A MICROPROCESSOR CONTROLLED DATA ANALYZER FOR SINGLE-BEAM SPECTROPHOTOMETERS

by

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ABSTRACT

Analysis of absorption data gathered from the employment of single-beam spectrophotometers is based on the construction of a standard, or calibration, curve. The method of construction used to date is manual preparation of a plot based on experimental data recorded for a series of standard solutions. While yielding satisfactory results, use of manual plots is inefficient in terms of the time needed to perform an analysis.

The topic of this thesis is the design and development of a microprocessor based instrument which, by direct interface to single-beam spectrophotometers, resolves this problem. First we relate the nature of the problem to the physics of absorption. Next, a brief overview of the technical approach employed and the work accomplished is given. This is followed by a more detailed discussion of the work completed. Finally, the conclusions reached are given.

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Spectrochemical Analysis and the Nature of Light

Spectrochemical analysis, in its broadest sense, comprises all the methods of chemical analysis that depend upon the measurement of the wavelength and intensity of spectral lines or absorption bands, and encompasses all frequencies in the electromagnetic absorption spectrum. In more common usage, spectrochemical analysis refers to 1) ultraviolet and visible emission spectroscopy and 2) ultraviolet and visible absorption spectrophotometry. Both methods characterize the qualitative and quantitative composition of matter. In the following treatment, however, we will only address the subject of absorption spectrophotometry since it is our primary concern.

In order to understand the basic principles of absorption spectrophotometry, we first need to explore the nature of light and its interaction with matter. Light is of a dual nature: under certain conditions it is useful to speak of light as an electromagnetic wave; in other situations it is advantageous to consider light as packets of energy, called photons. In fact, light possesses only one nature, but because the analogies we draw from the world are inadequate, we need to describe light in two distinct manners.

The dual nature of light is evident in Planck's Equation, which relates the energy of a photon of electromagnetic radiation to the frequency of the radiation:

$$E = h\nu \qquad \text{(E-1)}$$

where E is the energy of the photon, ν is the frequency of the radiation, and h is Planck's constant (6.624×10^{-27} erg sec).

Although a beam of light normally includes waves of many frequencies (or photons of many energies), it is possible to separate light waves of a desired frequency from the rest. This can be accomplished by the employment of a diffracting prism or a diffraction grating; when light is passed through such a prism the waves of different frequencies are deflected by varying amounts so that the resultant beam is "spread out" in space. (Figure 1.) If the broadened beam is filtered through an opaque barrier with a slit in it, only the narrow band of frequencies that falls at the slit opening will pass through the barrier. By rotation of the prism or grating, various frequencies will be allowed to pass through the barrier. As we shall see, this fact is very important in relation to the success of absorption spectrophotometry.

The band of light which exits the opaque barrier in Figure 1 is usually characterized by its central frequency, saying for instance, that the light has a frequency of 3 x 10^{14} $\sec^{-1}$. We should realize, however, that frequencies both above and below this value are always present, even though their radiant power is less than the power of the central frequency. Consequently, to accurately describe the situation we must indicate the band pass or actual range of frequencies within specified power limits* which are present, of the beam exiting the opaque barrier. As discussed later, this band pass is often of considerable importance in absorption spectrochemical measurements and can affect both the qualitative and quantitative validity of an analysis.

At this point, we should pause in our treatment of the nature of light and mention a possible source of confusion which may result from the improper use of the terms radiant power and intensity. In all spectrochemical measurements, it is important to determine the amplitude and frequency of the electromagnetic radiation which is employed. Unfortunately, the accurate measurement of both amplitude and frequency is practical only for radiation at microwave frequencies or lower due to the limited frequency response of available detectors.

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*The limits for the band pass is usually set at the half-power, or 3db, point, although other limits may occasionally be used.

In spectral regions of higher frequency the variable that can be measured is radiant power (P), which is proportional to the square of the wave amplitude. Radiant power is the amount of energy transmitted per unit time. Thus, if E is the energy of a single photon, radiant power can be expressed as

$$P = E\Phi = h\nu\Phi \qquad \text{(E-2)}$$

where Φ is the photon flux (i.e. the number of photons).

The radiant power of a beam of electromagnetic radiation is often incorrectly referred to as intensity. Intensity is properly defined as radiant power from a point source per unit solid angle, typically in units of watts per steradion. Although it is correct to speak of the intensity of a source of radiation, it is incorrect to describe radiation striking a sample in terms of intensity. This is especially true if the radiation is collimated; that is, if the radiation appears to originate from an infinitely distant source. Unfortunately, the term intensity is widely used as an equivalent to radiant power in available literature on spectrochemical analysis. To avoid such ambiguities, the term intensity will only be used in its correct sense in the present treatment.

The Interaction of Light and Matter

If a beam of electromagnetic radiation passes through a chemical substance, it is possible for the sample to absorb a portion of the radiation. This type of phenomenon is depicted in Figure 2, which shows a beam of radiant power P_o being directed into a sample of concentration C, and the corresponding energy band diagram of the process. Each specific frequency $(\nu_1, \nu_2, \ldots \nu_n)$ which comprised the beam of incident radiation will, by equation E-1, have its own energy $(h\nu_1, h\nu_2, \ldots h\nu_n)$. If the chemical sample contains a species whose energy states differ by any of these exact energies, the sample will absorb radiation at those frequencies. This behavior is illustrated in the energy band diagram of Figure 2, in which a chemical species having energy levels G and E is portrayed. If the species, which is typically a molecule, exists in the lower, or ground, state G before its encounter with the beam of radiation, it can be excited to the upper state E. In the excitation, the species must absorb a quantity of energy h which is exactly equal to the difference in energy between states G and E. The energy h cannot be divided in the process; either it is absorbed in total or it does not affect the sample.

The energy state of a molecule is the result of a combination of three primary intramolecular motions:

rotational, vibrational, and electronic. Since molecules are three-dimensional, they can rotate or tumble in space. The frequency of rotation is defined as the number of complete rotations per second around some molecular axis defined by the position of the atomic nuclei. Only certain frequencies, corresponding via equation E-1 to certain energies of rotation are allowed. The spacing between rotational energy levels is small, so low energy photons, in the microwave region, cause transitions between these rotational levels to occur. In fact, this type of absorbance is the basis of microwave spectroscopy.

The atoms that make up molecules are in continuous motion, so the molecule is continuously vibrating. Again, only certain vibrational frequencies are allowed to any molecule, and hence only certain energies, corresponding to the energy spacings between vibrational quantum states, can be absorbed from radiation interacting with the molecule. These energy spacings are larger than rotational energy spacings and the transitions require correspondingy higher photon energies; thus absorption of energy occurs in the higher frequency infrared region of the electromagnetic spectrum.

A molecule is of course both vibrating and rotating at the same time, so one can add the energies and obtain a series of vibration-rotation energy levels for each vibrational state. This is schematically represented in Figure 3.

The separation of electronic energy states, due to the motion of electrons in the molecule, are still greater than the vibrational states. Consequently, electronic energy transistions are responsible for the absorption of energy in the visible and ultraviolet regions of the electromagnetic spectrum. Again superimposed on each electronic energy level are many vibrational levels and their concomitant rotational levels, so that the absorption of light, especially absorption by molecules containing more than three or four atoms, usually appears as relatively wide bands. This type of absorption is the basis of ultraviolet and visible absorption spectrophotometry.

As should be expected from the discussions so far, the energy states of a molecule may only take certain frequencies of light will be able to excite the molecule from a lower to higher state. Stated in other terms, only those frequencies capable of being absorbed will be attenuated in a purely absorbing sample; all other frequencies will pass through the sample with no power loss. This suggests the possibility that components in the sample can be identified from the absorption spectrum; that is, from the frequency components of the radiation which are absorbed. Understandably, the diminution in the radiant power of the beam at each frequency should be related to the number of absorbing chemical species present in the sample. This leads to the discussion of Beer's Law.

Lambert-Beer Law of Absorption

The fundamental law of absorption spectrophotometry, a relation between radiant power, concentration, and rate of absorption, is known as the Lambert-Beer Law, or more simply Beer's Law. The relation, derived in Appendix A, can be expressed mathematically as

$$\log (P_o/P) = abc \qquad \text{(E-3)}$$

where P_o is the power of the radiation incident upon a sample, P is the power of the radiation transmitted through the sample, a is the absorptivity* of the sample, b is the length of the light path through the sample, and c is the concentration of the absorbing substance. If the parameter b, commonly known as the sample path length, is expressed in centimeters and the concentration factor, c, in grams per liter, then the absorptivity constant has units liter $\text{grams}^{-1}\text{cm}^{-1}$. The logarithmic term is referred to as the

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*Absorptivity is a constant dependent of the wavelength of the incident light and the nature of the absorbing material.

absorbance of the sample while the argument of the logarithm is known as the transmittance.

Several features of equation E-3 have experimental significance. First, it can be seen that the concentration of an absorbing species can be determined from a measurement of relative beam power with and without the absorber in the beam path. Absolute measurements of power are not necessary, a fact which considerably simplifies absorption spectrophotometry procedures. Second, it appears that the absorptivity and sample path length must be known or determined before the concentration can be deduced. We shall shortly see, however, that only the product of these two factors is determined through the use of an experimentally constructed curve, but first it is important to discuss some further aspects of Beer's Law.

Deviations from Beer's Law

Deviations from Beer's Law are classified as being either real or apparent. Real deviations are the result of changes in the refractive index of the sample. The amount of light that passes through a sample and is lost at the interfaces between the sample and its container is determined largely by this refractive index. Since the index varies nonlinearly with concentration, the amount of light lost at the interface will also vary nonlinearly with concentration. Such effects are, however, generally unimportant except for very concentrated solutions. Consequently, they are more of a theoretical concern than a practical interest and are therefore neglected in the majority of absorption spectrochemical analyses.

Apparent deviations, which cannot be neglected in most spectrochemical analyses, result from 1) the occurrence of chemical reactions by the species of interest and 2) the use of polychromatic instead of monochromatic radiation. These deviations are called apparent deviation since the application of the law, and not the law itself, is at fault.

If the compound of interest chemically reacts with some other species in the solution, the actual concentration of the compound of interest may be reduced to an amount that is not a linear function of the quantity of compound initially introduced. Thus the absorbance will not appear to be linear in concentration for such samples. Such chemical reactions are common and one should therefore be on guard for their effects.

Use of nonmonochromatic radiation may also cause apparent deviations from Beer's Law. The derivation of the law tacitly assumes the use of monochromatic radiation. In general, any "monochromatic" beam of light will include a range of energies, as previously mentioned in regard to band pass specification. Naturally one tries to minimize the band pass of "monochromatic" light, but it is difficult or unfeasible to obtain truly monochromatic light for spectral radiation. If a sample's absorptivity does not change to a great degree with frequency, then Beer's Law will hold quite well even if the band pass of the radiation being employed is a moderately large fraction of the wavelength region. On the other hand, if the absorptivity cannot be treated as a constant over the band pass of the incident radiation then deviations are likely to appear.

Quantitative Analysis of Absorption Data

The applicability of Beer's Law can be determined for a particular system if one measures the absorbance of a series of known concentration of the the absorbing species. If Beer's Law is obeyed, a plot of the resultant experimental data in terms of absorbance versus concentration will yield a straight line passing through the origin, with a slope equal to the absorptivity-path length product of equation E-3. More often than not, however, deviations, although they may be slight, will exist. When the absorbance versus concentration for a particular system exhibits a nonlinear relation, no matter what the cause, quantitative analysis is still possible. Here, however, the concentration is obtained directly from the plot of measured absorbance values versus concentration of solutions containing known concentrations of absorbing species. This plot, commonly referred to as a standard or calibration curve, must be verified at frequent intervals for best results. In fact, practice dictates the construction of a standard curve covering the entire range of concentrations to be encountered whether or not the solution obeys Beer's Law. If the solution does not absolutely conform with the law, which is usually the case, nothing is forfeited in the curve construction; if the solution does obey the law the determination of the absorptivity-path length product for the system is realized. Therefore, the chief requirement for successful absorbance spectrophotometry is that the radiation and absorbing properties of a system be measurable and reproducible. This requirement is realized by the careful construction of the standard curve and proper design of absorption measuring instruments.

Construction of the Standard Curve

The standard curve is constructed, as mentioned before, from experimental data obtained from a series of prepared samples containing known concentrations of the absorbing species. It is therefore evident that some method of fitting a curve to experimental data points must be employed. To this end, a simple linear regression algorithm based on least square computations is most often used. This method admittingly assumes that the experimental data adheres to a linear relationship (i.e. Beer's Law), and one might question the validity of such an assumption in light of the previous discussion concerning deviations from Beer's Law. While it is true that deviations usually appear in the experimental data, these deviations are usually minor. It is therefore appropriate to assume a linear relation from the outset. If the relation between absorbance and concentration is not linear the least square algorithm will reflect the fact in the regression coefficient it yields. In such a case, more advanced curve fitting techniques could be subsequently employed. A more detailed discussion of the least square technique appears in Appendix B.

Elements of a Spectrophotometer

The instrument used to gather absorbance data is called a spectrophotometer. Although it is not necessary to discuss the subtleties of spectrophotometer design, it is advantageous to understand the basic components of spectrophotometers in general, and single-beam spectrophotometers specifically.

The basic elements common to all spectrophotometers, in one form or another, are shown in block diagram fashion in Figure 4. Typically, a radiant energy source is broken down into component colors by a prism or grating, as previously mentioned. The principle requirements of the energy source are that it emit a sufficient intensity of radiation in a reproducible and controlled fashion. The dispersed radiation emerging from the prism or grating is swept past through a slit until the desired emission line or band of frequencies falls on the sample positioned behind the slit. The slit is adjustable to allow any bandwidth to be chosen. The source, prism and slit form the monochrometer of the instrument. The sample, if it contains an absorbing species at the frequencies of interest, will absorb a portion of the radiation emerging from the monochrometer; the remainder is transmitted through the sample and strikes a light detector. This detector, usually a photocell, converts the incident light energy into a small electrical signal which in turn is amplified. The amplified signal is used to drive a display which is usually calibrated to record absorbance, although at times transmittance may be shown (transmittance is defined a the ratio of incident to transmitted radiant power, while absorbance is the logarithm of transmittance). The calibration of the unit is accomplished by allowing all the radiant energy and none of the radiant energy to reach the light detector, corresponding to zero and infinite absorbance, respectively. In practice, the condition of infinite absorbance is accomplished by including some sort of occluder in the light path which prevents light from reaching the detector. Zero absorbance is realized by the insertion of a blank sample; that is the insertion of a sample identical to the actual samples in all respects except that it contains none of the analytic species. There is, however, a caution which must be realized in order not to introduce errors. The blank sample should scatter, refract, and reflect radiation in exactly the same manner as the actual samples. This requires that the sample and blank cells should be matched cells which are kept free of dust, fingerprints, or other foreign materials.

The single-beam spectrophotometer is characterized by a single light path from the source to the detector, and is primarily used for the quantitative determination of a single component of chemical species when a large number of samples are to be analyzed. The instrument, which is relatively inexpensive, enables the accumulation of reliable absorption spectra data conveniently and reproducibly. Consequently, it has become a common clinical laboratory in use today.

The Problem

We have seen that the accumulation and analysis of absorption data is a relatively simple task. It is because of this fact that the use of absorption spectrophotometry is so widespread. There exists, however, a problem related to the analysis task. The realization of quantitative results is based on a standard curve, a curve which must be prepared each time an analysis is undertaken. Although we have seen that the construction of such a curve is usually a straightforward process, there is no denying the fact that such a construction can become a tedious and time consuming task. Yet the curve, which should be checked at periodic intervals to insure that the least possible error is realized, must be prepared with care since they form the foundation of future results.

A solution to this dilemma can now be realized due to the major advances in the field of microelectronics made in the last several years. It is now economically and technically feasible to construct a microprocessor controlled data processing unit which, when interfaced with a single-beam spectrophotometer, could generate and store a standard curve in a fraction of the time and with less error than the employment of manual methods. The terms generate and store are loosely used implying that certain statistical parameters which fully characterize the curve are calculated and retained. The design and construction of such a data processing unit was attempted; the results and conclusions arrived at from the work accomplished to date constitutes the remainder of this paper.

Overview of Technical Approach and Work Accomplished

The heart of the data processing unit is a microprocessor. A microprocessor is ideally suited for the control and arithmetic processing functions required. The unit, under programmed control of the microprocessor, is designed to perform the following tasks:

1. Process a standard sample, associating a value of absorbance with concentration.
2. Generate a standard curve using the processed standard data values.
3. Display parameters reflecting the nature of the standard curve.
4. Process an unknown sample by displaying the concentration calculated using the stored curve.

The hardware needed to perform the above tasks has been designed, built, and tested. A conceptual block diagram of the unit is shown in Figure 5. Although each of the blocks in Figure 5 will be discussed in further detail at a later point, we present here a brief description of each. The voltage-to-frequency converter, in conjunction with the digitizer, realizes the task of an analog-to-digital converter. That is, these two functional blocks transform the analog information available from the spectrophotometer into a digital form which the central processor can manipulate. Read-only-memory, being nonvolatile, supplies the storage medium for the software routines needed to instruct the microprocessor, while read/write memory offers run-time parameter storage. The keyboard and display interface serves two related functions. First, it allows data and commands to be entered via an alphanumeric keyboard. Second, it drives a six digit LED display. Finally, the serial interface provides a standard RS232C communication link, usuable for a CRT display or printer.

The prototype was constructed around a nucleus provided by a commercially available system design kit. This kit provided some of the basic hardware features needed such as memory, keyboard and display control, and central processing capability. Other hardware features, however, had to be incorporated into the kit. These features, to be discussed in detail later, were voltage-to-frequency conversion, memory expansion, and serial communication interface. Flow diagrams illustrating the necessary sequences to realize these four tasks are included in Appendix D.

The software needed to realize the design objectives, unlike the hardware, is not completed. Approximately one-third of the software needed, however, is written and debugged. This includes a custom monitor, I/O drivers for the serial and voltage-to-frequency interfaces, and specification of a commercially available floating-point package. Programs for curve fitting data, logarithmic conversion, and overall functional control of the interrelated tasks need to be coded. These programs will not, however, be written in connection with this thesis.

The Microprocessor Family

A number of factors, such as applicability to the problem, ease of use, software and hardware support, and availability, were considered in the determination of the microporcessor family to be employed. In the final analysis, the MCS-85 family of components, manufactured by Intel Corporation, was chosen.

Although a complete description of the MCS-85 microcomputer system is not necessary, it is appropriate to briefly discuss some aspects of the family. For more detailed information, one should consult Reference 1.

The MCS-85 family, introduced by Intel in 1977, is generally considered as a second generation of that firm's popular MCS-80 family. The basic components of the MCS-85 are shown in block diagram form in Figure 6. The components represented by dotted lines are those components which are not relevant to the design and construction of the data processor, and consequently will not be discussed.

The heart of the MCS-85 system is the 8085A, a single chip 8-bit N-channel metal-oxide-semiconductor microprocessor. The 8085A is a second generation 8080A, an earlier 8-bit microprocessor introduced by Intel and widely second sourced. The 8085A, while being totally software compatible with the 8080A, integrates many functions that are auxillary to an 8080A based system. Functions such as clock generation, system control, and interrupt prioritizing are directly realized by the 8085A. Thus, 8085A based designs tend to be more compact then 8080A based designs, yet the 8085A may still draw upon the wealth of existing software which has been developed for 8080A systems. Since software design is in most cases the determining factor for success this fact is very important. The basic hardware functions inherent to the 8085A are shown in Figure 7.

Before mentioning the other components of the family which are relevant to the data processor design, it is important to briefly discuss the nature of the bus structure supported by the 8085A. Naturally, the 8085A, being the central processing unit of the MCS-85 family, coordinates all bus transfers and operations and executes the instruction set. The 8085A, however, employes a rather unique multiplexed bus system. This bus system, explained in detail in Reference 1, is such that the lower eight bits of the sixteen bit address bus is time multiplexed with the eight bit data bus. The main reason for employment of this type of structure was to free device pins in order to integrate more on-chip functions while maintaining the standard forty pin package.

Naturally, since the information on the data bus is time multiplexed, some type of signal must be generated in order that peripheral devices know what type of information is on the bus. This signal, generated by the 8085A itself, is referred to as ALE, standing for Address Latch Enable. When the ALE line is active, peripheral devices know that address information is present on the bus which must be latched, either internally or externally, in order to properly complete the machine cycle.

The reason for the brief highlight of the MCS-85 bus structure will now become apparent. There are three peripheral components which are specifically designed to be directly interfaced to the multiplexed bus structure of the 8085A. In other terms, these three components possess on-chip logic which enables the internal latching of address information. The components are:

1. 8155/8156 RAM, I/O and Timer
2. 8355 ROM and I/O
3. 8755 EPROM and I/O

Block diagrams illustrating the basic features of each device are shown in Figures 8-10. Out of these three devices, the employment of the 8155 and 8755 chips was originally envisioned. The 8755, for reasons to be later discussed, was not employed. The 8155, however, played a major role, especially in regard to its use in the analog-digital conversion scheme employed. We will say more on this when the actual conversion scheme is discussed.

The last component of the MCS-85 family which was employed in the design of the data processor is the 8279 Programmable Keyboard/Display Interface chip. Data input and display are an integral part of many microprocessor designs; the data processor we are considering is no exception. It was obvious from the start that some form of keyboard entry and data display would be needed. While it is true that the function of keyboard entry and data display may be controlled by the CPU itself, such a task places a large burden on the device. The 8279, however, provides this function for eight bit microprocessors, thereby relieving the CPU from scanning the keyboard and refreshing the display.

The Spectrophometer Interface

It is not only desirable to employ a simple interface between the data processor and the spectrophotometer, it is a necessity if the unit is to be used by nontechnical individuals. Fortunately, this type of interface is easily accomplished for the majority of single-beam spectrophotometers due to the use of data accumulation devices. Chart recorders, data loggers, and other accumulation devices also need a simple and clean interface and spectrophotometer manufacturers, realizing this fact, include several output lines on essentially every instrument marketed. These lines supply an analog voltage which is proportional to the transmittance of the sample under study. Typical, almost universal, values for the voltage are one and ten volts full scale. Consequently the data processor can achieve the same type of interfacing as accumulation devices by simply accepting an input voltage proportional to transmittance.

Analog to Digital Conversion Scheme

It was apparent, given an analog input, some form of analog-digital conversion technique was required. Before a review of the basic types of conversion schemes was undertaken, a determination of the desired resolution was in order. The resolution that was arrived at was one part in ten thousand. It was therefore necessary to employ a 13-1/2 bit A/D converter. Although conventional conversion, schemes such as successive-approximation and integration could yield the desired resoltuion, the cost of modules based on such techniques would be prohibitive for our purposes. It was obvious that some alternative technique, which would be more economically sound, had to be found.

Fortunately, an alternative technique was found in the form of a voltage-to-frequency converter based design. Such a design employs a voltage-to-frequency converter and a digital counter.

Voltage-to-frequency converters, generally speaking, are signal processing devices that accept an analog input and convert it to a train of pulses having fixed width, fixed height, and a rate that is directly proportional to the input voltage or current. For an arbitrary voltage input, 0 V_{in} V_{max}, and a corresponding full-scale frequency, f_{max}, the frequency of the output pulse train, f, is

$$f = f_{max} \; {}^{V}in/V_{max} \tag{E-4}$$

The frequency f_{max} may either be fixed by the design of the device or adjustable over a decade or so by the specification of an external resistor-capacitor network.

Since the relationship between analog input and the output frequency is inherently monotonic, it is possible to construct V/F based A/D converters of extremely high resolution, exhibiting no missing codes, even in the presence of widely varying ambient temperatures. Noise rejection is high, first because of the V/F's internal integrator, and second because of the integrating efect of the counter. Also, since the input is tracked continuously, there is not need for clock pulses, convert commands, or any other form of external control logic. But the main advantage of a V/F based conversion technique is, from the viewpoint of the present treatment, the relative low cost for a given resolution compared to other viable forms of conversion. An initial estimate of the cost of a 14 bit A/D conversion based on a V/F was about twenty dollars.

There are naturally limitations on the use of V/F based converters. These limitations, surprisingly enough, are due to only two significant factors:

1. They are slow in conversion
2. They require a counter to interpret the output digitally.

The speed of conversion is determined by the full scale count and the amplitude of the signal being measured.

$$\text{Conversion Time} = \frac{\text{Desired full scale count}}{\text{Maximum input measured}} \cdot \frac{\text{Full scale input}}{\text{Maximum frequency}}$$

We desire a resolution of 1 part in 10,000, with an input range of zero to ten volts. Using a 100 KHz V/F, the conversion time is $(10{,}000/10)(10/10^5) = .1$ seconds. In the present application, this conversion time is totally acceptable, but it is easy to see that real-time processing could not tolerate such an excessive conversion length.

The need of an external counter is not a problem in the present application either. The reason is due to the fact that the 8155 component includes a 14 bit programmable binary counter/timer. It becomes evident that the 8155 can serve as the necessary counter to complete the conversion circuit. All that is necessary to convert the analog input, from the CPU's point of view, would be:

1. Program the 8155 timer for the proper mode
2. Start the count
3. Delay for .1 seconds in a software loop
4. Halt the count
5. Interpret the count result

Operator Interface

The operator interface must naturally provide straightforward control of the instrument and easy entry and display of required data. Parameters and commands that require frequent manual entry are designed to be entered through a front panel keyboard. Besides the standard numerical keys, the following functions were committed to a single key each:

1. Standard - processes a standard curve point
2. Curve - generate a standard curve from stored data points
3. Slope - display slope of the last standard curve to be calculated
4. Intercept - display intercept point of the last standard curve to be calculated
5. Review - display the standard data points previously entered
6. Unknown - display concentration value for the present input

7. CE - clear entry key

8. Reset - reinitialize the state of the unit.

The display consists of six seven-segment LEDs. Four of the LEDs are committed to arithmetic data, one for an arithmetic sign, and one for command prompting.

The requirement of a keyboard input and LED display is easily realized through the employment of the previously mentioned keyboard/display interface chip, the 8279.

Although transmittance usually is the type of information presented to the unit, it is possible that absorbance information could appear. Consequently, a switch is provided which informs the unit on the nature of the input data. The status of the input is important since transmittance requires the employment of a logarithm, while absorbance does not.

Memory Requirements

There are two types of memory devices which were needed; RAM and ROM. The ROM portion of the memory, being nonvolatile in nature, incorporates the software routines needed to provide the basic functions. Although the determination of unwritten software memory length is a tricky task, it was originally estimated that about 2K words of ROM memory would be needed.

Today, it appears that this is a very close estimate. In any event, the 8755 EPROM was originally specified to contain the ROM based software during development. For reasons to be discussed later, the 8755 EPROM was never employed, but a similar type of EPROM, the 2716, was used.

The RAM portion of the memory, being volatile but rewritable, constitutes the area for data storage and scratch pad computations. The length requirements for this form of memory, being more easily defined, was set at about .25K words. Therefore, the 8155 static RAM, already being used in the A/D circuit, was employed.

Power Requirements

All the components used required either +5 and/or 15 volts. It was estimated that the five volt supply would need to be able to provide two amperes, while the current requirements for the fifteen volt supply would be minimal. Therefore, two self-contained logic power supplies were purchased from Analog Devices:

1. AD205 +5 volts at 2A
2. AD215 ±15 volts at 15mA

Selection of a V/F Converter

There are two basic types of V/F converters available; namely module units or integrated circuit devices. A module unit, while slightly more expensive than an IC device, is totally self contained and therefore it is easier to employ. An IC device, however, is a more versatile device in regard to input and output characteristics. Versatility led to the selection of an IC device.

The actual device selected was Analog Devices' AD537. The chip and external components necessary to realize a TTL compatible 100 KHz output are shown in Figure 11. A complete discussion of the device and design information regarding component specifications is given in Reference 2. It is worth noting the high input impedance (i.e. 250 M) and versatile output characteristics (i.e. 12 TTL Fanout) the device displays.

The SDK-85 Design Kit

After the selection of the V/F converter the task of hardware design and development was started. At the outset it was apparent that a "hands on" familiarization with the components involved in the design would be beneficial in the long run. Yet, it was also desirable to begin the fabrication of a device which could serve as a hardware prototype.

Fortunately, both of these goals could be reached through the purchase of a system design kit manufactured by Intel Corporation. The kit, named the SDK-85, contained a minimal amount of components to assemble a complete 8085 microcomputer system on a single printed-circuit board. The board also contained a wire-wrap area for custom designed circuitry, an obvious advantage from out point of view.

The kit is described in detail in Reference 3. A functional block diagram of the SDK-85 is shown in Figure 12. The components enclosed in dashed boxes were not included in the original kit, although spaces for such components were provided on the printed-circuit board. Only after the basic kit was assembled and functioning properly was the expansion attempted.

The assembly of the basic kit went smoothly and the system functioned properly upon its initial power-up. After a period of familiarization, the expansion components were purchased. As can be seen in Figure 12, expansion is of two types. First, an additional 8155 and 8355 or 8755 may be installed for increased memory and I/O capabilities. Since facilities to program 8755 devices were not available, only the extra 8155 was obtained.

The second type of expansion was related to the bus structure. The 8085 requires some form of buffering for intensive systems, and the bus expansion circuitry provided this buffering function. Also, in order to interface to standard memory components, the bus must be demultiplexed, and the expansion components also provide this demultiplexing function.

It was apparent that some form of EPROM based storage would be needed in the development of software. While the basic kit provided EPROM based storage in the form of a 8755, facilities to program such a device were not available. Facilities to program other standard EPROMs, such as the 2708 and 2716, were available. Consequently, it was decided to incorporate such a standard EPROM into the kit, but the expansion circuits had to be present to accomplish this feat. It was felt that once the software had been developed to a sufficient point a new prototype more closely resembling the desired unit could be assembled employing 8755 devices programmed by Intel. While such techniques are obviously not optimal with regard to development, circumstances dictated their use.

The expansion of the basic kit, the design of which was provided in the SDK-85, was also accomplished with no initial problems. After apparently functioning properly for a period of a few weeks, however, the kit developed a malfunction. The problem appeared to be related to the keyboard and display circuitry. The keyboard and display, as mentioned previously, is controlled by the 8279 component. Basically, this chip signals the 8085 that keyboard data is available by raising one of its output lines high, which in turn is connected to one of the restart interrupt request lines of the CPU. A full treatment of the 8085's interrupt structure is beyond the scope of this paper; for more detailed information the reader is referred to Reference 1. In simple terms, however, if the 8085 allows the interrupt to be recognized (this function is under software control), the CPU will execute a vector subroutine call to a specific address in memory. The subroutine called, in this case, contains the necessary code to read the data available from the 8279 controller.

The problem noticed after a few weeks of operation was that entered data would not always be acknowledged. That is, at times the keyboard entries would be totally ignored. The problem was solved only after a discussion with an Intel service engineer took place. I was informed at that point that the initial design for the 8085 CPU contained a few minor and subtle flaws, one of which resulted in a possible malfunction of the interrupt system. Since the 8279 controller communicates with the 8085 via the interrupt system, it became evident that the problem was due to the malfunctioning of the original CPU received. The original 8085 was replaced with a 8085A, and the problem disappeared.

Custom Modifications to the Kit

The SDK-85 possesses the capability of communicating with a teletypewriter, employing the serial input and output lines incorporated in the 8085A. Although at the time no serious software development or debugging was being done, it was evident that the use of a serial communication channel could be beneficial in such endeavors. Therefore, the 8085A serial I/O lines were connected to a dump video terminal operating at 110 baud in a current-loop mode via the teletypewriter interface. Although the serial data link functioned properly, the use of 110 baud was too restrictive to be of much practical use. In fact, I/O functions could still be performed at a faster rate using the SDK-85 keyboard!

It should be noted that there was nothing inherent in the hardware design of the serial interface which constrained the baud rate which could be employed. The problem was firmware related. The timing for the serial communication channel was set by the SDK-85 monitor residing in the 8355, and the parameters involved were constants enabling the use of 110 baud only. If these parameters were alterable, which was not the case, then varying baud rates could be used.

A new monitor was therefore written to replace the original SDK-85 monitor. Not only did this enable the employment of I/O routines able to establish higher communication rates, but a more varied repetoire of useful monitor functions become available. A comparison of the functions for the two monitors is presented in the section concerning system software. Source listings of the monitors are included in Appendix.

During the same time period, a custom memory expansion circuit was designed, built, and tested. This expansion, as mentioned beforehand, was used to develop and debug system softward. The expansion provides an additional 4K words of EPROM based storage by employing two 2716 devices. The new monitor was mapped into this expansion memory space, so 3K words of EPROM based storage was left for software development. The inclusion of 1K RAM storage is also provided for by the custom expansion. The schematic of the custom memory expansion circuit is included in Appendix C.

The inclusion of a standard RS232C interface was also accomplished. This necessitated the disconnection of the provided current-loop interface, and the addition of RS232C line drivers in its place. The circuit for this modification is shown in Figure 13.

SYSTEM SOFTWARE

The coding of the system software is approximately one-third complete. The tasks completed to date are:

1. Specification of a Floating-Point package
2. Custom monitor coded and debugged
3. Serial I/O drivers for RS232C interface coded and debugged
4. Voltage-to-frequency I/O drivers coded and debugged

We proceed to discuss these accomplishments in detail.

Floating Point Arithmetic

The first software task which was addressed was the coding of a floating point package. To this end, several previously written packages were purchased through a software library. An attempt was made to modify one of the purchased packages to be compatible with the SDK-85 kit. This attempt failed miserably for several reasons; namely the complexity of the problem did not lend itself to an easy solution and the documentation and coding techniques displayed in the purchased software was, at best, fair. In simple terms, the attempt at modification of one of the purchased packages became a Kludge. After a considerable effort, this approach was forfeited and alternatives were considered.

About the same time, Intel marketed their own version of a floating-point software package. This package was fashioned so the user had no need to be concerned with the actual code. All that was required to employ the package was a familiarization with the interfacing routines, and only those functions which were desired need be included in the final object program. Also, the memory requirements for the use of the package were modest, easily lending themselves for use with the SDK-85 board. It was therefore decided to use Intel's floating point package in place of any attempt to code a custom package. Complete information concerning Intel's package can be found in Reference 4.

Custom Monitor and Serial I/O

As previously mentioned, a custom monitor was coded which provided the following:

1. an increased variety of monitor functions
2. serial input and output capabilities for a standard RS232 interface.

The original monitor provided with the SDK-85, although well documented and easy to use, contained a minimal amount of functions. The custom monitor which was written incorporated all the commands of the SDK-85 monitor in addition to some very useful commands for debugging purposes. The following is a list of the commands available in the new monitor along with a brief description of their use. Commands marked with an asterisk are unique to the custom monitor.

*1. Display Memory Command

Selectable areas in memory are accessed and their contents displayed.

2. Substitute Memory Command

Selectable areas in memory are accessed and their contents displayed on an individual basis, with the option of modifying the contents.

*3. Examine Register Command

The contents of the CPU's working registers are collectively displayed.

4. Examine/Modify Command

The contents of an unique working register is displayed, with the option of modifying the contents.

*5. Move Memory Command

A selectable area in memory is accessed and its contents are moved to a destination specified.

*6. Fill Memory Command

Selectable areas in memory are filled with a specified character.

7. Go Command

   Control is transferred from the monitor to a user program.

*8. Read Input Port Command

   The contents of a specified input port is read and displayed.

*9. Output Data to Port Command

   Outputs data to specified output port.

*10. T Command

   Executes a jump to the address 9000H for easy interfacing between monitor and any user program.

The serial input and output routines incorporated into the custom monitor are based on the algorithms presented in Intel Application Note AP-29, "Using the Intel 8085 Serial I/O Lines" (Reference 5). Although the actual code performing the algorithms differ as a result of hardware interface differences, the discussion of the software package contained in that Application Note applies. The differences in the actual code result from opposite polarity signal conventions.

Analog-to-Digital Conversion Driver

A driver routine to complete the V/F based A/D conversion was coded. Unfortunately, difficulties were encountered. As mentioned beforehand, the general scheme needed to complete an A/D conversion is

1. Program the 8155 timer for the proper mode
2. Start the count
3. Delay for .1 seconds in a software loop
4. Halt the count
5. Interpret the count result

The problem encountered was related to the last step above; interpreting the count result. It was not known at the time, nor was it mentioned in available literature the timer circuit incorporated on the 8155 was designed to be a square-wave timer, not an event counter. To achieve this, the timer counts down by two's in completing a cycle. It is therefore a more involved process which must be employed in interpreting the count then just subtracting the final count from the initial count setting. A discussion of the problem between the author and an Intel technical representative provided the answer. The author was informed of an Intel publication (Reference 6) concerned with the use of the 8155 programmable timer which included an example of the task trying to be accomplished; consequently the program which was subsequently used was taken directly from this source.

CONCLUSIONS

The specification and design of a microprocessor based data analyzer for single-beam spectrophotometers was completed. A hardward prototype capable of realizing such an instrument was built, tested, and found to function properly. The task of software development was undertaken and, although not complete at this point, work on a floating point package, custom monitor, and serial and voltage-to-frequency I/O drivers was completed. Software for curve fitting the experimental data, logarithmic conversion, and functional control of the unit remains to be done.

REFERENCES

1. MCS-85 Users Manual, Intel Corporation, Santa Clara, California, 1978.

2. Integrated Circuit Voltage-to-Frequency Converter AD537, Analog Devices, Inc., Norward, Massachusetts, 1976.

3. SDK-85 Users Manual, Intel Corporation, Santa Clara, California, 1978.

4. 8080/8085 Floating Point Arithmetic Library Users Manual, Intel Corporation, Santa Clara, California, 1978.

5. "Using the Intel 8085 Serial I/O Lines," Application Note AP-29, Intel Corporation, Santa Clara, California, 1978.

6. "Using the Intel 8155," Application Note AP-38, Intel Corporation, Santa Clara, California, 1978.

7. Memory Design Handbook, Intel Corporation, Santa Clara, California, 1977.

8. Peters, Dennis G., Hayes, John M., and Hieftje, Gary M., A Brief Introduction to Modern Chemical Analysis, W.B. Saunders Company, Philadelphia, Pennsylvania, 1976.

APPENDIX A

Derivation of the Beer-Lambert Law

Imagine an infinitesimal layer, of thickness db, of a sample which is perpendicular to an incident light beam, as shown in Figure 14. Let the radiant power of the incident light be P. Since the radiation may react with the sample, some of the incident light may be absorbed. Let us therefore represent the radiant power of the emerging light be P-dP, where dP represents the infinitesimal amount of power absorbed by the layer.

Several factors determine how many photons will be absorbed, and consequently how large dP will be. First are the concentrations of the two interacting species; the more photons that enter the layer and/or the more sample molecules there are in the layer, the greater will be the number of photons that interact. It seems reasonable to assume that the number of photons absored is directly proportional to the number of photons entering the layer, and to the concentration of absorbing molecules in the layer, c. This assumption, along with the preceeding statement, are by no means obvious; they are only justifiable through experiments verifying the conclusions to which they lead.

Second, we must realize that all energy absorptions are not equal probable, so there will have to be an energy (frequency) dependent parameter, a', introduced to account for the probability of photons of a particular energy being absorbed. Although this parameter can in principle be calculated if the quantum mechanical description of the system is complete, it is almost always operationally defined as the proportionality factor which will yield valid results based on the theoretical model.

If we combine these two factors into one equation that allows the determination of the decrease of radiant power of the incident beam due to absorption in a layer of thickness db, we arrive at

$$- \frac{dP}{db} = a'c\ P \qquad \text{(A-1)}$$

$$- \int_{Po}^{Pt} {}^{dP}/_{P} = a'c \int_{o}^{b} db \qquad \text{(A-2)}$$

$$\ln\ {}^{Po}/_{Pt} = a'c\ (b-o) \qquad \text{(A-3)}$$

$$\log\ {}^{Po}/_{Pt} = abc,\ a = {}^{a'}/_{2.303} \qquad \text{(A-4)}$$

Equation A-4 is the integrated form of the Beer-Lambert Law of Absorption.

APPENDIX B

Linear Regression and Least Squares Computation

Regression analysis is a means of studying the variation of one quantity (dependent variable) at selected levels of another quantity (independent variable). The word regression was first used by Sir Francis Galton in describing certain genetic characteristics which appeared to return from extreme values toward a mean value. Linear regression is characterized by the regression of extreme values toward a straight line.

The general equation for a straight line can be written as

$$Y = a + b\,(x-\overline{x}) \qquad \text{(B-1)}$$

where b is the slope, Y is the evaluated dependent variable, x is the independent variable, and $\overline{x}$ is the mean of the independent variables. The left hand side of equation B-1 is written as capital Y to emphasize that the evaluation of the equation yields the calculated value of the dependent variable in a regression analysis. This calculated value will in general differ from the observations at the same value of the independent variable, unless the observation happens to lie exactly on the calculated line.

The true regression equation, assuming the line to be really straight, can be written, for any specified value of x, as

$$\eta = \alpha + \beta (x-\overline{x}) \qquad \text{(B-2)}$$

where α and β are the true parameters, of which the statistics a and b are estimates made from a sample of observations. If we assume that the value of x can be measured with negligible error (which is the case in absorption analysis since this variable corresponds to the concentration of prepared solutions), there is no distinction between the observed and true values of x.

In order to determine the parameters a and b in equation B-1 by the method of least squares it is necessary to find the line that will minimize the badness of fit as measured by the sum of squares of deviations of the dependent variable from the line. That is, one minimizes the sum of the squared deviations

$$S = \sum_{j=1}^{N} d_j^2 = \sum (y_j - Y_j)^2 \qquad \text{(B-3)}$$

where yj is the observed and Yj the calculated value of the dependent variable. The square of the deviations is used, instead of the deviation itself, since deviations may be both positive or negative values.

The terms a and b can now be specified as follows. First, substitute the calculated value of Y at the jth value of x, which, from B-1 is Yj = a + b (xj-$\bar{x}$), into B-3 giving

$$S=\sum_{j=1}^{N}[y_j-a-b(x_j-\bar{x})]^2 \qquad \text{(B-4)}$$

Squaring the bracketted term yields

$$S=\sum_{j=1}^{N}[y_j^2+a^2+b^2(x_j-\bar{x})^2-2ay_j-2y_jb(x_j-\bar{x})+2ab(x_j-\bar{x})] \qquad \text{(B-5)}$$

This reduces to

$$S=\sum_{j=1}^{N}[y_j^2]+Na^2+b^2\sum_{j=1}^{N}(x_j-\bar{x})^2-2a\sum_{j=1}^{N}y_j-2b(x_j-\bar{x})\sum_{j=1}^{N}y_j+2ab\sum_{j=1}^{N}(x_j-\bar{x}) \qquad \text{(B-6)}$$

The last term in B-6 is zero, since (x-$\bar{x}$) = 0. The object is to find, for the particular values of x used and the particular values of y observed, the values of a and b which make S a minimum. We therefore use the usual procedure in calculus for finding a minimum by differentiating and equating to zero. Thus, to find the least squares value for a, we differentiate B-6 treating b as a constant

$$\frac{\partial S}{\partial a}=2Na-2\sum_{j=1}^{N}y_j=0 \qquad \text{(B-7)}$$

therefore

$$Na = \sum_{j=1}^{N} y_j$$

so

$$a = \Sigma y/_{N} = \overline{y} \qquad \text{(B-8)}$$

Similarly, to find the least squares estimate of b, we differentiate B-6, treating a as a constant

$$\frac{\partial s}{\partial a} = 2b\sum_{j=1}^{N}(x_j-\overline{x})^2 - 2\sum_{j=1}^{N} y_j\,(x_j-\overline{x}) = 0 \qquad \text{(B-9)}$$

therefore

$$2b\sum_{j=1}^{N}(x_j-\overline{x})^2 = 2\sum_{j=1}^{N} y_j\,(x_j-\overline{x})$$

so

$$b = \frac{\sum_{j=1}^{N} y_j(x_j-\overline{x})}{\sum_{j=1}^{N}(x_j-\overline{x})^2} \qquad \text{(B-10)}$$

or equivalently

$$b = \frac{\sum_{j=1}^{N}(y_j-\overline{y})\,(x_j-\overline{x})}{\sum_{j=1}^{N}(x_j-\overline{x})^2} \qquad \text{(B-11)}$$

Besides the determination of the parameters a and b we need to determine the extent to which the calculated line represents the data. To this end, a coefficient of linear regression may be calculated. Although the derivation of the relation is beyond the scope of the present treatment, it can be shown that the term r^2, given by

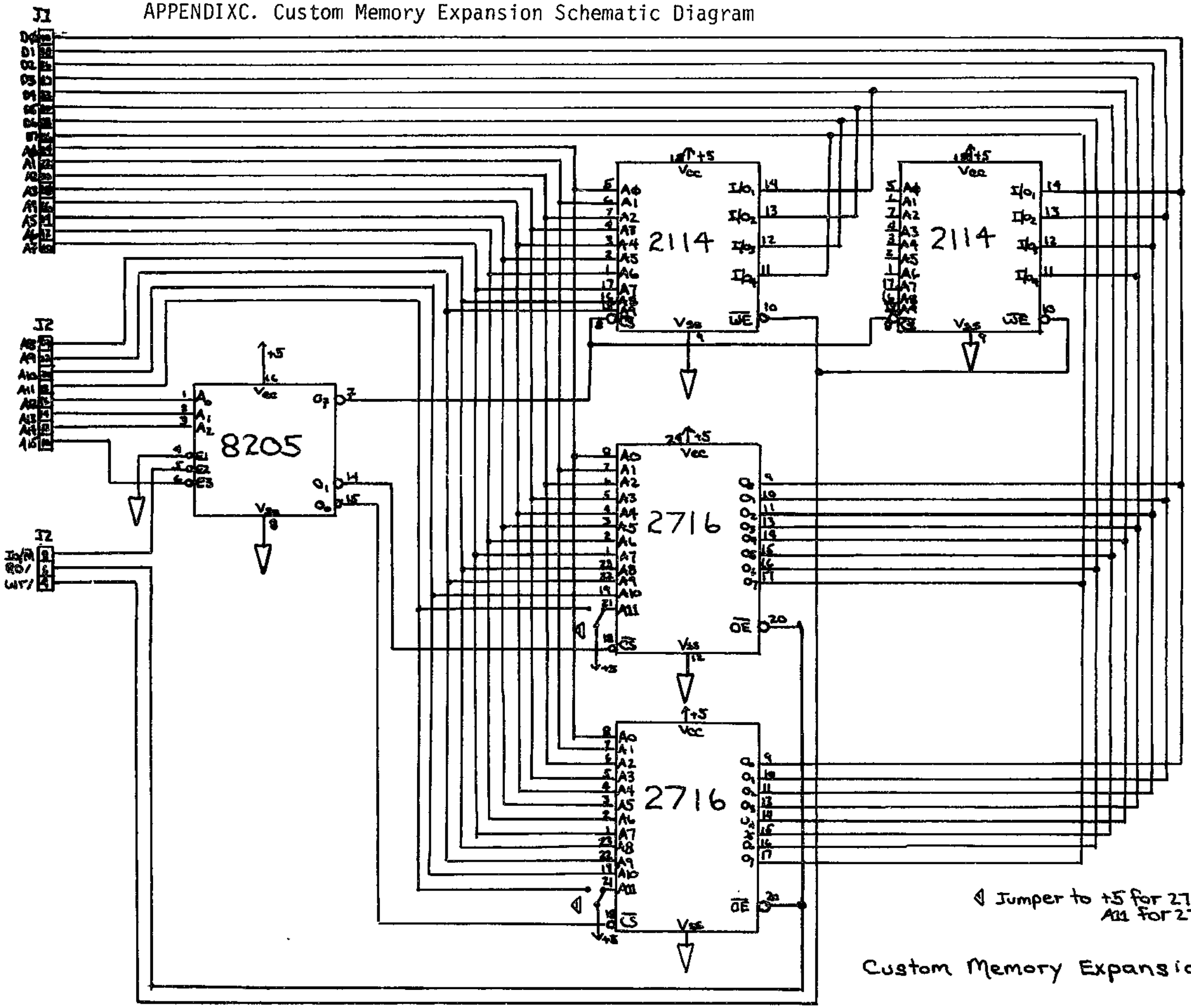
$$r^2 = \left(\sum_{i=1}^{N}(y_j-\overline{y})^2 - \sum_{i=1}^{N}\left(y_j - a - b(x_j-\overline{x})\right)^2\right)\Big/\sum_{i=1}^{N}(y_j-\overline{y})^2 \qquad \text{(B-12)}$$

represents such a regression coefficient. The value of r^2 is such that $0 \leq r^2 \leq 1$. The closer the value r^2 is to unity, the more linear the data.

APPENDIX C

CUSTOM MEMORY EXPANSION SCHEMATIC DIAGRAM

APPENDIXC. Custom Memory Expansion Schematic Diagram



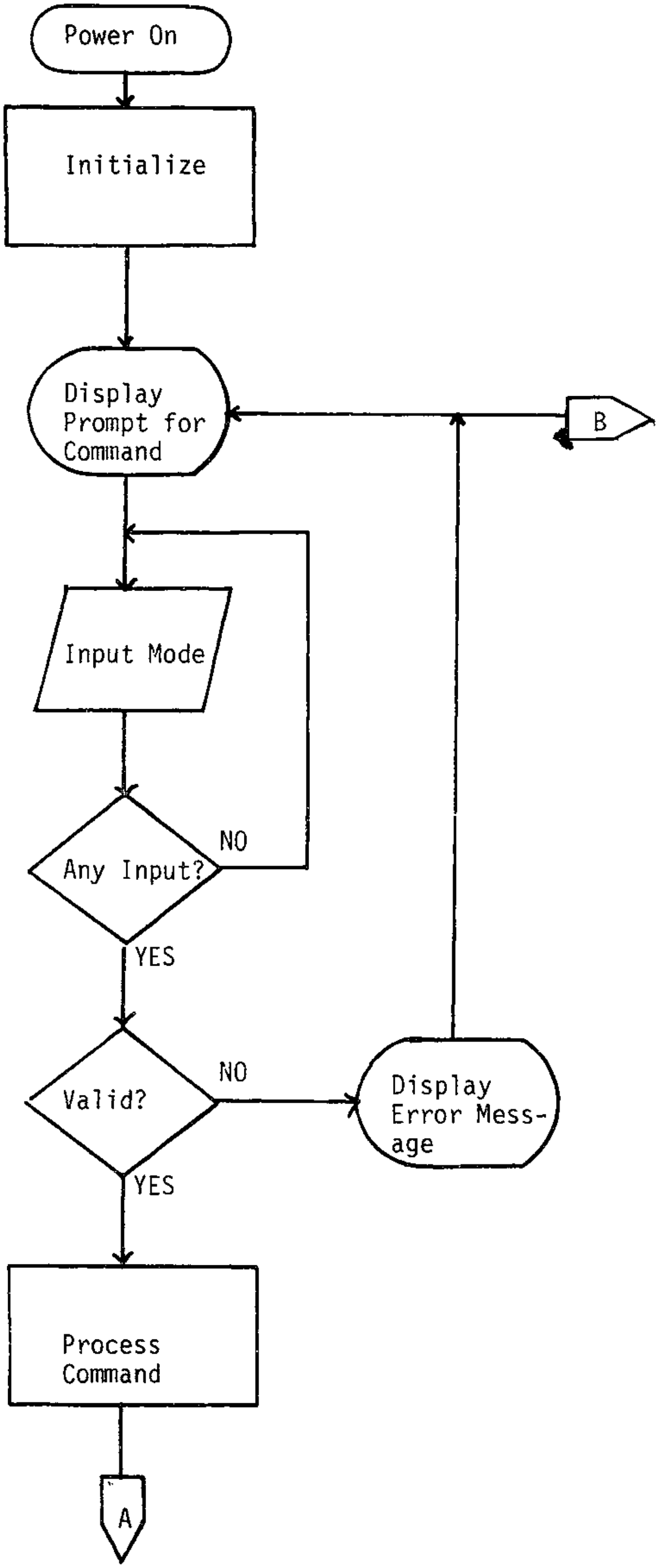
◁ Jumper to +5 for 2716
A11 for 2732

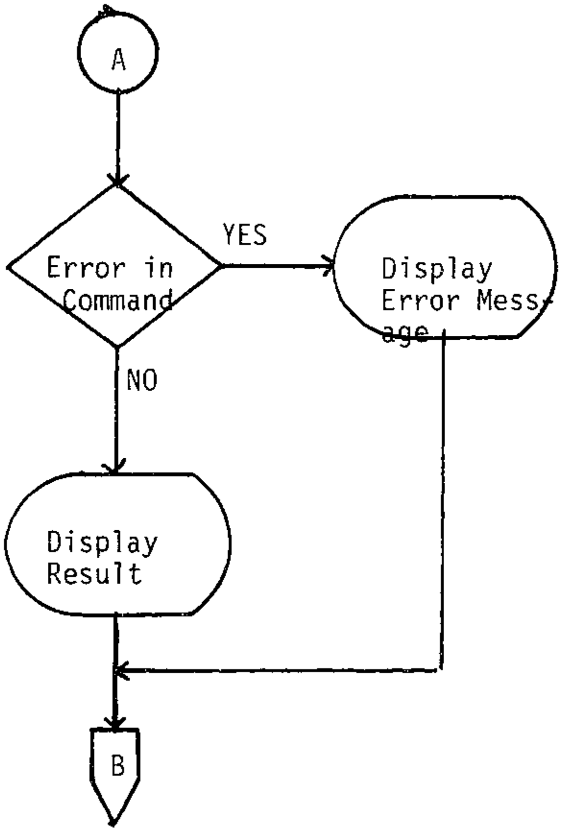
Custom Memory Expansion

APPENDIX D

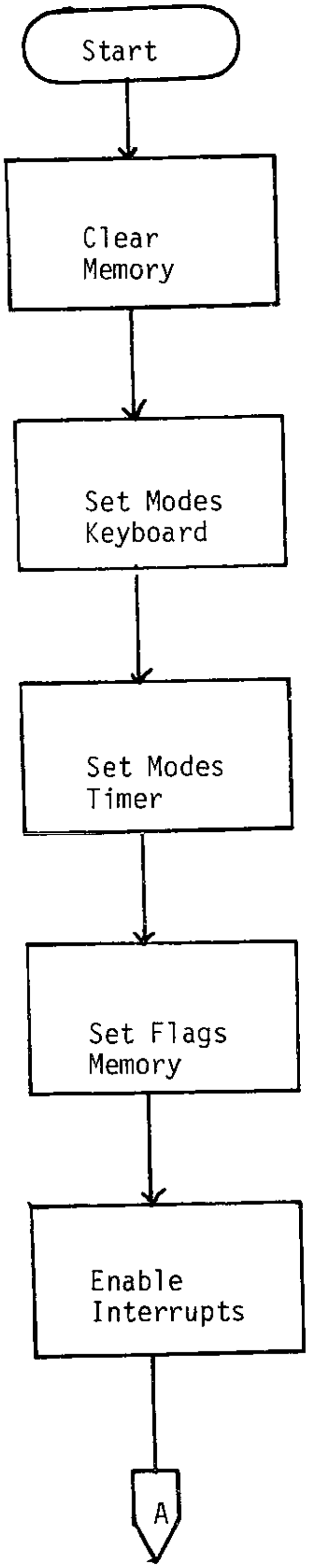
FLOW DIAGRAMS OF TECHNICAL APPROACH

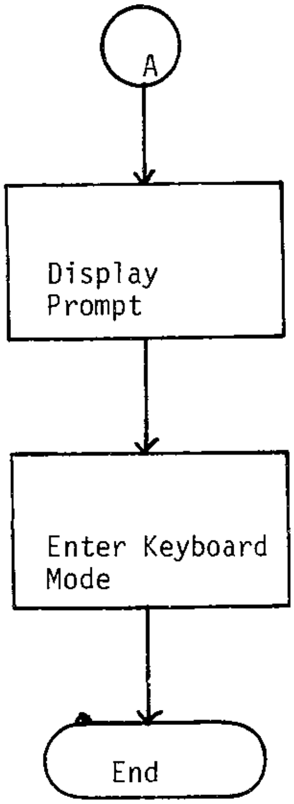
OVERALL SEQUENCING of UNIT



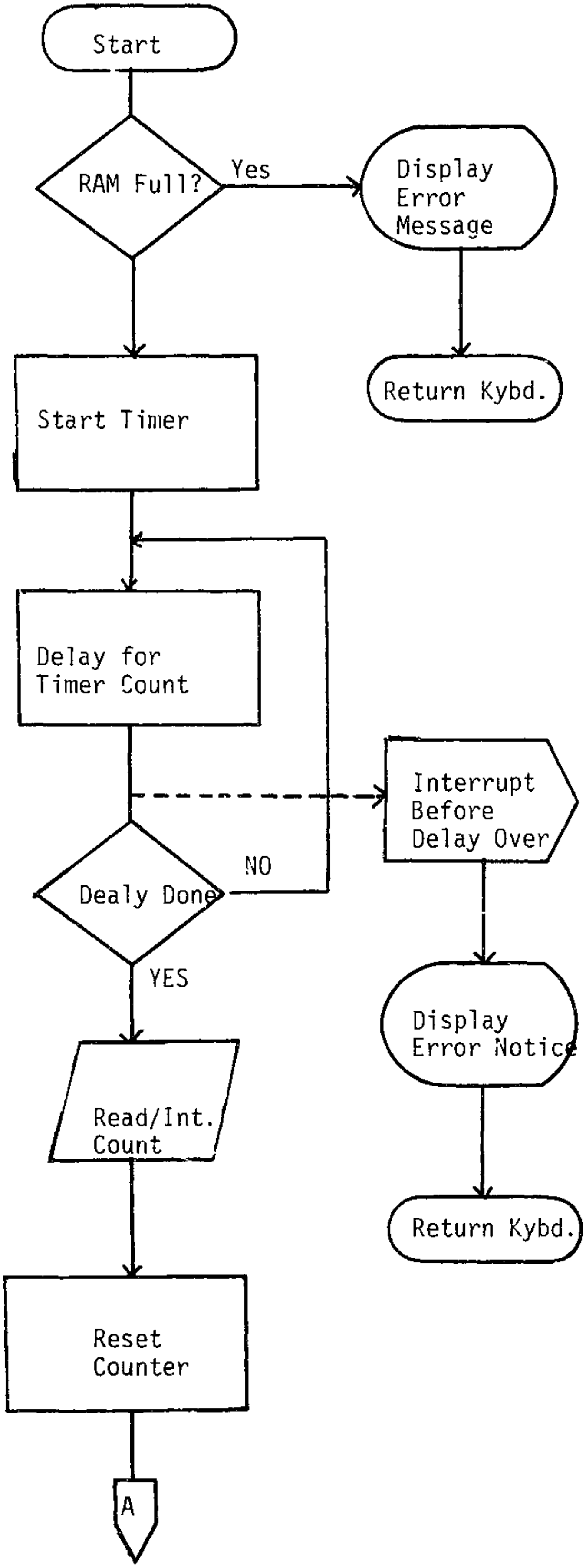

A
Error in Command
YES
Display Error Message
NO
Display Result
B

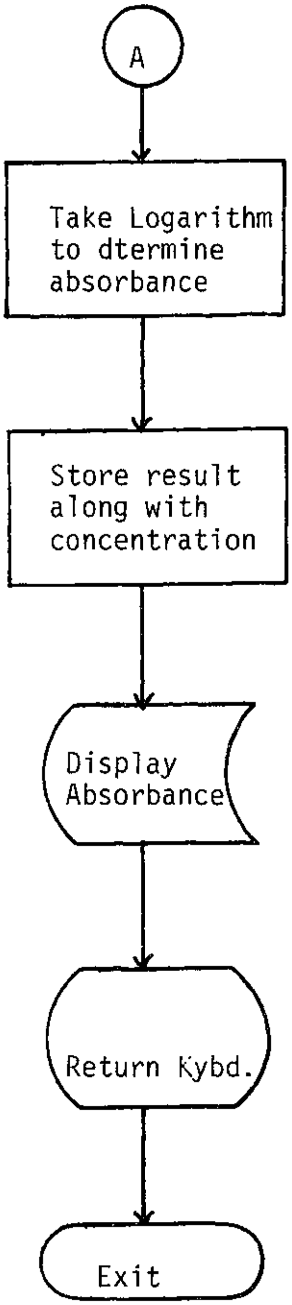
INITIALIZATION



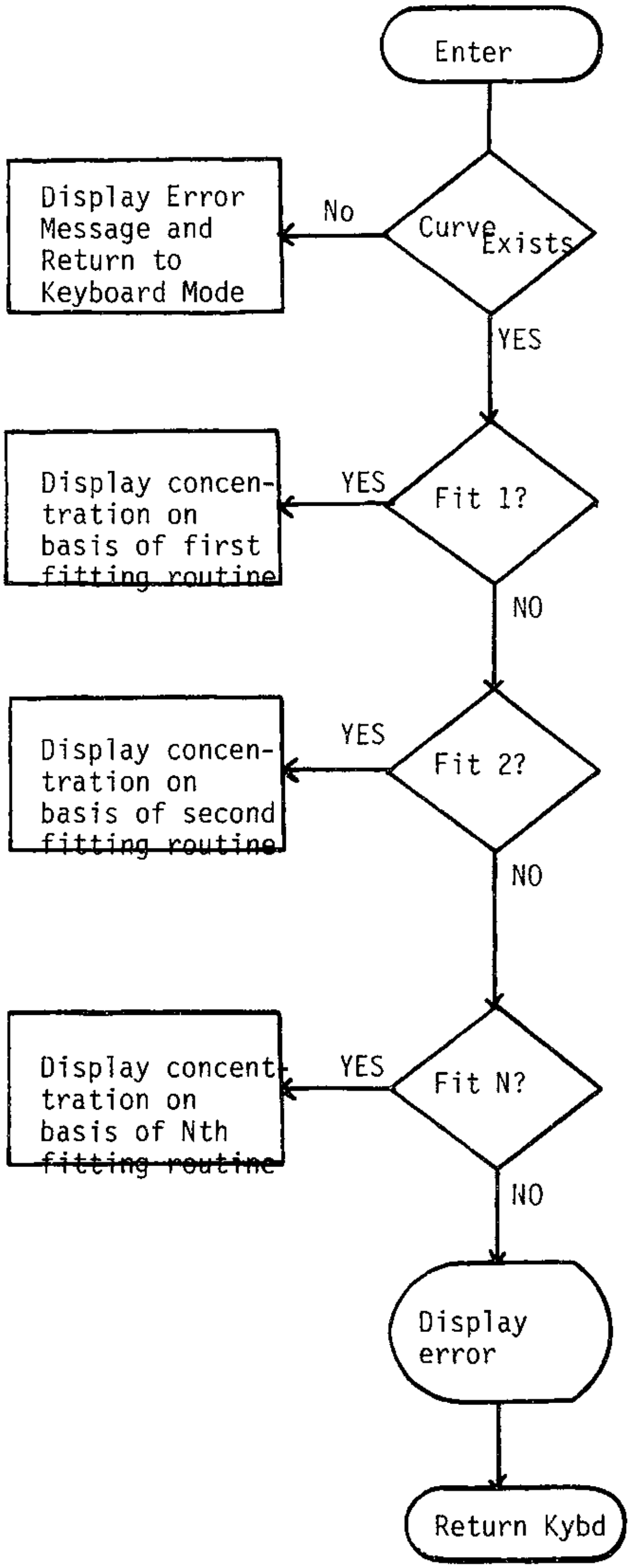

A
Display Prompt
Enter Keyboard Mode
End

Processing Standard




A
Take Logarithm to dtermine absorbance
Store result along with concentration
Display Absorbance
Return Kybd.
Exit

Processing
Unknowns



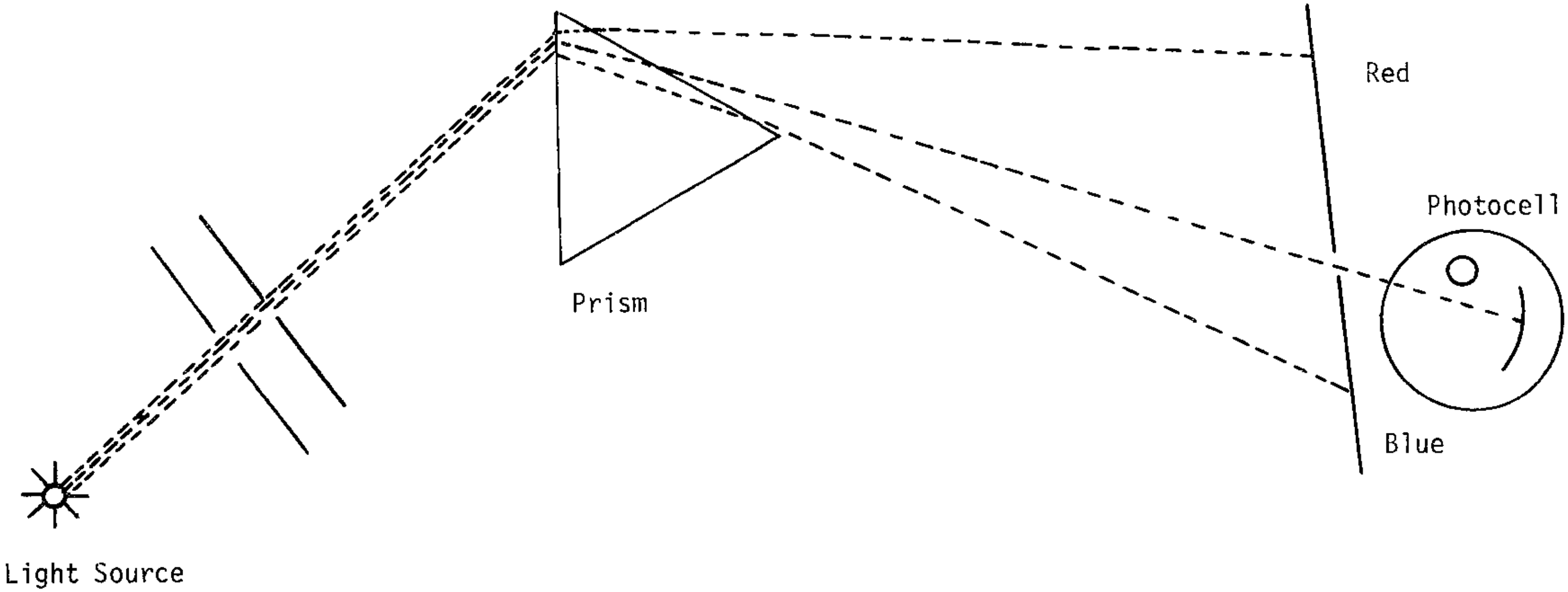


Figure 1. Dispersion of Light by a Prism

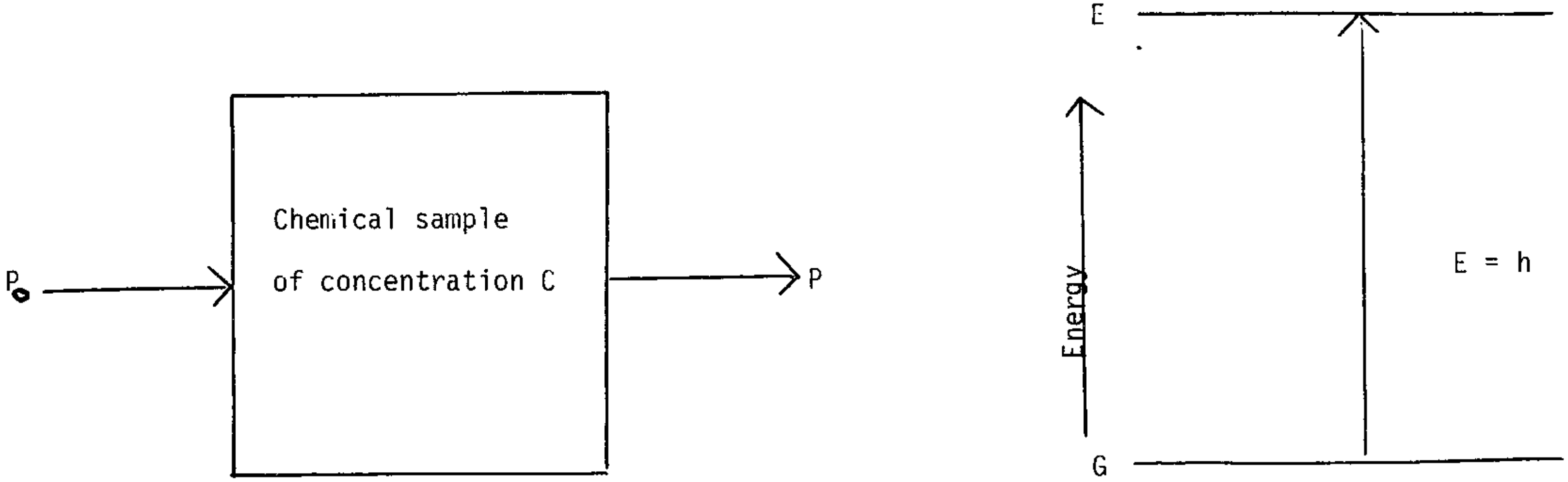


Figure 2. Absorption of Radiant Power

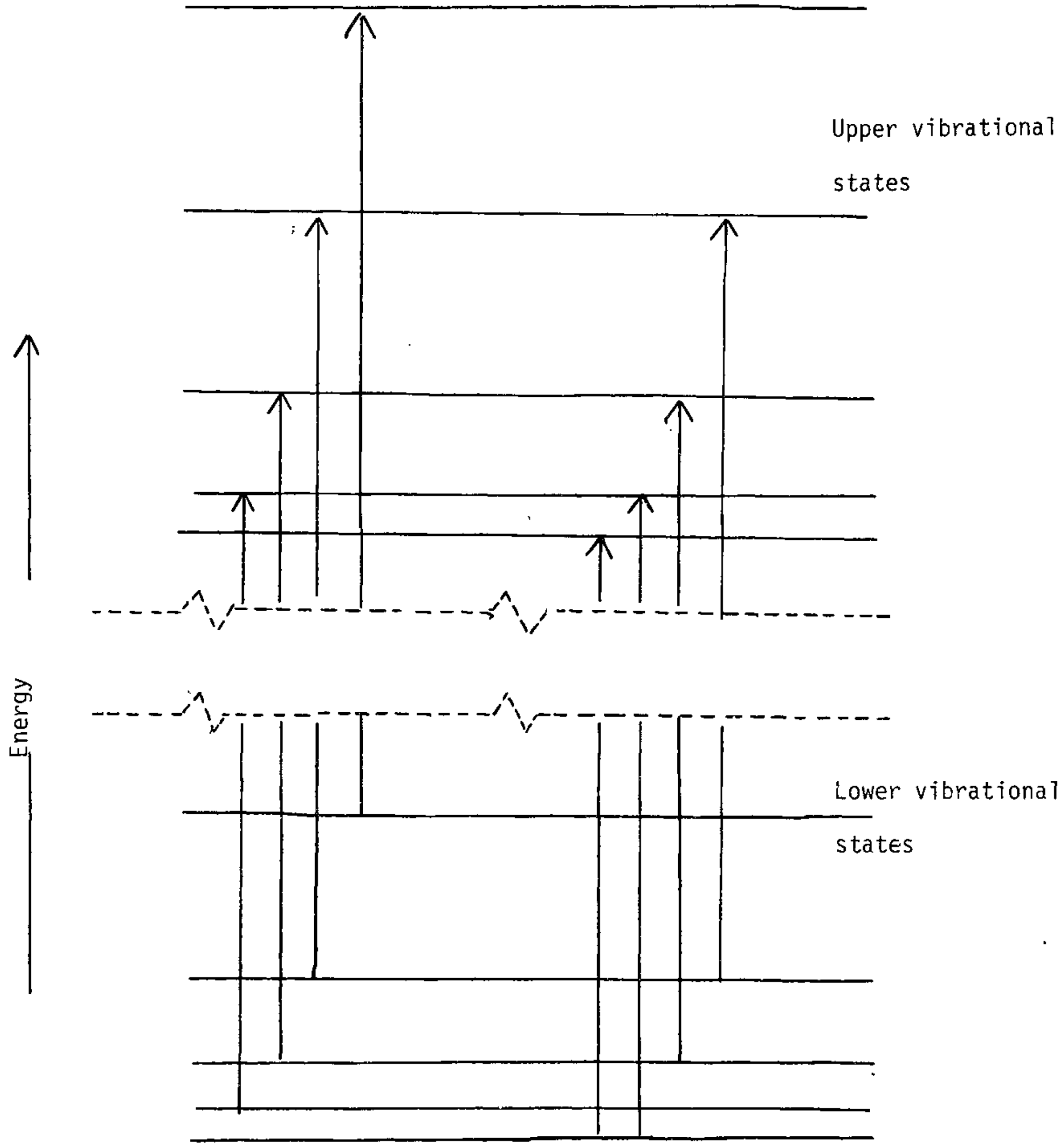


Figure3.Representation of Vibrational-Rotational Energy Levels

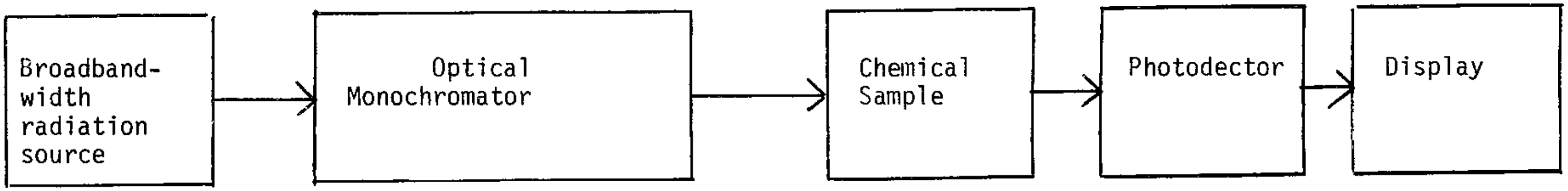


Figure 4. Block Diagram of Basic Absorption Spectrophotomater

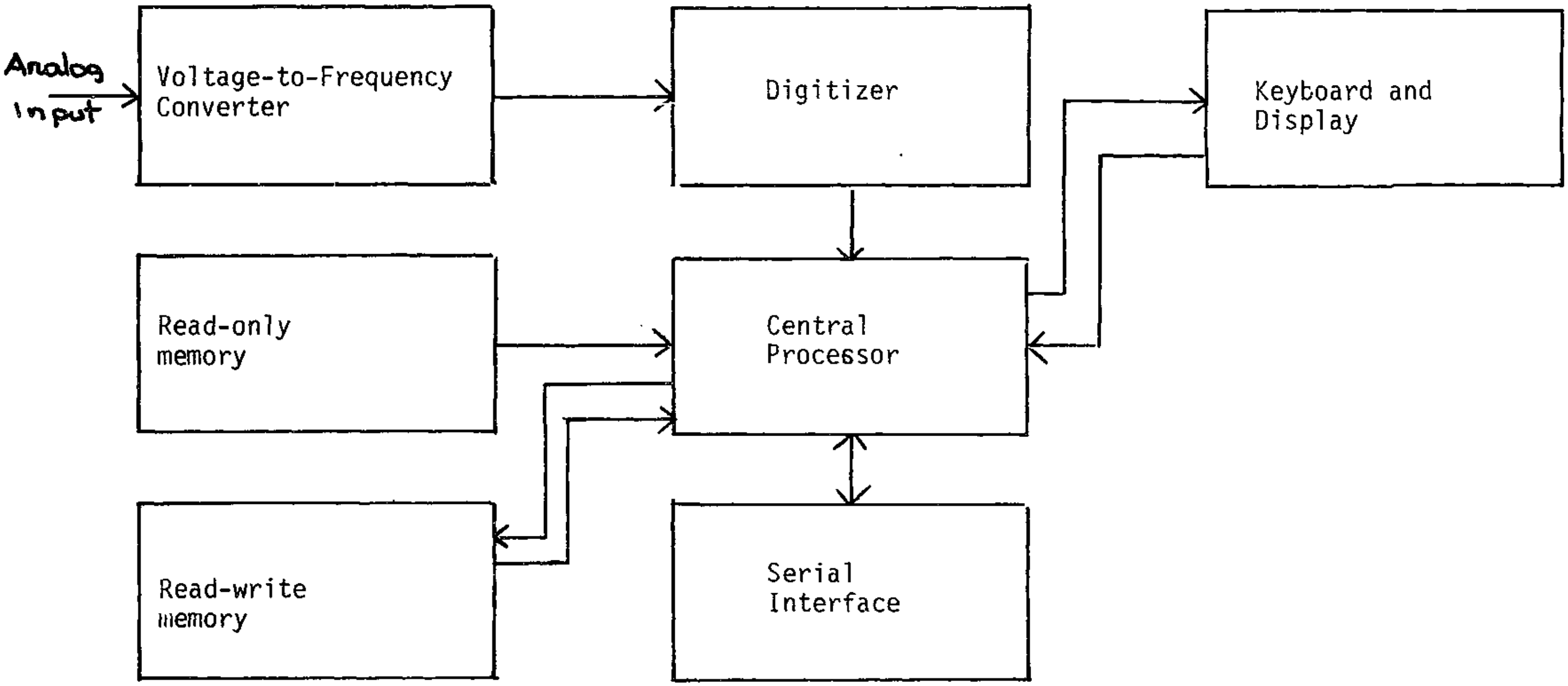


Figure ~~4~~5. Basic Diagram of Hardware Prototype

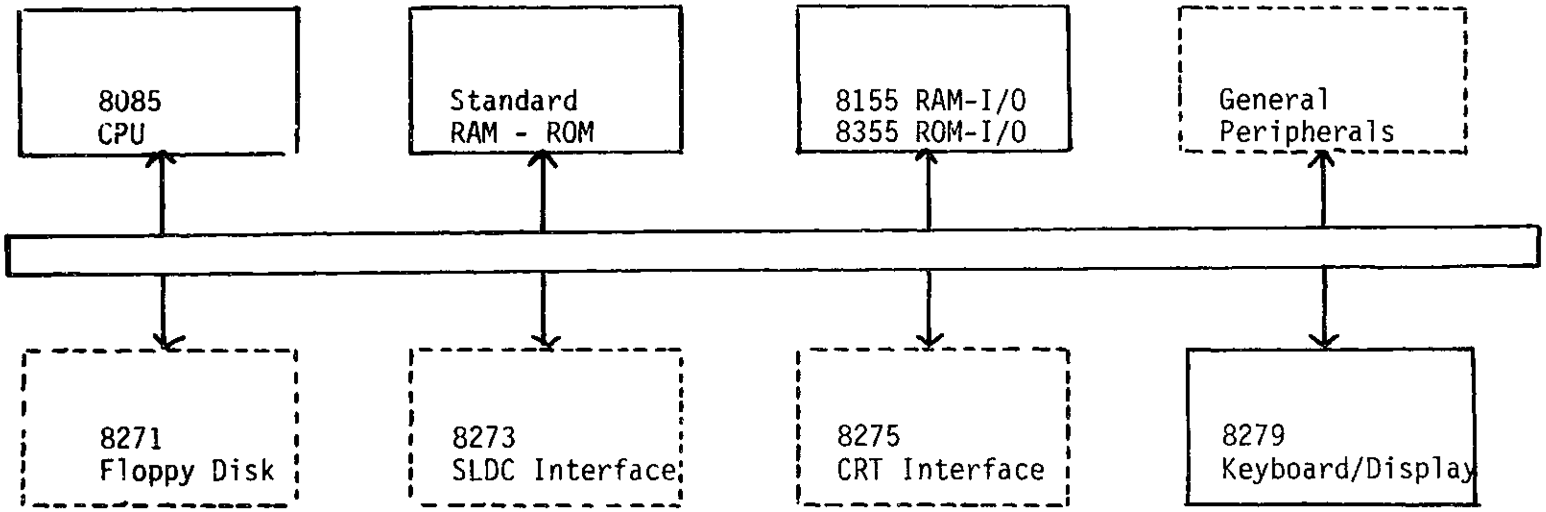


Figure 6. Basic MCS-*85 System of Components

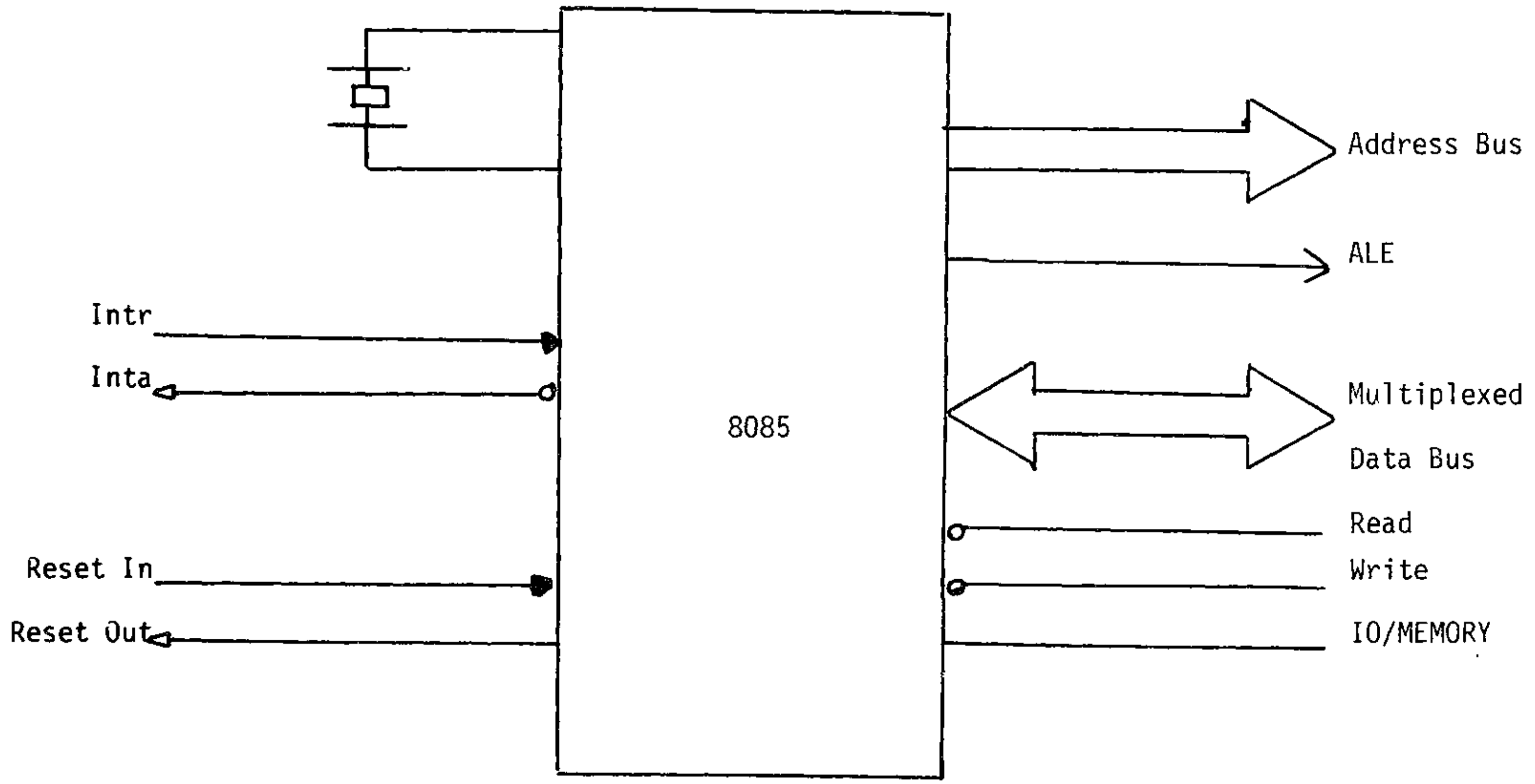


Figure 7. Block Diagram of 8085

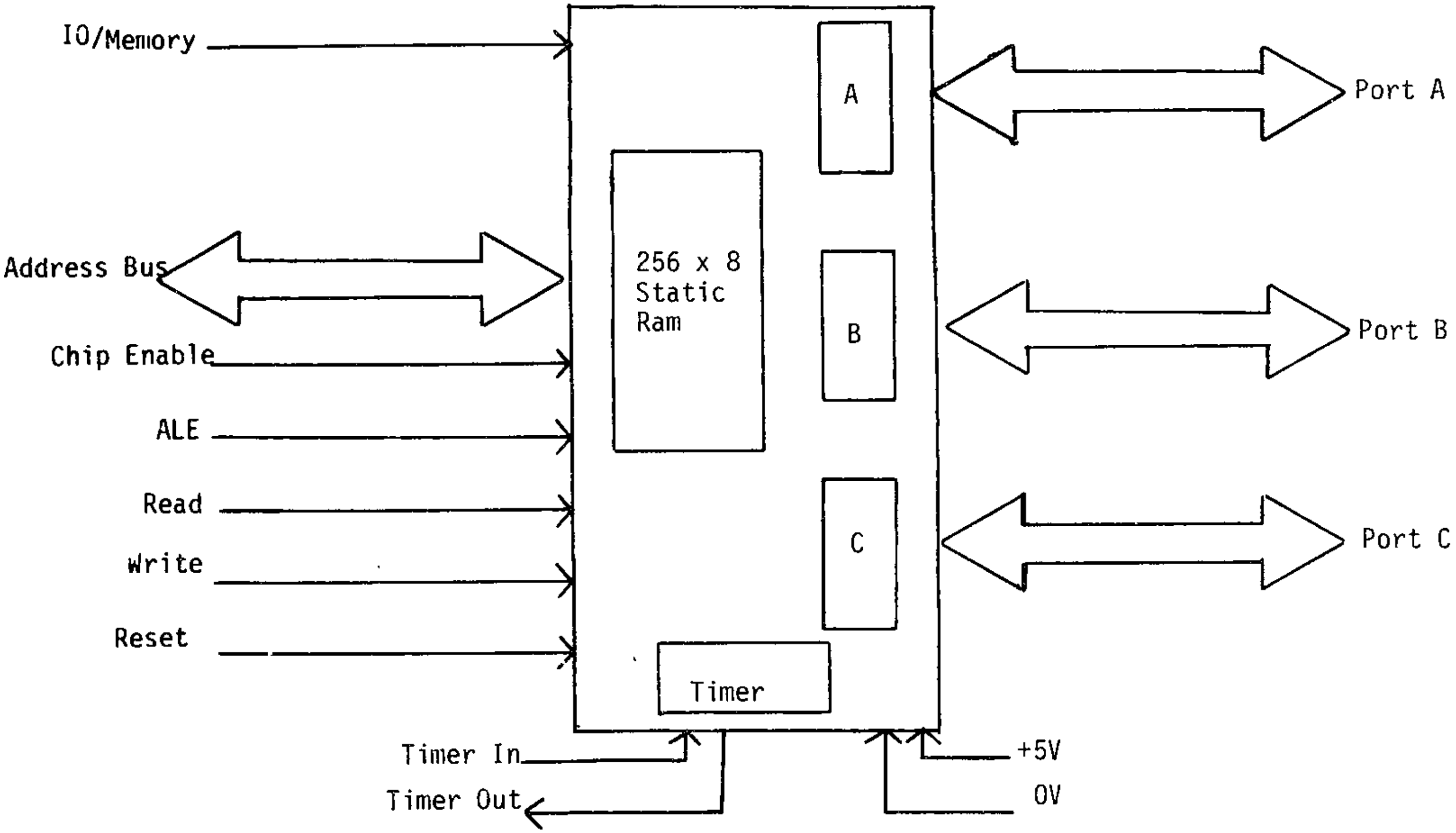


Figure 8. Block Diagram of 8155/8156

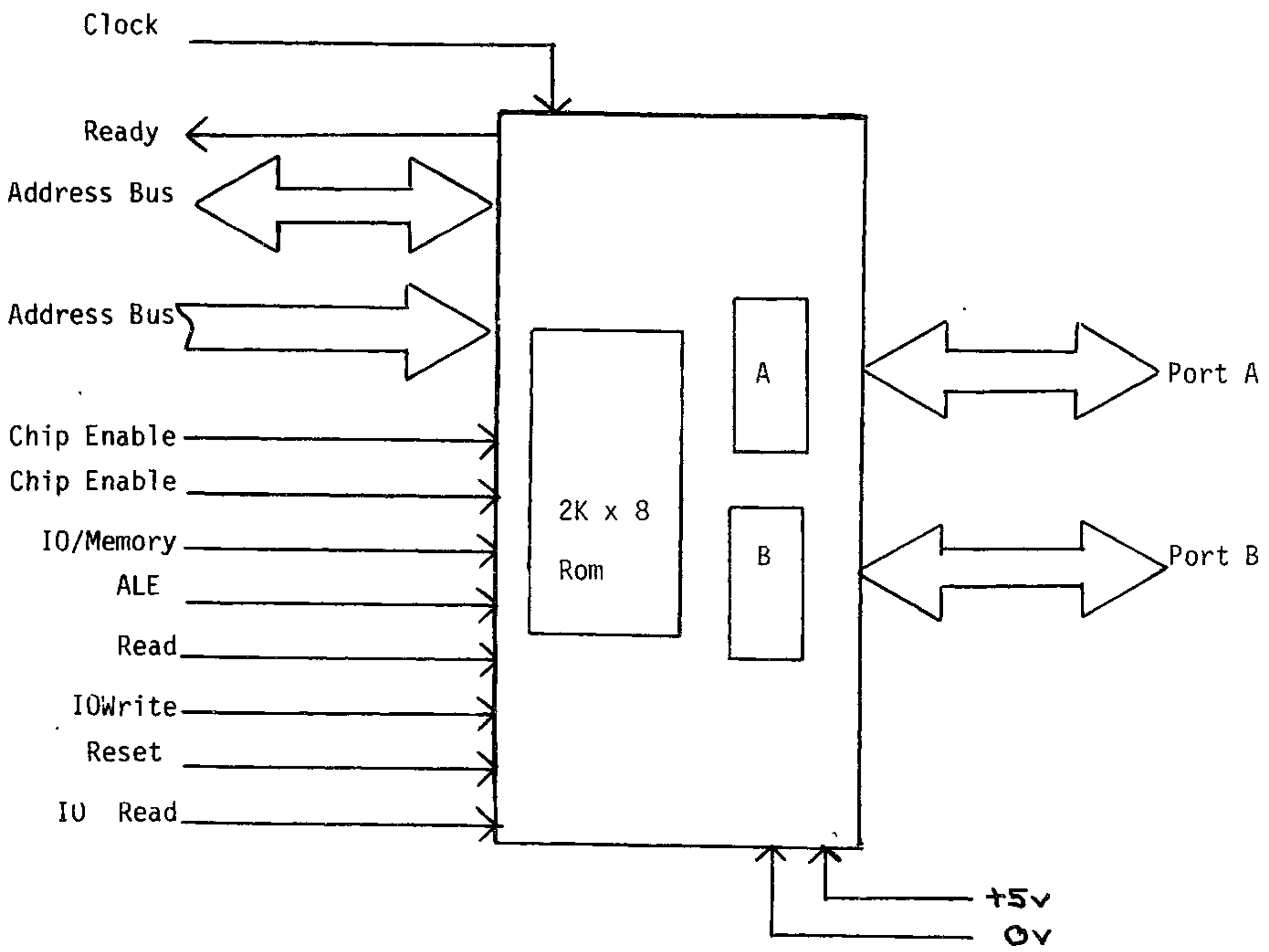


Figure 9. Block Diagram of 8355

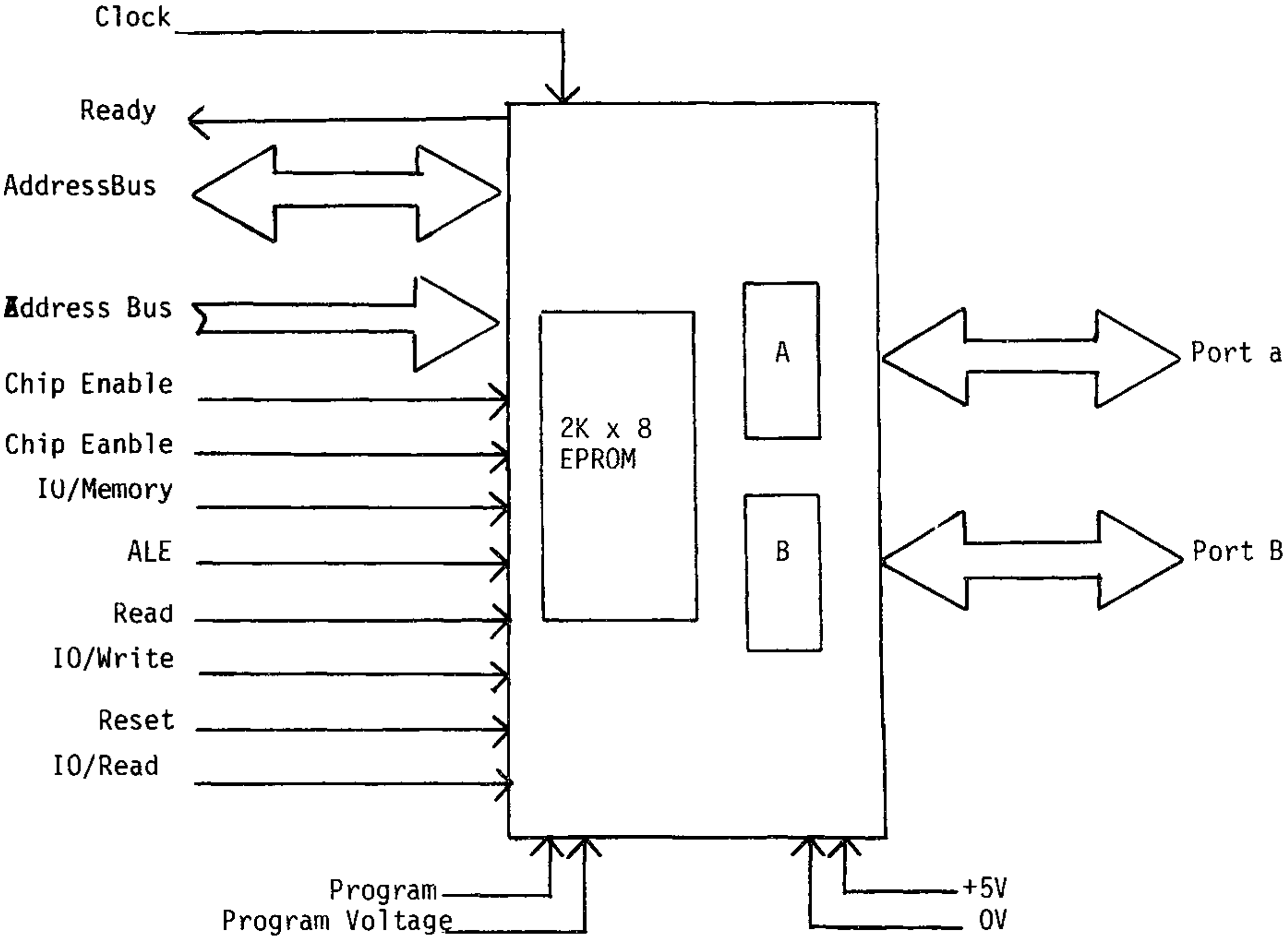
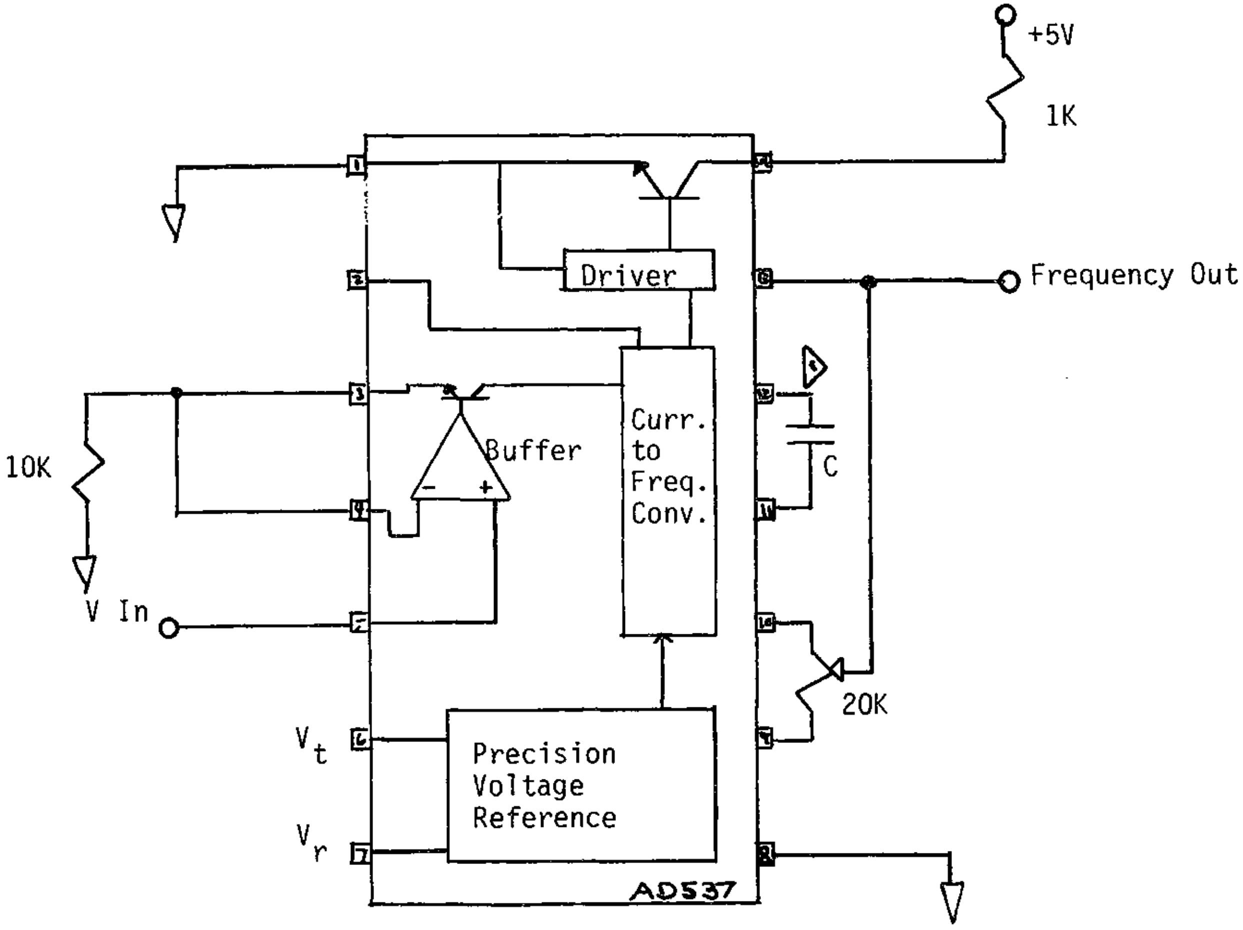


Figure 10. Block Diagram of 8755



[1] Polystyrene
.0001μf

Figure 11. Voltage-to-Frequency Voltage Realization

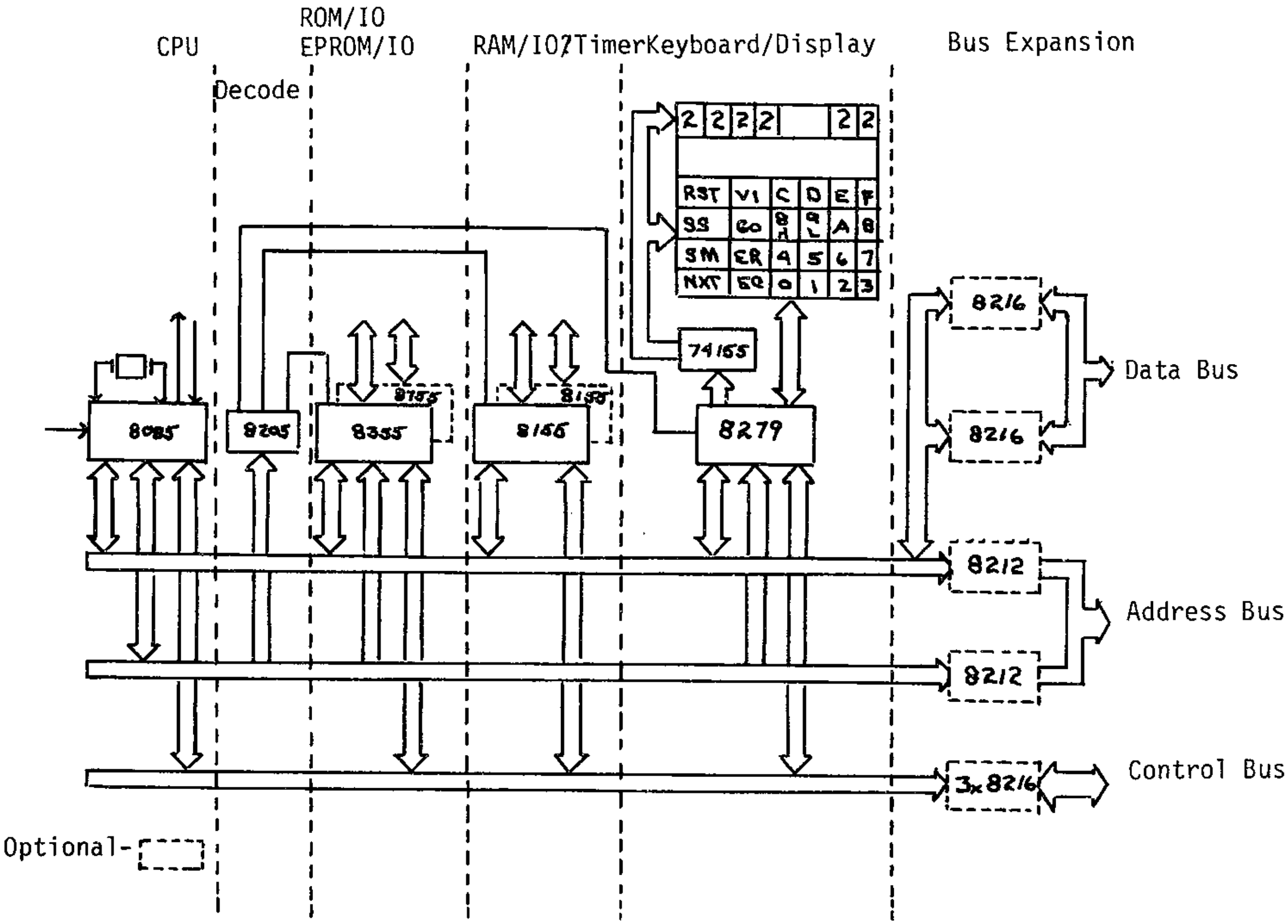


Figure 12. Block Diagram of SDK-85

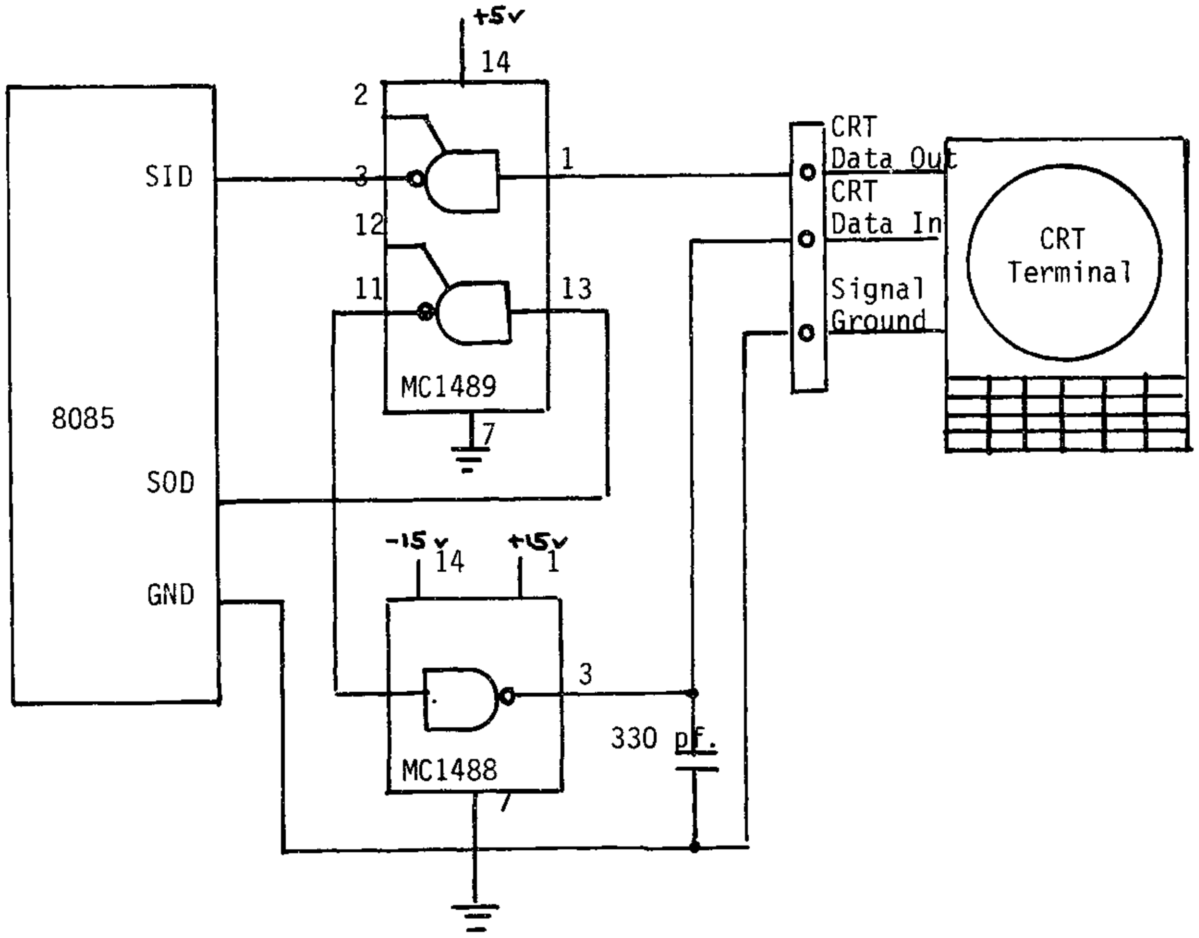


Figure 13. RS232C Interface Circuit

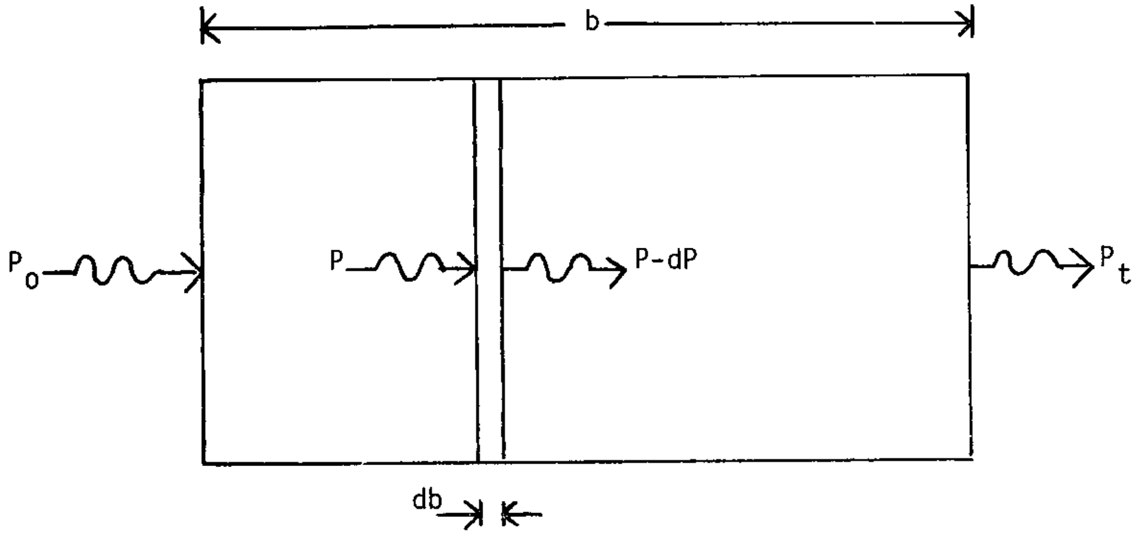


Figure 14. Absorption and Beer-Lambert Law