00
15001  PRINT
15002  INPUT "WOULD YOU LIKE TO RUN ANOTHER CONSTANT TEMPERATURE ELECTROP
     HORESIS? (Y OR N)";YN$
15003  IF YN$ = "Y" GOTO 2030
15010  END
```

SQUARE WAVE PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
19  REM  SQUARE WAVE SUBROUTINE
20  INPUT "WHAT BASELINE VOLTAGE WOULD YOU LIKE?";V2
29  PRINT
30  INPUT "WHAT POSITIVE VOLTAGE WOULD YOU LIKE TO PULSE TO?";V3
35  INPUT "WHAT VOLTAGE BELOW BASELINE WOULD YOU LIKE TO PULSE TO?";V1
41  PRINT
42  INPUT "HOW LONG IN SECS. DO YOU WANT TO WAIT BETWEEN PULSES?";T1
43  PRINT
50  INPUT "HOW LONG IN SECS. WOULD YOU LIKE YOUR PULSE TO LAST?";T2
60  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
70 D% = TIMELEC * 600: CALL 880
80  CALL 908
85  IF D% <  = 0 GOTO 2200
87 FUG = 1300
90 V = V2
95 D% = ((2048 / 893) * V) + (FUG / 10): & A01
100 D% = 32767: &  TO 5
110  & TI5:T = D%
120 RD = T - T1
140  & TI5: IF D% > RD GOTO 140
150  & TI5:T = D%
160 V = V2 + V3
161 D% = ((2048 / 893) * V) + (FUG / 10): & A01
163 D% = 32767: &  TO 5
164  & TI5:T = D%
165 PD = T - T2
180 D% = ((2048 / 893) * V) + (FUG / 10): & A01
190  & TI5: IF D% > PD GOTO 190
192 V = V2
193 D% = ((2048 / 893) * V) + (FUG / 10): & A01
194 D% = 32767: &  TO 5
195  & TI5:T = D%
196 RD = T - T1
198  & TI5: IF D% > RD GOTO 198
200 V = V2 - V1
210 D% = ((2048 / 893) * V) + (FUG / 10): & A01
213 D% = 32767: &  TO 5
214  & TI5:T = D%
215  & TI5:T = D%
220 PD = T - T1
230  & TI5: IF D% > PD GOTO 230
250  GOTO 80
2200 D% = 0: & A01
2201  PRINT
2202  INPUT "WOULD YOU LIKE TO RUN ANOTHER SQUARE WAVE ELECTROPHORESIS? (
     Y OR N)";YN$
```

```
2200 D% = 0: & A01
2201  PRINT
2202  INPUT "WOULD YOU LIKE TO RUN ANOTHER SQUARE WAVE ELECTROPHORESIS? (
     Y OR N)";YN$
2203  IF YN$ = "Y" GOTO 20
2210  END
```

POLARITY REVERSAL PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
19  REM   SAWTOOTH WAVE SUBROUTINE
20  INPUT "WHAT BASELINE VOLTAGE WOULD YOU LIKE?";V2
29  PRINT
30  INPUT "WHAT POSITIVE VOLTAGE WOULD YOU LIKE TO RAMP TO?";V3
41  PRINT
42  INPUT "HOW LONG IN TENTHS OF SECS. DO YOU WANT TO WAIT BETWEEN RAMPS?
     ";T1
43  PRINT
60  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
65  INPUT "WHAT FREQUENCY OF RAMP DO YOU DESIRE?";FRQ
70 D% = TIMELEC * 600: CALL 880
80  CALL 908
85  IF D% <  = 0 GOTO 2200
87 FUG = 1300
90 V = V2
95 D% = ((2048 / 893) * V) + (FUG / 10): & A01
100 D% = 32767: &  TO 5
110  & TI5:T = D%
120 RD = T - T1
140  & TI5: IF D% > RD GOTO 140
154 SCAL = 20
155 DV = FRQ * SCAL
156  FOR V4 = 0 TO V3 STEP DV
160 V = V4 + V2
161 D% = ((2048 / 893) * V) + (FUG / 10): & A01
190  NEXT V4
600  GOTO 80
2200 D% = 0: & A01
2201  PRINT
2202  INPUT "WOULD YOU LIKE TO RUN ANOTHER SQUARE WAVE ELECTROPHORESIS? (
     Y OR N)";YN$
2203  IF YN$ = "Y" GOTO 20
2210  END
```

SAWTOOTH PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
19  REM   SAWTOOTH WAVE SUBROUTINE
20  INPUT "WHAT BASELINE VOLTAGE WOULD YOU LIKE?";V2
29  PRINT
30  INPUT "WHAT POSITIVE VOLTAGE WOULD YOU LIKE TO RAMP TO?";V3
35  INPUT "WHAT VOLTAGE BELOW BASELINE WOULD YOU LIKE TO RAMP TO?";V1
41  PRINT
42  INPUT "HOW LONG IN TENTHS OF SECS. DO YOU WANT TO WAIT BETWEEN RAMPS?
     ";T1
43  PRINT
60  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
65  INPUT "WHAT FREQUENCY OF RAMP DO YOU DESIRE?";FRQ
70 D% = TIMELEC * 600: CALL 880
80  CALL 908
85  IF D% <  = 0 GOTO 2200
87 FUG = 1300
90 V = V2
95 D% = ((2048 / 893) * V) + (FUG / 10): & A01
100 D% = 32767: &  TO 5
110  & TI5:T = D%
120 RD = T - T1
140  & TI5: IF D% > RD GOTO 140
154 SCAL = 20
155 DV = FRQ * SCAL
156  FOR V4 = 0 TO V3 STEP DV
160 V = V4 + V2
161 D% = ((2048 / 893) * V) + (FUG / 10): & A01
190  NEXT V4
192  FOR V4 = V3 TO 0 STEP  - DV
195 V = V4 + V2
196 D% = ((2048 / 893) * V) + (FUG / 10): & A01
198  NEXT V4
350 V = V2
360 D% = ((2048 / 893) * V) + (FUG / 10): & A01
370 D% = 32767: &  TO 5
380  & TI5:T = D%
390 RD = T - T1
400  & TI5: IF D% > RD GOTO 400
430  FOR V5 = 0 TO V1 STEP DV
440 V = V2 - V5
450 D% = ((2048 / 893) * V) + (FUG / 10): & A01
460  NEXT V5
500  FOR V5 = V1 TO 0 STEP  - DV
510 V = V2 - V5
520 D% = ((2048 / 893) * V) + (FUG / 10): & A01
530  NEXT V5
600  GOTO 80
```

```
2203  IF YN$ = "Y" GOTO 20
2210  END
```

SAWTOOTH PLUS PROGRAM

```
1  HIMEM: 36095:D% = 0: DIM C%(5),Q%(5),D%(100): PRINT  CHR$ (4)"BRUN QUI
     CKI/O"
3  REM  ENABLE ANALOG HANDSHAKE
5  POKE 36259,1
10  LOMEM: 24576:D% = 0: PRINT  CHR$ (4)"BLOAD TIMOBJ1,A$370"
19  REM   SAWTOOTH WAVE SUBROUTINE
20  INPUT "WHAT BASELINE VOLTAGE WOULD YOU LIKE?";V2
29  PRINT
41  PRINT
42  INPUT "HOW LONG IN TENTHS OF SECONDS DO YOU WANT TO WAIT BETWEEN NEGA
     TIVE PULSES?";T1
43  PRINT
50  INPUT "HOW LONG DO YOU WISH TO REVERSE POLARITY";T2
60  INPUT "HOW LONG SHOULD THE ELECTROPHORESIS LAST?";TIMELEC
70 D% = TIMELEC * 600: CALL 880
80  CALL 908
85  IF D% <  = 0 GOTO 2200
87 FUG = 1300
90 V = V2
95 D% = ((2048 / 893) * V) + (FUG / 10): & A01
100 D% = 32767: &  TO 5
110  & TI5:T = D%
120 RD = T - T1
140  & TI5: IF D% > RD GOTO 140
160 D% = 1: & D00: & D01
170 D% = 32767: &  TO 5
180  & TI5:T = D%
190 PD = T - T2
200  & TI5: IF D% > PD GOTO 200
210 D% = 0: & D00: & D01
600  GOTO 80
2200 D% = 0: & A01
2201  PRINT
2202  INPUT "WOULD YOU LIKE TO RUN ANOTHER SQUARE WAVE ELECTROPHORESIS? (
     Y OR N)";YN$
2203  IF YN$ = "Y" GOTO 20
2210  END
```

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