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RESEARCH AND DEVELOPMENT OF AN INSTRUMENT FOR DETECTION OF EXTRATERRESTRIAL LIFE BY OPTICAL ROTATORY DISPERSION

FOURTH QUARTERLY REPORT

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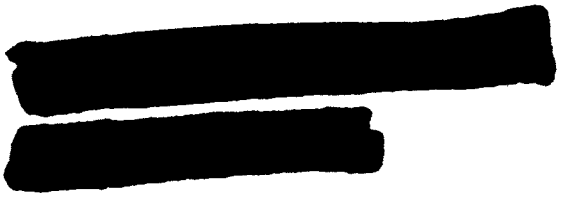

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1. INTRODUCTION

This document is submitted as the Fourth Quarterly Status Report on the NASA Contract NASw-842. The purpose of this contract is to provide NASA with a research effort, the anticipated outcome of which is the development of an instrument for detection of extraterrestrial life by means of optical rotatory dispersion.

For the sake of convenience, the statement of work is reproduced below. The work during the period covered by this report has been devoted to items A and B. This is in keeping with the schedule set forth in paragraph 4 (Future Work) detailed in the Third Quarterly Report.

2. STATEMENT OF WORK

A. Design, fabricate, and test a breadboard model of an instrument for the detection of extraterrestrial life by means of optical rotatory dispersion.

B. Upon completion of the breadboard phase, and after tests have been conducted to prove design feasibility, prototype units will be designed.

C. Upon completion of the prototype designs, two models will be constructed for proof testing.

D. Deliver one flight prototype instrument that has been fully tested.

3. WORK PERFORMED DURING FOURTH QUARTER (November 25,1964 - February 24, 1965)

A. Debugging of the optical electronic breadboard has been completed.

B. Design of the mechanical and processing portions of the breadboard has been initiated.

C. Functional analysis and design of the complete system for automatic operation has been initiated and is approaching completion.

D. The spectral bandwidth of the radiation used to illuminate the sample has been investigated. In terms of the nucleic acids the requirements are very severe at 254 mμ, but are considerably relaxed in the neighborhood of 365 mμ. Operation at 365 mμ entails little or no loss in sensitivity and eases, or eliminates, many of the instrumentation problems. Its effectiveness for the extracts of soil samples remains to be evaluated.

E. An investigation has been initiated for determining the optimum conditions for extraction of optically active products from soil samples. One of the techniques employed in this investigation utilizes radioactive tracers to determine the efficiency of extraction. This has, thus far, been applied only to nucleic acids. The work is still in its early stages, but nucleic acids have been successfully extracted from soil samples. The efficiency of the process is being checked by means of the radioactive tracer technique. Efficiencies attained to date are not considered optimum and work is continuing to improve the extraction process. The restrictions imposed by the mission for which this instrumentation is intended are continually kept in mind in choosing or modifying extraction procedures. Consideration is also being given to organic constituents of soil other than nucleic acids.

Details of the work performed will be found in Section 5 (Discussion).

4. FUTURE WORK (FIFTH QUARTER)

The principal effort during the next report period will be concerned with the construction and evaluation of an advanced breadboard system adequate for critically evaluating the scientific and technical aspects of optical rotatory dispersion as one means for investigating the presence of

extraterrestrial life past or present. The paucity of opportunity and the expense and labor involved in an extraterrestrial mission for conducting a biological investigation emphatically require that the probability of successful operation of any experiment sent on such a mission be as high as possible. This has been, and continues to be, the guiding principle in the development of an instrument for detection of extraterrestrial life by optical rotatory dispersion. In terms of this principle both the scientific and technical aspects of the experiment may be considered of equal importance. However, the extent to which the technical effort supports the goals of this experiment, no matter how good the effort, is limited, in the final analysis, by the scientific understanding of the phenomena to be detected. Further, it may be considered important to limit or expand the amount of information to be obtained from this experiment. This decision can obviously best be made on the basis of the fullest possible understanding of the phenomena of interest vis-a-vis the other factors which pertain. Even though we are limited to terrestrial biology and our best decision-making efforts result in guesses, it is nevertheless true that the most educated guess possible is required. For these reasons, the future work on this project will concern itself with those aspects listed below.

A. Complete the design and construction of an advanced breadboard instrument; this breadboard to be used to evaluate the technical aspects and the operational strategy which has been chosen.

B. Continue the investigation of soil extraction procedures.

C. Investigate and define the constituents of soil extracts with particular attention being paid to the optically active components.

D. Insofar as possible, incorporate new insights and new information into the design and construction of the advanced breadboard.

E. Upon completion of construction of the advanced breadboard, evaluate it with respect to its scientific capability and operational strategy.

F. Until the advanced breadboard is completed and operational, utilize the present breadboard for determinations of optical activity.

G. Make recommendations with regard to the scientific capabilities and functional behavior to be expected from the next generation of this kind of instrument; these recommendations to be based upon the results obtained with the present and the advanced breadboards, as well as the investigation of the soil extraction procedures and the contents of soil extracts.

5. DISCUSSION

5.1 Introduction

The device being developed on this project for detecting extra-terrestrial life by optical rotatory dispersion in an exobiological environment is based upon novel instrumentation principles for detecting and measuring this phenomenon. Theoretical analysis has shown that these principles are sound and experimental data has confirmed this result. Nevertheless, in keeping with the principle enunciated in paragraph 4, namely, providing the highest possible probability of success, the instrumental development program has been kept continually sensitive to the results of experiment and the acquisition of new knowledge. This freedom to change can obviously not be allowed to endure indefinitely. Indeed, as shown in paragraph 4, it will be drastically curtailed and finally eliminated during the next report period.

5.2 Summary of Work Performed

The work performed on the various aspects of this project during this report period is summarized in this section. More detailed exposition appears in the succeeding sections. The calculations for the hydraulic system are given in the appendix.

5.2.1 Extraction Process

Of basic importance to the scientific aspects of this experiment and to the detailed design of the instrument is the question of the nature of the sample which will be presented, and the functional characteristics of the instrument required to detect and measure this sample. Since this experiment is to be performed in an exobiological environment, it is obvious that any specification as to the nature of the sample it will operate on is an assumption. Nevertheless, the design, development and evaluation of an instrument requires the introduction of samples which are known both quantitatively and qualitatively. In this regard, it has become apparent, in the course of using the existing breadboard, that soil samples can not well be compared with each other, even if taken from the same locale when only simple extraction procedures are utilized and no attempt is made at chemical analysis. Thus an effort was initiated to investigate the extraction process and better characterize the nature of the extract.

5.2.2 Electro-Optical

When nucleic acid solutions were introduced into the breadboard instrument for measurement, it was found that operation at 254 mμ, with the spectral band defined by optical filters, gave poor or negative results. However, operation at 360 mμ gave results which agreed exactly with those reported in the literature. This behavior, in the neighborhood of 254 mμ,

is due to the rapid change of optical rotation with wavelength (this is in the Cotton region) coupled with the relatively broad spectral bandwidth of optical filters and the shape of the absorption spectrum. In contrast, in the neighborhood of 365 mμ the optical rotatory dispersion of nucleic acids is a slowly varying function, is reduced by only a small factor, and the optical density approaches zero. For a given optical pathlength, light source, and phototube sensitivity the greatly reduced optical density means that the concentration of solute may be much greater when measurements are made in the neighborhood of 365 mμ than when they are made in the neighborhood of 254 mμ. This situation makes it entirely possible that operation in the neighborhood of 365 mμ may result in equal or enhanced sensitivity when compared with operation in the neighborhood of 254 mμ (larger soil samples or more complete extraction possible). (Alkaline extracts of soil samples have also been observed to exhibit much greater optical density in the neighborhood of 254 mμ than in the neighborhood of 360 mμ. On the basis of this analysis, it was decided to utilize the 360 mμ mercury line for instrumental operation rather than the 254 mμ mercury line.

5.2.3 Hydraulic System/Mechanical System

In making the soil extracts it was noted that any filters utilized very quickly clogged with sediment from the soil. This would obviously severely limit the amount of extract that could be obtained and also increase the power burden due to the filtration by necessitating higher differential pressures and long filtration times. Provision for keeping the filter surface free of sediment and simultaneously providing strong agitation of the reaction mixture is being incorporated into the design to minimize or eliminate this difficulty.

Detailed attention was also given to the variable pathlength cell and to an alternate method of achieving the same goal which consists of diluting the filtrate by approximately the same factor as would be true for the change in pathlength of the variable pathlength cell. The clogging of the filter upon filtration of the soil extract-extracting fluid mixture necessitates the use of a positive mixer to keep the surface of the filter clear of sediment insofar as possible. The presence of this mixer makes possible the use of additional solvent as diluent to perform the same function as would have been performed by the variable pathlength cell. Dilution is considered preferable to changing the pathlength of the sample cell and the design of the advanced breadboard presently being carried forward is incorporating a mixer and mixing chamber and provision for diluting the mixture.

5.2.4 Electronics

An operational strategy has been chosen. Detailed design is essentially completed for automating the instrument in accordance with this strategy. Included are the electronic, electromechanical, and logic functions.

6. DETAILED DISCUSSION

6.1 Extraction of Soil Samples

6.1.1 Introduction

Ideally, the extraction procedure for soil samples should yield only products which have optical rotatory dispersions which are additive in the region of the spectrum where the measurement is made. Interfering substances which detract from the amplitude of optical rotation should be discriminated against. Choosing an extraction procedure for the instrument must, therefore, be based upon detailed knowledge of the optical rotatory

dispersion behavior of substances, or their degradation products, which are to be found in soil. As opposed to large molecules such as the nucleic acids relatively small molecules when found in soil may provide some clues tothe parent molecules. This knowledge will be useful not only for choosing an extraction procedure and a spectral region within which to operate, but may also permit refinement of the estimate of the kinds of substances, in terms of their optical activity, one might expect to find in Martian soil. Thus the overall objective of this effort is to characterize chemically and in terms of optical rotatory dispersion the constituents of soil extracts. Corollary to this is the need for knowing, qualitatively and quantitatively, the chemical and optical history of known substances after they have been introduced into soil samples and have been treated in a manner designed to simulate the natural processes.

Since nucleic acids are ubiquitous in terrestrial biology and since they are also readily available in pure form, they have been chosen for the initial studies. It should be noted that assessment of the adequacy of the percent yield (obtained with radioactive tracer techniques) depends upon the specific rotation of the substance in question. Thus a substance with relatively low yield but high specific rotation may be a more sensitive index than one high yield, but low specific rotation. Such assessment and comparison coupled with an estimate of probability of occurrence is the ultimate goal of this effort. The specific objective for the work performed during this reporting period is given below.

6.1.2 Work Performed

6.1.2.1 Specific Objective and Rationale

The primary objective of the current experiments is to investigate non-destructive extraction procedures for nucleic acids present in soils -- procedures which will solubilize both undegraded ribonucleic acids (RNA) and undegraded deoxyribonucleic acids (DNA) in good yield, but which will solubilize only a minimum quantity of interfering compounds such as polypeptides and proteins. The need for non-destructive procedures is based on the fact that although the optical rotatory dispersion curves of RNA and DNA are qualitatively the same, the constituent nucleotides not only exhibit considerably less optical rotation than do the intact nucleic acids, but different nucleotides rotate light of a given wavelength in opposite directions Mixtures of nucleotides, therefore, or of nucleotides and nucleic acids, might appear to contain no optically active molecular species. Similarly, proteins and polypeptides are levorotatory at wavelengths at which nucleic acids are dextrorotatory. (See above for discussion of relative importance.)

Many procedures which have been used more or less routinely for the analysis of nucleic acids in bacterial or tissue samples are either specific for DNA or RNA, or cause the complete or partial hydrolysis of nucleic acids to nucleotides and nucleosides. Extraction with aqueous phenol[1] for example, solubilizes RNA without appreciable degradation, but does not dissolve DNA. This solvent system would probably also be unsuitable because of the instability of aqueous phenol and because of the difficulties inherent in the handling of highly corrosive solutions. In the Schmidt-Thannhauser[2] method, in which the material to be extracted is treated with 1 N alkali at 37°C for 16-20 hours, hydrolysis of RNA to its constituent nucleotides

is almost complete, whereas DNA is not greatly degraded. Trichloroacetic and perchloric acids[3,4] which coagulate proteins have also been used, but both of these acids tend to degrade RNA and DNA. Milder procedures, such as extraction with hot 10% NaCl or with citrate-EDTA (tetraethylenediamine acetate)[5] have been used successfully for the extraction of intact nucleic acids from microorganisms, but will also extract proteins and peptides. Because of the requirements for sterilization of all reagents employed, it would be preferable if a simple inorganic salt (or non-reactive mixture of inorganic salts) could be used.

Since the evaluation of extraction and purification procedures is frequently facilitated by the use of radioactive tracers, as well as by the determination of optical absorption curves, both of these procedures have been employed in the current studies.

6.1.2.2 Experimental

Material and Equipment

The soil used throughout this set of experiments was obtained in the vicinity of Melpar's Falls Church plant.

The tritium-labeled ribonucleic acid used as a tracer had been prepared from E. coli. A uracil-requiring mutant of E. coli (ATCC No. 9723-g) was grown under standard conditions in a medium containing uracil-H^3. After the washed cells were lysed with sodium lauryl sulfate, the RNA was extracted with aqueous phenol and subjected to purification on Sephadex G-25. The final solution contained 735 γ RNA/ml, with an activity of 2.01×10^6 dpm/ml, equivalent to 2,750 dpm/γ RNA.

All buffers were prepared from reagent grade, commercially obtained, salts.

Spectra were obtained with a Bausch and Lomb 505 recording spectrophotometer.

Radioactivities were determined in a fully automatic Packard Tri-Carb Liquid Scintillation Spectrometer, using Bray's counting solution, with a counting efficiency of 13.5%.

Extraction Procedures

a. Air-dried soil samples (5.0 g each) were agitated for 1 minute with 10.0 ml buffer solution and centrifuged for 10 minutes (desk top centrifuge, approximately 3,000 rpm). One series of soil samples was left in contact with buffer for 16 hours before centrifugation. The clear supernatant solutions were withdrawn and undiluted, as well as serially diluted, aliquots were used to determine absorption spectra in the near ultraviolet range (220 mμ to 350 mμ).

b. Tritium-labeled ribonucleic acid (0.1 ml solution containing 73 RNA) was added to 1.0 g soil samples. After thorough mixing, these soil samples were left to air-dry at room temperature. Each dried sample was agitated with 2.0 ml buffer solution for 1 minute and centrifuged for 10 minutes. Aliquots of the supernatant solutions, withdrawn immediately after centrifuging, were used to determine radioactivity and optical absorption in the near ultraviolet.

Results and Discussion

Typical absorption spectra obtained with both phosphate and borate buffer extracts, and with sodium hydroxide extracts, of untreated soil samples and of RNA-treated soil samples are shown in figures 1, 2, and 3. All of the extracts absorbed strongly between 220 mμ and 300 mμ, but no peaks assignable to nucleic acids were found. A possible exception was the pH 9.0 borate extract

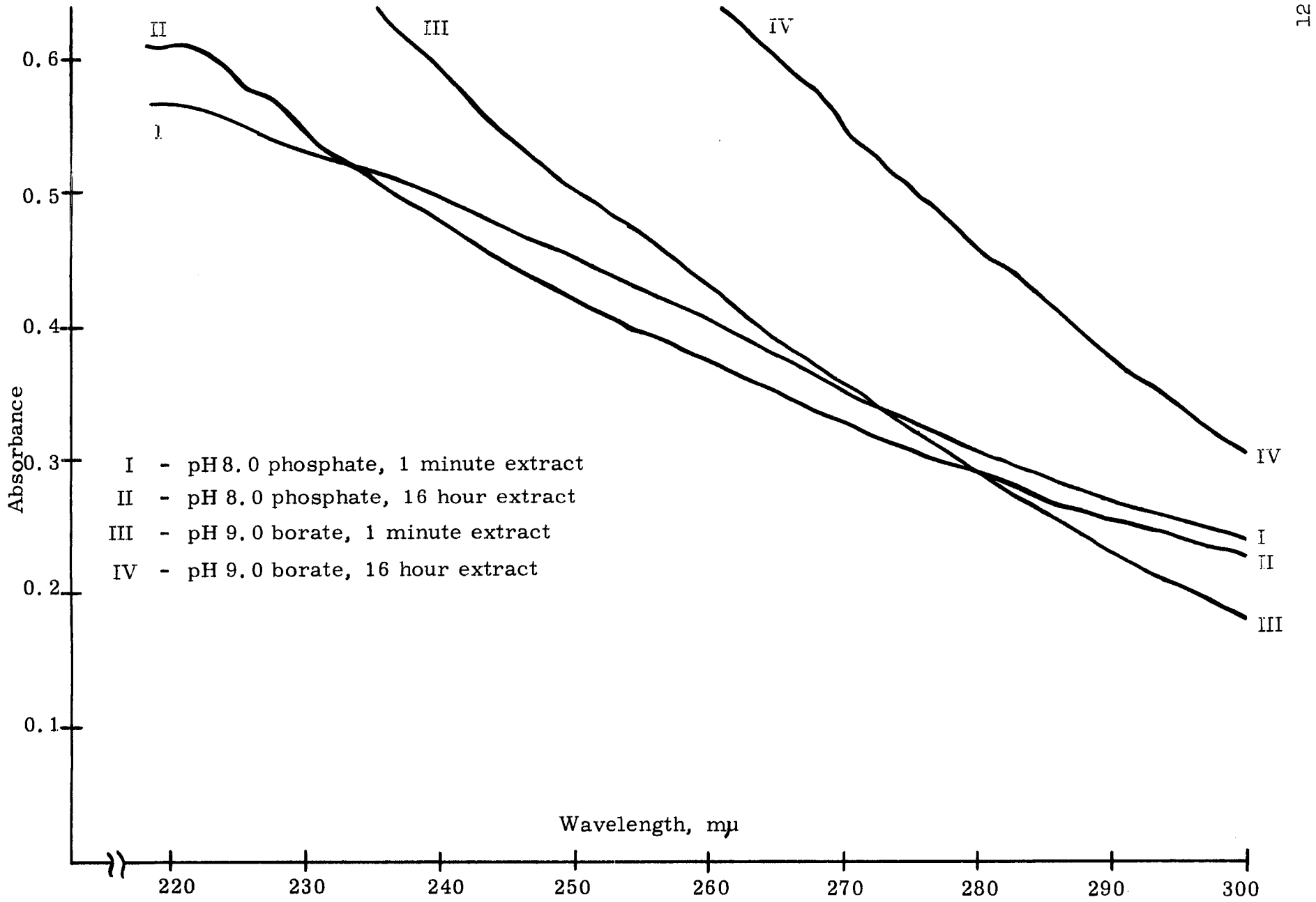


Figure 1. Absorption Spectra of Extracts of Untreated Soils

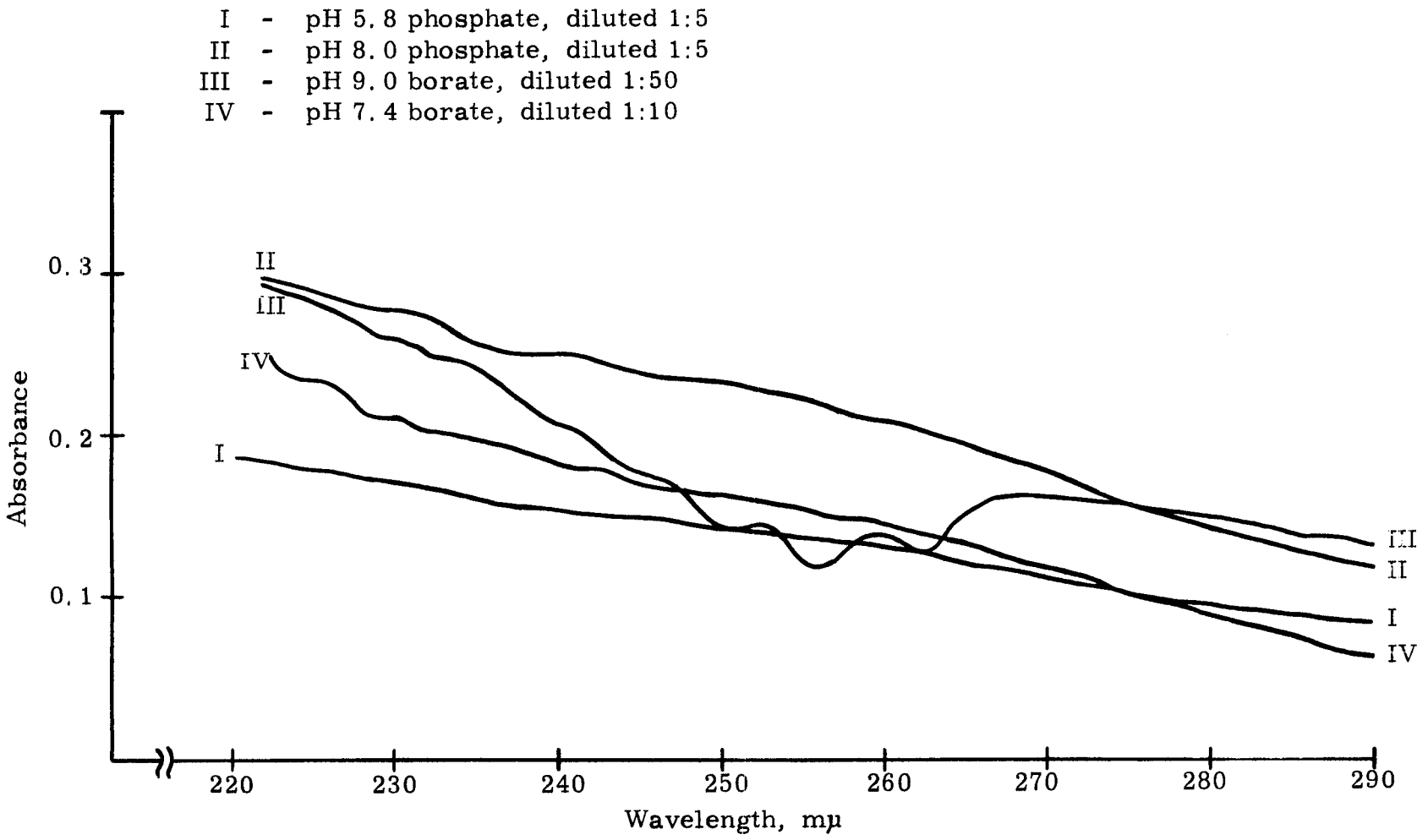


Figure 2. Absorption Spectra of Extracts of RNA-Treated Soils

I - 0.05N NaOH
II - 0.10N NaOH
III - 0.15N NaOH

} 1 gram soil + 2.0 ml solution; extracts diluted 1:500

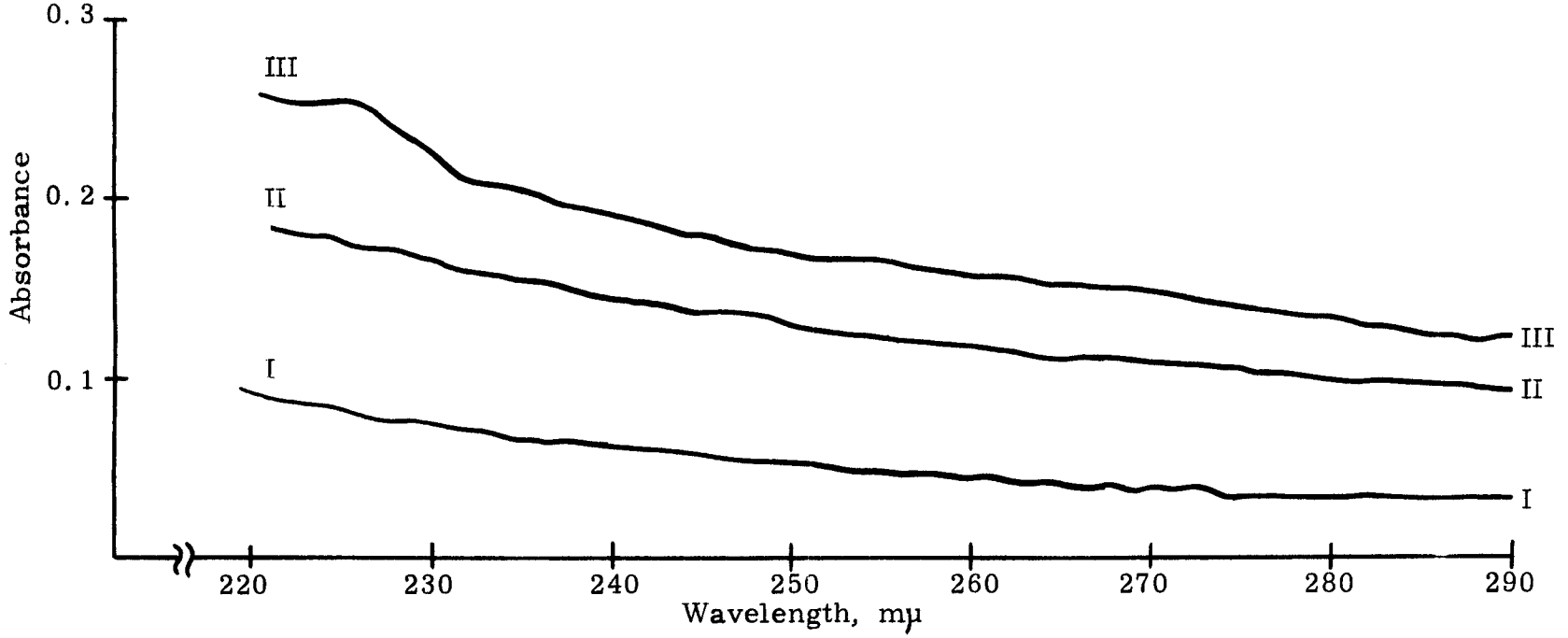


Figure 3. Absorption Spectra of Alkaline Extracts of Untreated Soils

of a soil sample to which RNA had been added (fig. 2).

Recovery data for tritium-labeled RNA added to dry soils are shown in table I. With both phosphate and borate buffers, extraction was more efficient at higher than at lower pH values, although none of the four conditions used gave high efficiency. Ribonucleic acid appears to be firmly held by the powdered soil sample.

The tracer results are in fair agreement with the spectral data; in both sets of experiments the pH 9.0 borate buffer was the best of the systems investigated. The spectral data also indicate that absorption curves on unpurified extracts are not a sensitive index of extraction efficiency, probably because of the complex mixtures of ultraviolet absorbing compounds present in soils.

Improved recoveries of added nucleic acids, as well as of nucleic acids present in soils, might be obtained by increasing solution to soil ratios, increasing extraction temperatures, or by the addition of chelating or surface active agents. Filtering, under either suction or pressure, of the soil-buffer suspensions, or recycling of the solvent, might well be a marked improvement over centrifugation.

6.2 Electro-Optical

One of the problems which have been encountered in the use of the existing breadboard has been the fact, using simple filters, it has not been possible to obtain optical rotation data at 254 mμ for nucleic acid solutions, whereas at 365 mμ results were obtained in exact agreement with the literature.[6] Investigation of these results leads to the following explanation and also indicates the methods which must be employed to correct this behavior.

TABLE I

EXTRACTION EFFICIENCIES OF PHOSPHATE AND BORATE BUFFERS

Sample	cpm	Counting Efficiency	dpm/0.1 ml Extract	RNA Recovered
		Phosphate Buffers		
5 λ Standard	11,000	13.5%		
0.1 ml pH 5.8 extract + 5 λ standard	10,900	12.6%		
0.1 ml pH 5.8 extract	525		4160	4.1%
0.1 ml pH 8.0 extract + 5 λ standard	8,500	9.3%		
0.1 ml pH 8.0 extract	905		9750	9.7%
		Borate Buffers		
5 λ Standard	12,000	13.5%		
0.1 ml pH 7.4 extract + 5 λ standard	10,700	11.9%		
0.1 ml pH 7.4 extract	218		1830	1.8%
0.1 ml pH 9.0 extract + 5 λ standard	8,700	9.2%		
0.1 ml pH 9.0 extract	555		6040	6.0%

Optical filters are, in general, not capable of isolating very narrow portions of a spectrum with large attenuation of the undesired portions of the spectrum. Interference filters which have a narrow passband do not have sufficient attenuation in the stop band to make them fully useful. The reasons for this are that in the neighborhood of 254 mμ the nucleic acids and also the extracts of soil samples exhibit appreciable optical density. This, in effect, serves to change the characteristics of the optical filter employed in such fashion that the filter's functional behavior approaches that characteristic of neutral density filters. In addition, any other wavelengths transmitted by the optical system through the sample which exhibit optical rotations different from that at 254 mμ will serve to alter the rotation of the plane of polarization at 254 mμ. The observed optical rotation is the vector sum of the rotations of all the wavelengths transmitted by the system. If the transmitted spectral band includes a region of little optical activity or optical activity of reversed sign, and if, in addition, the intensity of the radiation at these undesired wavelengths is appreciable, then the apparent optical activity will be reduced from the value at 254 mμ alone. Indeed, the observed value could go to zero or even change sign.

Figure 4 shows the optical rotatory dispersion of salmon DNA in the ultraviolet region as reported by Samejima and Yang[6]. Note that the spectral region in the neighborhood of 254 mμ, which shows levorotatory optical activity, is rather narrow with a sharp peak and steeply sloping sides. Even if the optical filter passband were limited to this levorotatory optical activity region of the spectrum, energy at wavelengths which experience small amounts of optical activity would add vectorally to the energy at 254 mμ with the result that the sinusoidal signal present in the output of a multiplier

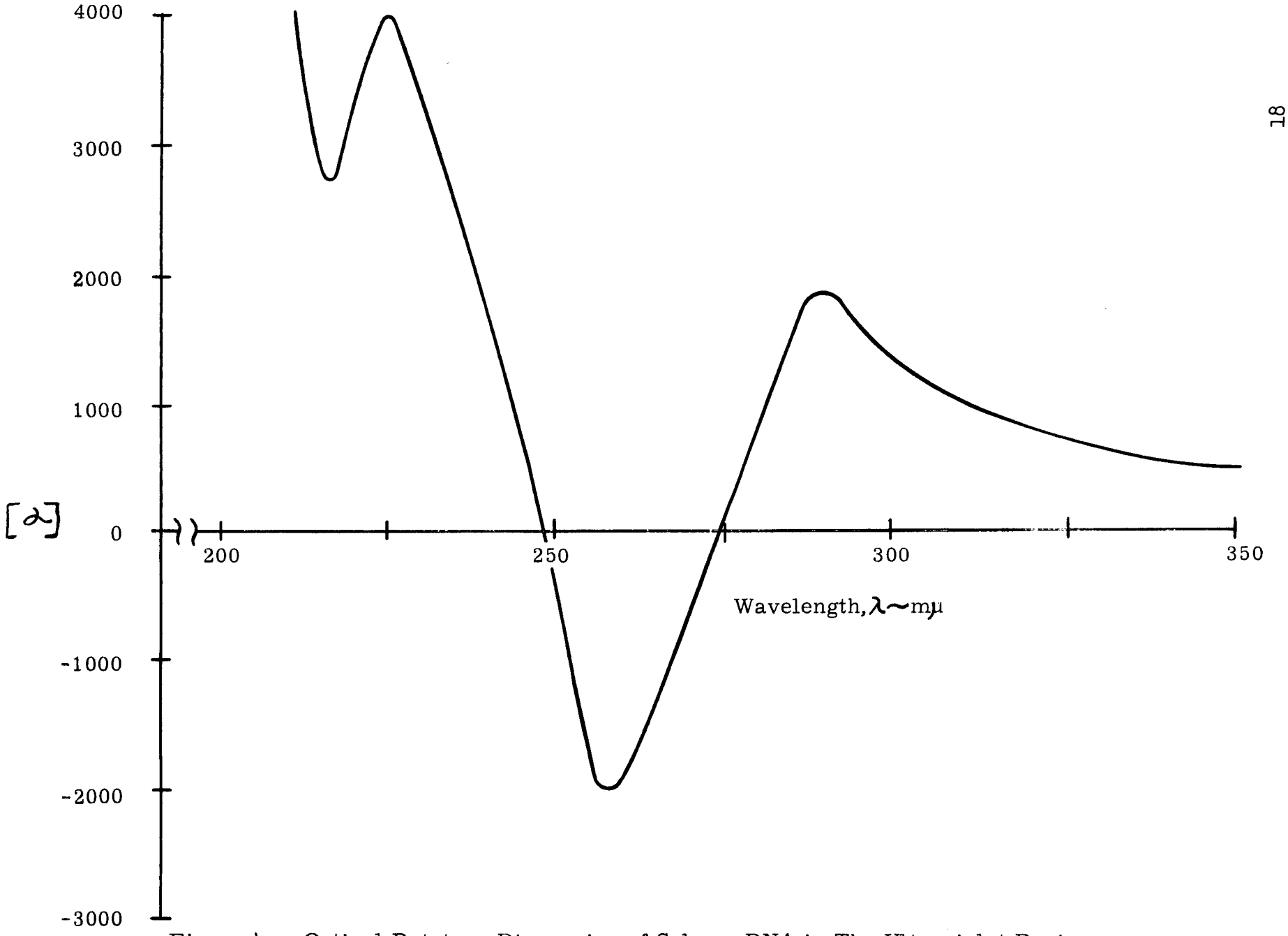


Figure 4. Optical Rotatory Dispersion of Salmon DNA in The Ultraviolet Region (Redrawn from Samejima and Yang, 1964)

phototube used as the detector would be shifted in phase by some amount less than that at 254 mμ for the given concentration of optically active substance.

This state of affairs is shown in figure 5. For simplicity, assume the light source emits white light (equal energy at all wavelengths) and that the spectral response of the phototube is uniform for all wavelengths. Then alterations in the intensity of the light passing through the system are due to absorption by the sample at that wavelength. The phototube senses only amplitude of the light which has passed through the sample and rotating analyzer without regard to wavelength.

The vectors shown in the figure represent the amplitude and phase of the sinusoidal modulation of the light intensity produced by the rotation of the analyzer prism with respect to the polarizer prism. The sample resides between these two prisms. The x-axis coincides with the reference from which optical rotation is measured and represents the spatial position of the plane of polarization in the absence of an optically active sample. Also

A_o = the amplitude of light at wavelength, λ_o

λ_o = wavelength at which the sample has maximum optical activity and strong absorption

ϕ_o = optical rotation of λ_o by sample

A_1 = the amplitude of light at wavelength, λ_1

λ_1 = wavelength at which the sample has optical activity less than maximum and weak absorption

ϕ_1 = optical rotation of λ_1 by sample

V_A = the vector sum of V_o and V_1

A_A = the amplitude of V_1

ϕ_A = the apparent optical rotation

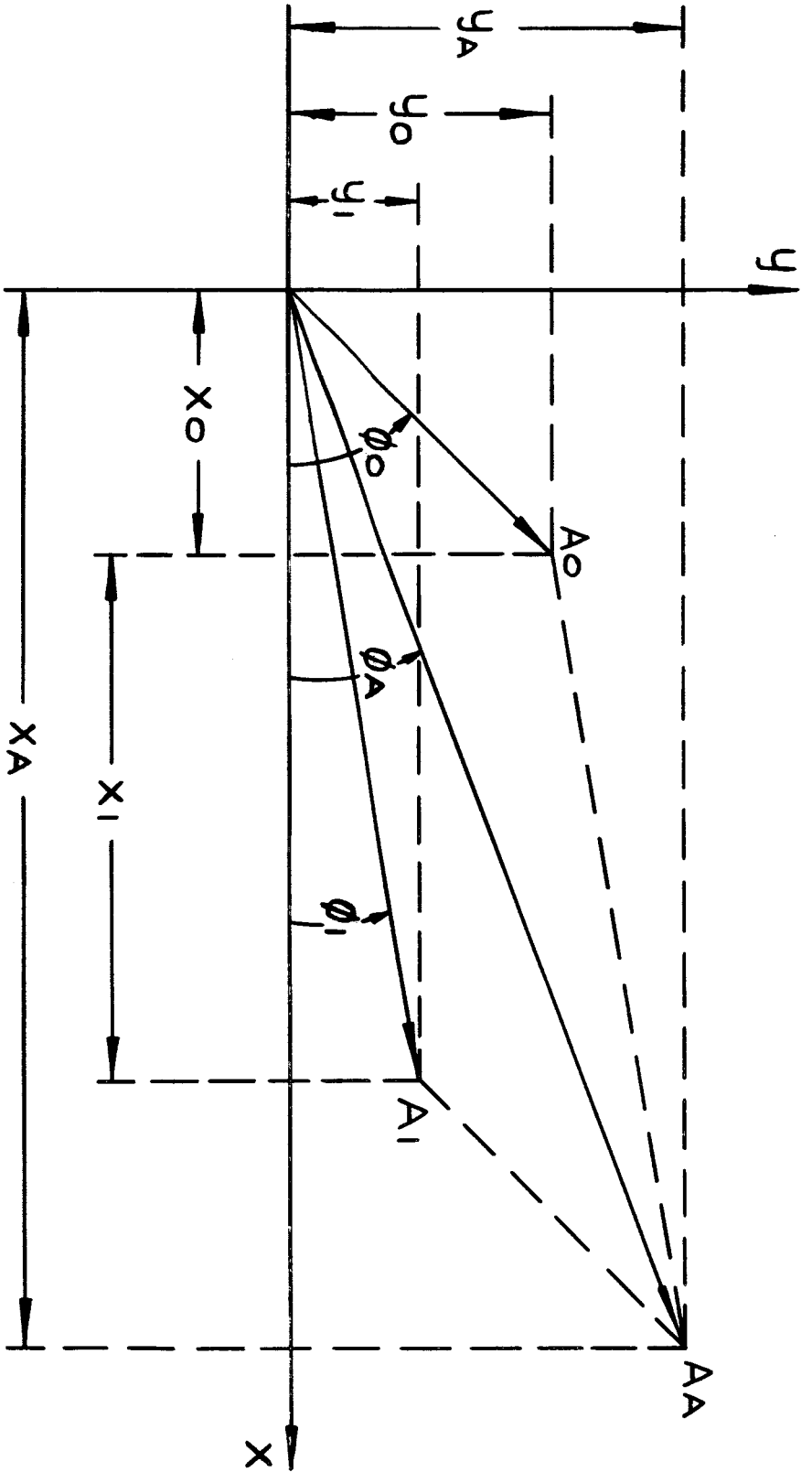


Figure 5. Vector Representation of Signals Seen By Phototransducer

Inspection of figure 5 shows that ϕ_A, the apparent optical rotation is less than ϕ_o, the maximum optical rotation.

The angle, $\phi_A = \tan^{-1} \frac{y_a}{x_a} = \tan^{-1} \frac{y_o + y_1}{x_o + x_1}$

and $\phi_A = \phi_o = \tan^{-1} \frac{y_o}{x_o}$ when $x_1 = y_1 = 0$

This is the condition for maximum observed rotation.

y_o = y-axis projection of v_o

y_1 = y-axis projection of v_1

x_o = x-axis projection of v_o

x_1 = x-axis projection of v_1.

The figure shows clearly that the apparent optical rotation is sensitive to the presence of wavelengths of smaller optical activity than the maximum. The magnitude of the effect depends upon the relative optical rotation of the interfering wavelength and its amplitude.

Returning to consideration of the nucleic acids in the neighborhood of 254 mμ. In this region of the spectrum, as shown in figure 4, the optical rotatory dispersion varies very rapidly with wavelength. Thus maximum sensitivity requires very small spectral bandwidth. This requirement cannot readily be satisfied with filters and resort must be had to a monochromator, a more complex system. At 365 mμ, again referring to figure 4, the slope of the optical rotatory dispersion curve varies gradually which minimizes the reduction in apparent optical rotation in the presence of relatively broad spectral bandwidth. It should be possible to satisfy the requirements at 365 mμ with simple optical filters. Indeed, the experimental results, as stated above confirm this result.

For these reasons and assuming that nucleic acids are the

substances whose optical activity is of prime interest in this application, 365 mμ has been provisionally chosen as the wavelength at which to conduct the experiment. This is an attractive portion of the spectrum in which to operate as a strong mercury line is available. It also moves the instrument out of the quartz-ultraviolet region of the spectrum. The material of interest may very well exhibit less optical density than at 254 mμ. While there is a small reduction in the optical activity of nucleic acids in this region of the spectrum compared with that observed at 254 mμ, the greatly increased transmittance means that for a given light source, optical efficiency and sensitivity of detection it is possible to introduct a much higher concentration of solute in the optical path than is the case at 254 mμ, for the same pathlength. The increase in concentration of solute which is possible, may result in an overall increase in sensitivity with respect to that which would be observed at 254 mμ. It should be emphasized that these conclusions are still subject to experimental verification. However, the other aspect of the situation is that if 254 mμ is to be used as the wavelength for measurement then much better spectral discrimination must be employed than is possible with optical filters. Some form of monochromator would have to be used which would require a much more complex optical system with the disadvantages that attend any increase in complexity. In any event, characterization of the soil extracts and correlation with their optical behavior is a necessity.

6.3 Hydraulic/Mechanical System

6.3.1 Introduction

With reference to figure 6, the Melpar Optical Rotatory Dispersion Unit consists of four inter-dependent sub-systems in the following categories:

(a) Electro-Optical

(b) Electrical (Control)

(c) Mechanical

(d) Hydraulic

Categories (a) and (b) are discussed separately in the report.

6.3.2 Mechanical System

The mechanical layout, shown schematically in figure 6, has evolved as an integration of sub-systems grouped on the optical system axis.

Symmetry with respect to the optical system axis has several advantages: Among these are the ease of component assembly and adjustment. Symmetry is also desirable from the environmental point of view, particularly when thermal growth and vibration are present.

Effort has been placed on component simplification to ensure reliability without compromising performance. The optical sample cell design approach is a case in point.

The sample cell of an early design concept incorporated a variable optical path-length feature. The feasibility of the approach has been proven with the Melpar breadboard unit; however, design investigations indicated an improved construction was possible.

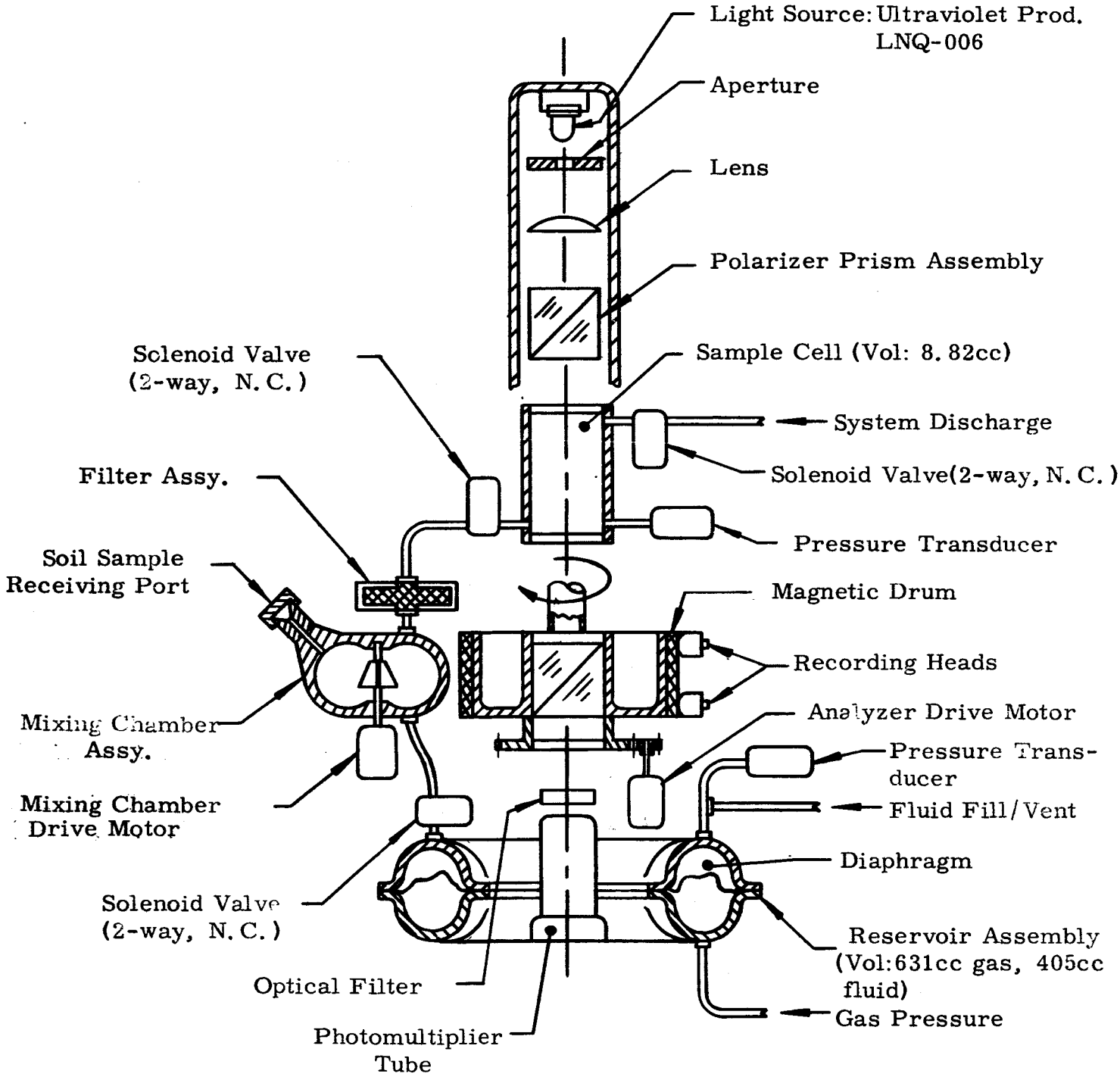


Figure 6: Hydraulic/Mechanical System Schematic

To achieve the effect of reducing the solute concentration, an hydraulic approach has been selected, thus eliminating the original cell drive motor, seals, gears, limit switches, and controls. These are to be balanced against an increase in fluid reservoir volume and weight. Significant reduction in package size and mounting problems are additional advantages.

6.3.3 Hydraulic System

6.3.3.1 General Description

With reference to figure 7, the system consists of a pressurized fluid reservoir, mixing chamber, filter, and sample cell. Also indicated by the schematic are three normally-closed solenoid valves and two pressure switches.

These elements sense and control system operation with respect to the over-all operational requirements. An analysis and performance prediction of the system is contained in the Appendix.

6.3.3.2 Operation (reference figure 7)

Actuation of the hydraulic system follows the completion of the three events given below:

1.3.2.1 Exhaust Residual Air to Ambient Low Pressure (Energize V_1, V_2)

1.3.2.2 Insert Sample

1.3.2.3 Close System (De-energize V_1, V_2)

With these events completed, the system is in a "ready" or "charged" condition.

The mixing section may now be filled by energizing Solenoid, VI. Gas under pressure in the one (1) liter (approximately) reservoir expulses

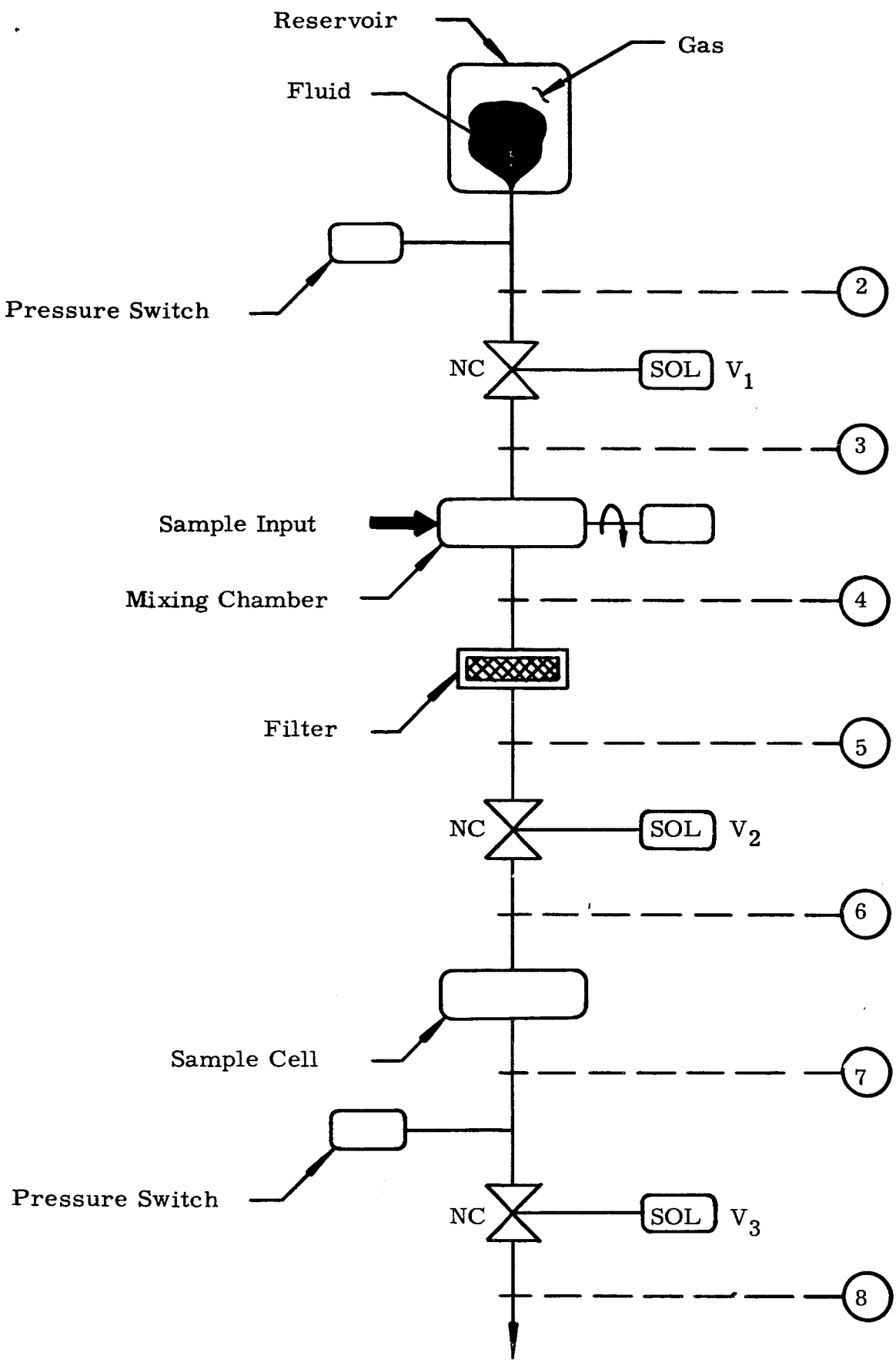


Figure 7. Hydraulic System Schematic

fluid into the mixing chamber. The mixing chamber pressure switch senses when this function is complete.

The mixing chamber is toroidal in shape with a fluid capacity of 88.2 cc. A motor-driven, open-face centrifugal impeller is located on the mixing chamber center line and provides a fast moving, swirling action to the fluid during the specified mixing time.

Upon completion of the mixing period, filtered fluid may be introduced to the sample cell from the mixing chamber by energizing V_2. The 5 micron filter, located centrally in the mixing chamber, is both removable and re-cleanable for convenience during test or check-out. The sample cell pressure switch provides the signal indicating that the 8.82 cc volume of the sample cell is completely charged.

A quiescent period following the sample cell charging event allows the fluid circulation within the cell to dampen out before optical activity of the fluid is sensed.

Subsequent operation of the optical system may indicate that the sample material concentration in the fluid is too great. Thus reducing the light level below a detectable level.

Reduction in cell fluid concentration is accomplished by energizing solenoid valve V_3 until a detectable light signal is obtained. In theory, the reservoir/mixing chamber volume relationship (reference Appendix) provides a capability for reducing the concentration by a factor of 100.

If all the fluid in the reservoir is exhausted while operating in this mode, both pressure switches will revert to the inactive state, thus indicating termination of the experiment.

6.4 Electronics

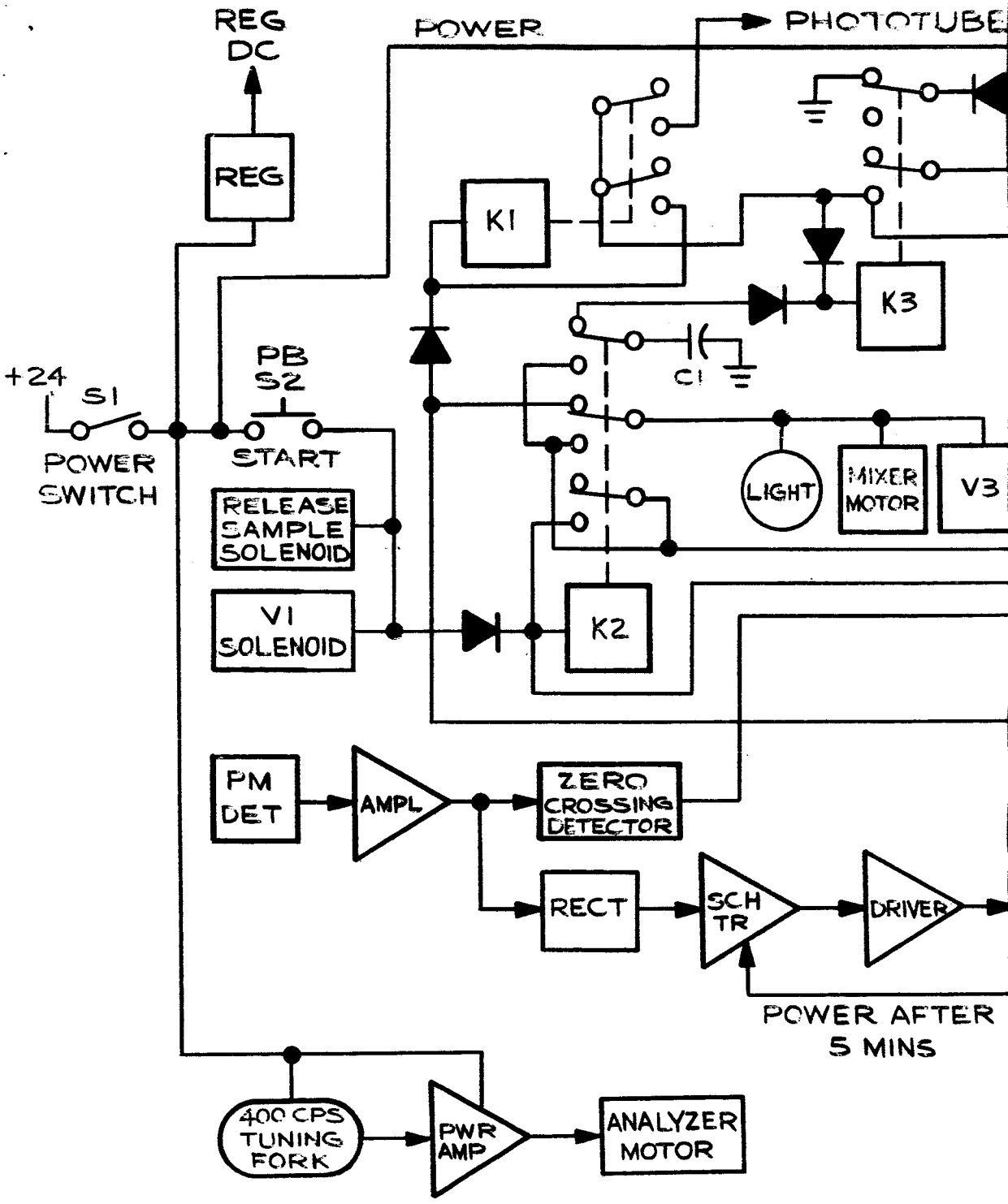
Figure 4 shows a functional diagram of electronic system to provide the detection, control, and readout functions for the experiment. The detection circuits have been described in a previous report, however, a brief explanation of the control system follows.

A push button switch has been selected to start the experiment in lieu of calling for an external start command.

When the main power switch, S1, is closed, power is applied to all of the electronic circuits except the optical density sensor circuits. The photomultiplier, amplifier, zero crossing detector, light source, the tuning fork oscillator, power amplifier, and analyzer drive motor are supplied with power.

Push button switch, S2, may be momentarily energized any time after power is applied. This switch will start the programming cycle of the experiment. The experiment may be considered to consist of five main functions, namely sample preparation, reference recording, sample introduction, sample recording and readout. These functions are performing in the listed order. By performing sample preparation first, all components are given a period of time to stabilize. The reference record and sample record periods may be run with a minimum time delay between the functions.

Push button S2 starts the sample preparation period. The sample solenoid and solenoid valve V3, are energized immediately. This places the sample to be analyzed and the liquid into the sample preparation chamber. Relays K_2 and K_5 are also energized and held. Relay K_2 applies power to the sample mixer motor, and solenoid valve V3 through the normally closed contact of time delay relay TD2. This starts the sample mixing process and vents the


REG
DC
REG
POWER
PHOTOTUBE
K1
K3
C1
+24
S1
POWER
SWITCH
PB
S2
START
RELEASE
SAMPLE
SOLENOID
V1
SOLENOID
K2
LIGHT
MIXER
MOTOR
V3
PM
DET
AMPL
ZERO
CROSSING
DETECTOR
RECT
SCH
TR
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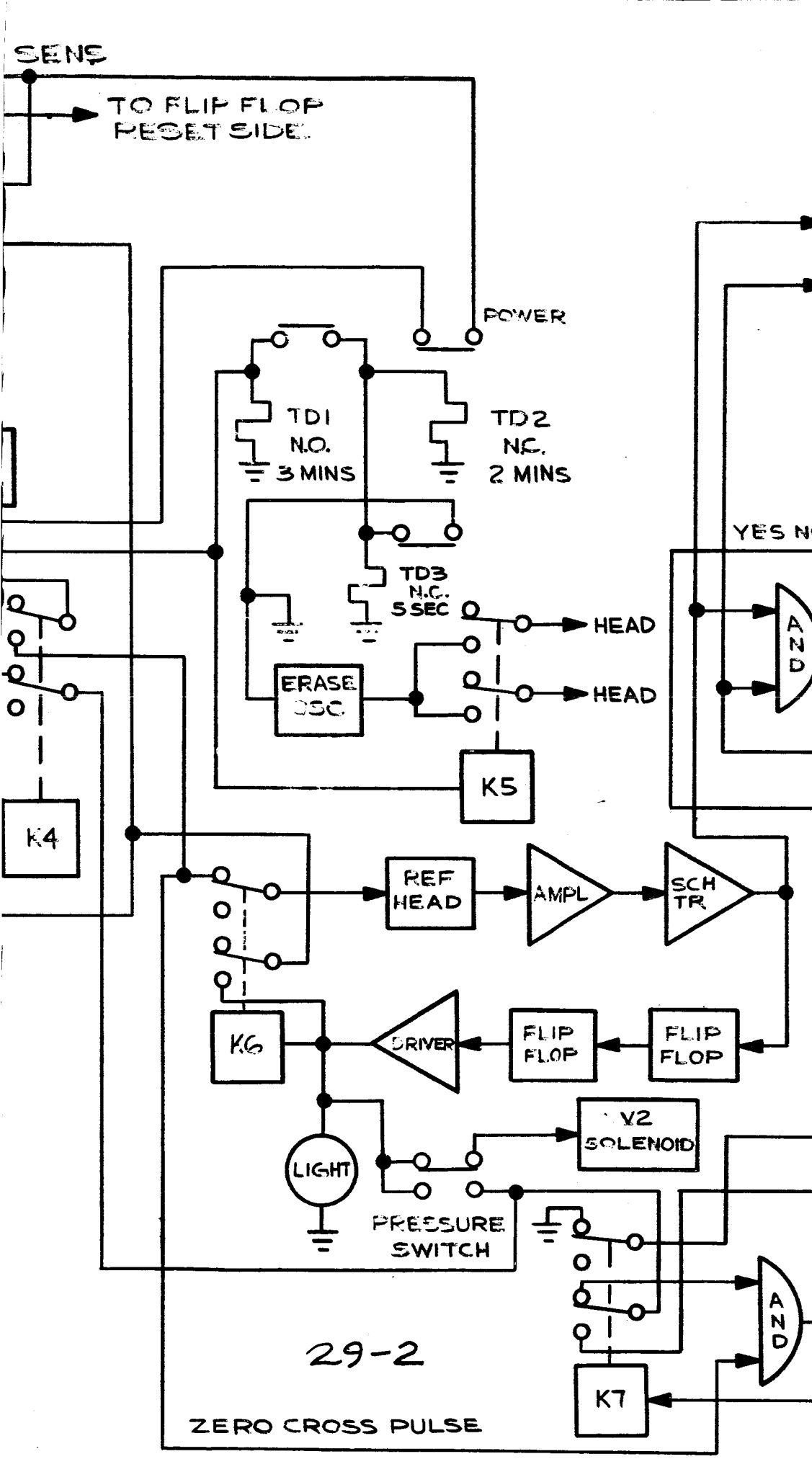
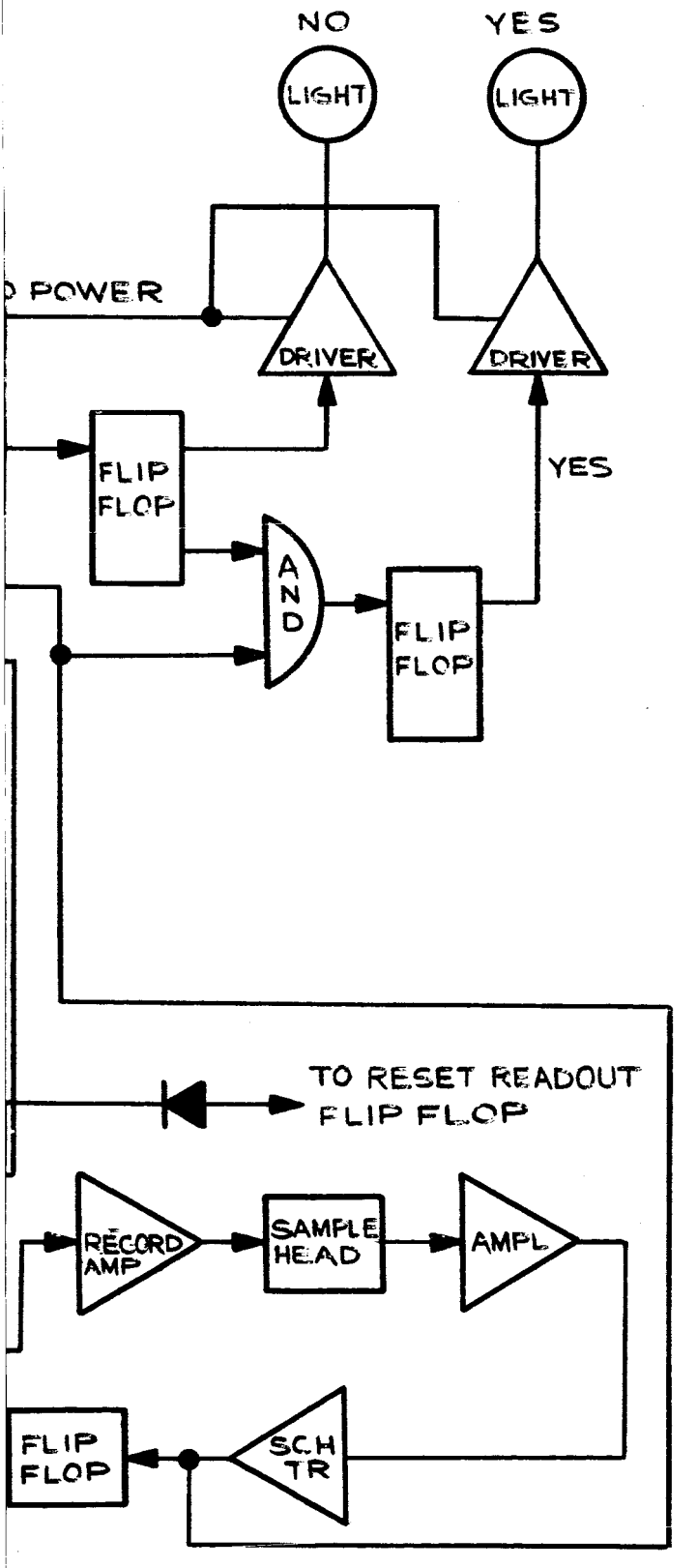
POWER AFTER
5 MINS
400 CPS
TUNING
FORK
PWR
AMP
ANALYZER
MOTOR


SENS
TO FLIP FLOP
RESET SIDE
POWER
TD1
N.O.
3 MINS
TD2
N.C.
2 MINS
TD3
N.C.
5 SEC
HEAD
HEAD
ERASE
OSC.
K5
K4
REF
HEAD
AMPL
SCH
TR
K6
DRIVER
FLIP
FLOP
FLIP
FLOP
V2
SOLENOID
LIGHT
PRESSURE
SWITCH
AND
AND
K7
ZERO CROSS PULSE
29-2

REF PULSE OUT

SAMPLE PULSE OUT



sample cell to the atmosphere through V_3. Capacitor C_1 is also charged through another contact of K_2. Time delay relay, TD1, which is normally open also starts its timing cycle. At the end of the present time delay, approximately 3 minutes, the contacts of TD1 are closed. This contact then applies power to TD2 and TD3. TD3 is normally closed and will remain closed for approximately 5 seconds. During this period, power is applied to the erase oscillator which is coupled to the recording heads through relay K_5. This makes certain that any information left on the recording drum is erased prior to the start of the recording cycles. The contact of TD2 is opened after two minutes and power is removed from V_3, K_2, K_5 and the sample mixer motor. The release of K_2 allows C1 to discharge through relay K_3 which is then held closed. Thus the 5 minute sample preparation period is completed and the reference record period may be started.

The closing of K_3 removes the reset function from the record pulse counters and applies power to the optical density (OD) sensor. The OD sensor relay, K_4, will now be energized if there is sufficient signal from the photomultiplier detector to proceed with the experiment. When K_4 is energized, the output of the zero crossing detector is coupled through K_4 and K_6 to the reference head of the recording drum. These recorded pulses are counted by two flip flops. The second recorded pulse will then energize and hold relay K_6 which prevents any additional pulses from being recorded on the reference track. This relay may also energize a lamp to give a visual indication that the reference recording cycle is complete.

When K_6 is energized, power is also applied to solenoid valve V2 which allows the sample mixture to be introduced into the sample cell in the optical path. After the sample cell is filled, which is detected by the

closing of pressure switch P1, AND gate 1 is enabled. If the optical density has not increased sufficiently to de-energize K_4, the sample pulse is recorded on a separate track on the recorder drum. However, if the optical density has increased sufficiently to de-energize K_4, corresponding to a reduction in amplifier output on the order of 10:1, power is applied to K_1, V3 and the mixer motor through the pressure switch sensor. When K_1 is energized the photomultiplier sensitivity is increased and held, and when V_3 and the mixer motor are energized, the sample mixture will be diluted by allowing additional liquid to flow through the optical path sample cell and out through V_3. This process will continue until relay K_4 is again energized by deciding the detected signal is of sufficient amplitude to record the sample zero crossing point. The sample introduction cycle is now complete and the sample recording period may be initiated.

One pulse corresponding to the zero crossing point of the signal will be recorded on the sample track of the recorder. The first pulse recorded will set a flip flop which in turn energizes relay K_7. Relay K_7 will then inhibit the recording of additional pulses and remove the reset condition from the readout flip flops. This indicates experimental measurements have been completed, the information is recorded, and the readout may be made.

The output of the two recorder head playback amplifiers are coupled through AND gate 2 to the set side of flip flop 1. If the two playback pulses occur at the same time, indicating no phase shift in the detected zero crossing points, flip flop 1 will be set indicating a "no activity" answer. If flip flop 1 is not set, AND gate 3 is enabled and flip flops 2 will be set indicating a "yes" (phase shift was detected) answer. If neither flip flop is set a malfunction is indicated.

7. APPENDIX

7.1 Analytical Relations

7.1.1 Mixing Chamber (Reference Figure 9)

The mass and volume relations for the mixing chamber at any time, t, are

$$W = W_1 + W_2 \tag{1}$$

$$V_{m/c} = V_1 + V_2 \tag{2}$$

The mixture density is obtained by dividing equation (1) by equation (2). This yields

$$\frac{W}{V_{m/c}} = \rho = \frac{W_1 + W_2}{V_{m/c}}, \text{ or}$$

$$\rho = \rho_1 \frac{V_1}{V_{m/c}} + \rho_2 \frac{V_2}{V_{m/c}} \tag{3}$$

The volumetric concentrations of elements (1) and (2) in $V_{m/c}$ may be defined as

$$\left.\begin{aligned} C_1 &= \frac{V_1}{V_{m/c}} \\ C_2 &= \frac{V_2}{V_{m/c}} \end{aligned}\right\} \tag{4}$$

Combining equations (3) and (4), we obtain

$$\rho = C_1\rho_1 + C_2\rho_2,$$

and since $C_1 + C_2 = 1$, the density may be expressed as

$$\rho = \rho_2 + (\rho_1 - \rho_2)\, C_1 \tag{5}$$

The mass flow relations for the volume, $V_{m/c}$, will now be derived for a time interval, Δt.

The mass flow entering $V_{m/c}$ is $\rho_a q_a \Delta t$; the mass flow leaving is $\rho_b q_b \Delta t$.

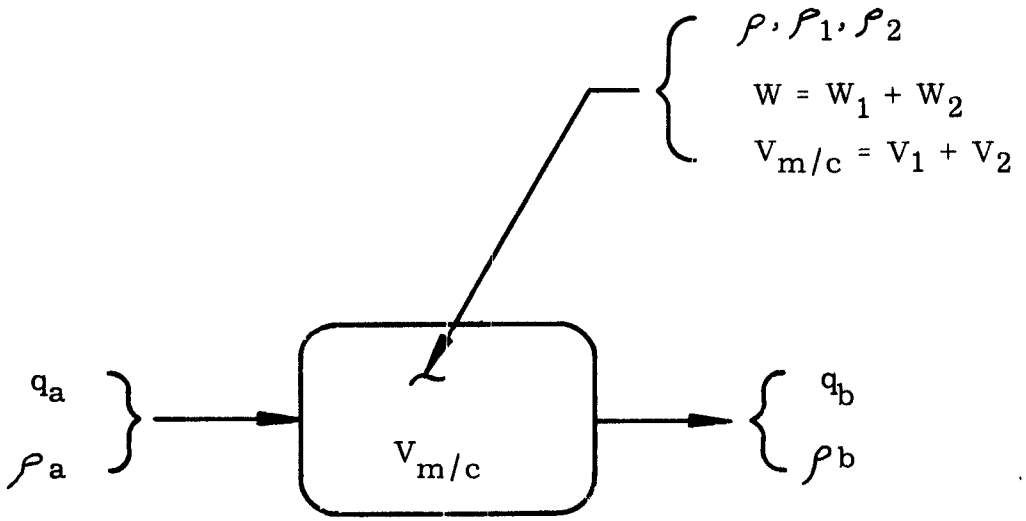


Figure 9. Mixing Chamber Block Diagram Illustrating Notation

Accumulation of mass in the volume, $V_{m/c}$, during the time interval, Δt, is

$$(W + \frac{\partial W}{\partial t} \Delta t) - W = \frac{\partial W}{\partial t} \Delta t.$$

Combining these immediate results yields

$$(\rho_a q_a - \rho_b q_b) = \frac{\partial W}{\partial t},$$

And assuming incompressible flow ($q_a = q_b = q$), we find

$$q (\rho_a - \rho_b) = V_{m/c} \frac{\partial \rho}{\partial t} \tag{6}$$

Assume that the liquid diluent entering $V_{m/c}$ is identical with the liquid phase in $V_{m/c}$ so that $\rho_a = \rho_2$; also, ρ_b is merely the mixture density leaving $V_{m/c}$ at any time, t, so $\rho_b = \rho$.

With this information equation (6) becomes

$$q (\rho_2 - \rho) = V_{m/c} \dot{\rho}, \tag{7}$$

where the subscript "2" refers to the solute in $V_{m/c}$.

Equations (4), (5) and (7) may be manipulated to give

$$- \frac{\dot{C}_1}{C_1} = \frac{q}{V_{m/c}} \tag{8}$$

The integration is simple, yielding

$$- \ln \left| \frac{C_1}{C_{01}} \right| = \frac{1}{V_{m/c}} \int_0^t q dt, \tag{9}$$

where C_{01} = initial concentration.

For illustration purposes, let us determine the reservoir capacity required to reduce the concentration by a factor of 100.

Accordingly,

$$V_f = - V_{m/c} \ln \left| \frac{1}{100} \right|,$$

$$V_f = 4.6 V_{m/c}. \tag{10}$$

About five times the mixing chamber volume is required to effect a 100:1 change in concentration.

7.1.2 System Reservoir

The total volume of the reservoir, V_r, consists of two sections separated by a membrane or diaphragm such that

$$V_r = V_f + V_g,$$

where subscripts "f" and "g" pertain to fluid and gas, respectively. This result may be differentiated with respect to time to give

$$\dot{V}_f = -\dot{V}_g,$$

or

$$q = -\dot{V}_g, \text{ the volumetric flow rate.}$$

From the first law of thermodynamics and the equation of state for a perfect gas undergoing an isentropic expansion (closed system; moving boundaries), we may write the following relation between pressure and volume:

$$\frac{p_g}{p_{gl}} = \left(\frac{V_g}{V_{gl}}\right)^{-\gamma} \tag{11}$$

Logarithmic differentiation of equation (11) with respect to time yields

$$\frac{\dot{p}_g}{p_g} = -\gamma \frac{\dot{V}_g}{V_g},$$

and from the preceding volume relations, this result becomes

$$\frac{\dot{p}_g}{p_g} = \gamma \frac{q}{V_g} \tag{12}$$

As another illustrative example, let us compute the total volume of the reservoir, V_r, for a 50% reduction in gas pressure at the end of the expansion process.

From equation (11), we have

$$\left(\frac{V_{g2}}{V_{g1}}\right)^{-\gamma} = \frac{1}{2},$$

Assuming a specific heat ratio, $\gamma = 1.4$, the volume ratio is

$$\frac{V_{g1}}{V_{g2}} = (0.50)^{0.715} = 0.609$$

At the end of the expansion process, all the fluid has been expulsed so that $V_{g2} = V_r$.

Therefore,

$$V_{g1} = 0.609\ V_r \qquad (13)$$

Adding equations (10) and (13):

$$V_{f1} + V_{g1} = 0.609\ V_r + 4.6\ V_{m/c} = V_r,$$

$$.391\ V_r = 4.6\ V_{m/c},$$

$$V_r = 11.77\ V_{m/c}. \qquad (14)$$

The total volume of the reservoir is about 12 times the volume of the mixing chamber, and combining equations (10) and (14) we obtain the useful relation

$$V_f = 0.391\ V_r, \qquad (15)$$

So approximately 40% of the reservoir capacity contains fluid; 60% contains gas, which undergoes an expansion such that the final pressure is one-half the initial pressure.

7.1.3 Hydraulic System Pressure Drop and Flow

With reference to figure 7, the pressure drop-flow relations for all valves, fittings, tubing, bends, etc. may be described by the following four equations:

$$\left.\begin{array}{ll} \text{Pipe, Bends} & : \Delta p = f \frac{L}{D} \frac{\rho V^2}{2g} \\ \text{Valves, Orifices:} & q = CA\Delta p^{1/2} \\ \text{Sudden Expansions, etc:} & \Delta p = K \frac{\rho V^2}{2g} \\ \text{Filter:} & q = CA\Delta p \ \exp\left(-t/\tau\right) \end{array}\right\} \quad (16)$$

The exponential term in the filter equation describes the time dependency of the filter resistance due to the solids build-up in the filter pores. Typical filter performance is shown in figure 10 with a first order exponential curve for comparison.

Equations (16) are not in a convenient common form for further development; so, with rearrangement, equations (16) become

$$\left.\begin{array}{ll} \text{Pipe, Bends} & : q = C_1 A\Delta p^{1/2} \\ \text{Valves, Orifices:} & q = C_2 A \Delta p^{1/2} \\ \text{Sudden Expansions, etc:} & q = C_3 A \Delta p^{1/2} \\ \text{Filter:} & q = C_F A_F \Delta p \ \exp\left(-t/\tau\right), \end{array}\right\} \quad (17)$$

Where

$$C_1 = \sqrt{\frac{2g/\rho}{f\, L/D}},$$

$$C_3 = \sqrt{\frac{2g/\rho}{K}}$$

Squaring the first three equations of (17) and adding the result to the fourth, we obtain

$$q^2 \sum \frac{1}{C^2 A^2} + \left[\frac{1}{C_F A_F \exp\left(-t/\tau\right)}\right] \cdot q = p_g - p_a, \quad (18)$$

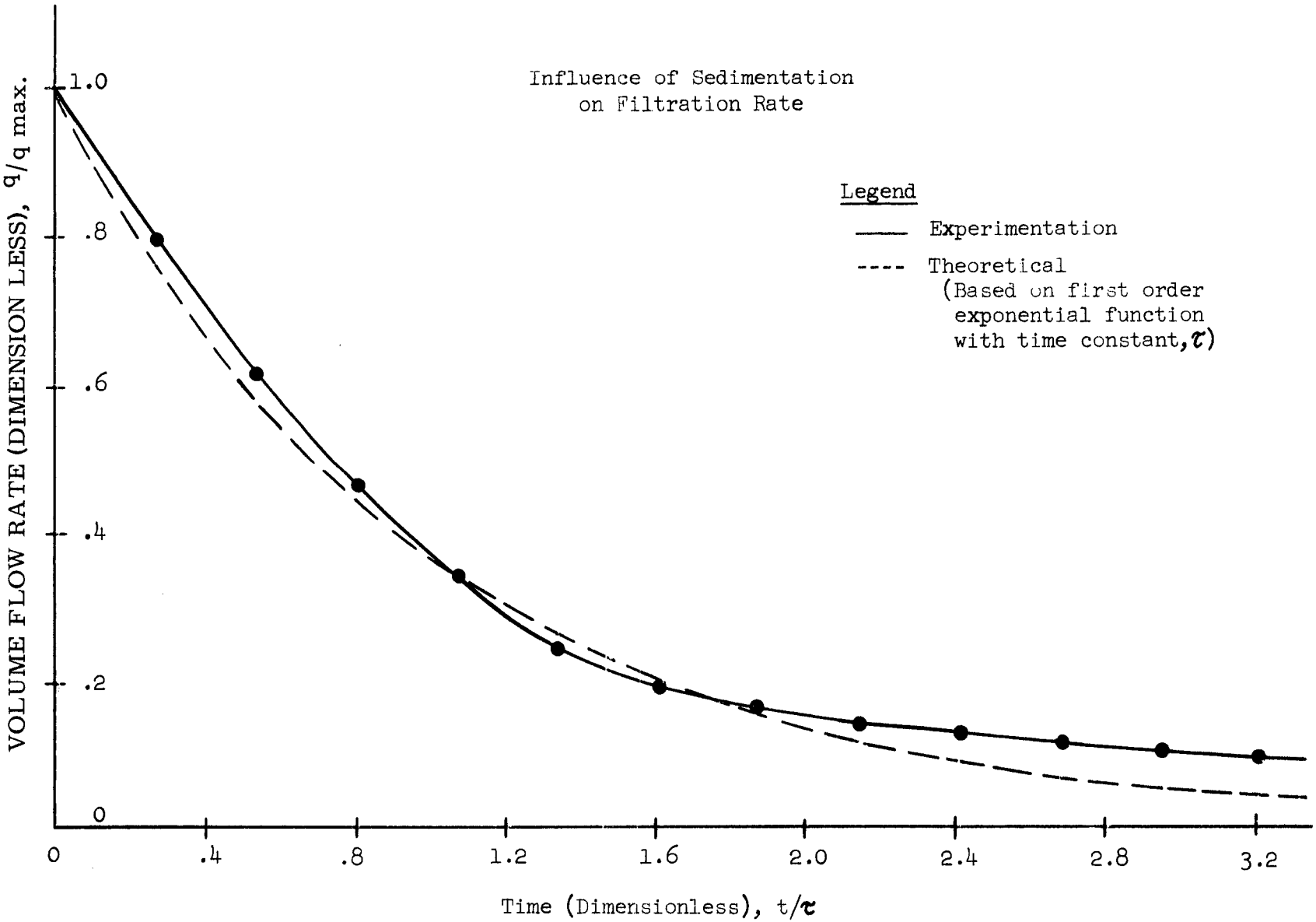

Influence of Sedimentation
on Filtration Rate
Legend
Experimentation
Theoretical
(Based on first order
exponential function
with time constant, τ)
VOLUME FLOW RATE (DIMENSION LESS), q/q max.
1.0
.8
.6
.4
.2
0
0
.4
.8
1.2
1.6
2.0
2.4
2.8
3.2
Time (Dimensionless), t/τ

Figure 10

Where

p_a = ambient pressure at station (8) (reference figure 7).

The summation process, $\sum \frac{1}{C^2A^2}$, is independent of the order of formation, so the component loss factors associated with stations (1) through (8) may be expressed conveniently as a system constant in the summation.

Solving equation (18) for q :

$$q = \frac{1}{2\, C_F\, A_F \exp(-t/\tau)\sum\frac{1}{C^2A^2}} \cdot \left\{ \sqrt{1 + 2\, C_F\, A_F \exp(-t/\tau)^2 (p_g - p_a)\sum\frac{1}{C^2A^2}} - 1 \right\} \quad (19)$$

Equation (19) represents the final expression for the volumetric flow rate in terms of the system parameters.

7.1.4 Calculation Procedure for System Flow Rate

The reservoir gas pressure and volume-time relationship may be estimated by the method of finite differences applied to equation (19) and those that follow below.

For some small increment in time, the reservoir gas pressure will be

$$(p_g)_{t+\Delta t} = (p_g)_t - \dot{p}_g \Delta t,$$

and from equation (12) we have

$$(p_g)_{t+\Delta t} = (p_g)_t - \gamma \left(\frac{q p_g}{V_g}\right)_t \Delta t \quad (20)$$

Similarly, the reservoir gas volume for this time increment will be

$(V_g)_{t+\Delta t} = (V_g)_t + \dot{V}_g \Delta t$, or

$$(V_g)_{t+\Delta t} = (V_g)_t + (q)_t \Delta t \qquad (21)$$

The fluid flow rate at any instant may now be estimated from equations (19), (20), and (21).

7.2 Design Calculations

7.2.1 Sample Cell

From prior work with the breadboard unit, a cell length of 5 cm. is a practical working figure.

For a 15 mm internal diameter that is commensurate with the working dimensions of the polarizer/analyzer prisms, we find the sample cell volume to be

$$V_{s/c} = 8.82 \text{ cc.}$$

7.2.2 Mixing Chamber

If the sample cell volume is 10% or less of the mixing chamber volume, there will be no significant change in the initial concentration when the sample cell is charged.

Accordingly, for 10%:

$$V_{m/c} = 88.2 \text{ cc}$$

7.2.3 Reservoir

From equation (14), the total volume of the reservoir, V_r, will be

$$V_r = 11.77\ V_{m/c},$$

or

$$V_r = 1036 \text{ cc.}$$

The fluid volume, V_f, is found from equation (15). This is

$$V_f = 405 \text{ cc.}$$

If we assume the fluid to be water, the weight is

W_f = 405 grams (14.3 oz.)

8. REFERENCES

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