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THE ESTABLISHMENT OF A SENSITIVE AND REPRODUCIBLE PROGRAM OF ANALYSIS OF BIOLOGICAL MATERIAL FOR METALLIC ELEMENTS

by

Stanley K. Derby

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Committee in charge:

Professor Ralph A. Wolfe, Chairman
Associate Professor Charles L. Dolph
Assistant Professor Paul V. C. Hough
Associate Professor W. Wallace McCormick
Associate Professor Marcellus L. Wiedenbeck

ABSTRACT

THE ESTABLISHMENT OF A SENSITIVE AND REPRODUCIBLE PROGRAM OF ANALYSIS OF BIOLOGICAL MATERIAL FOR METALLIC ELEMENTS

by Stanley K. Derby

The role of metallic elements in biological processes is acquiring increasing significance in medical research. These metals exist in the biological sample in such small percentages that a highly sensitive method of detection is required. This investigation deals with the establishment of a spectrographic program of analysis which is both sensitive and reproducible.

In order to facilitate the comparison of various methods and techniques, a large volume of hair solution was prepared. The concentration of this solution was set at 100 milligrams of hair per milliliter of solution. One hundred milligrams represents a rough average of the normal weights of the biological samples of skin, hair, and nails.

The influence of various methods and techniques upon the hair solution was studied through the spectral response of the elements iron, magnesium, and silicon. These elements represented respectively a high excitation potential element, a low excitation potential element, and an analytically troublesome element.

Best results for wet sample methods were obtained when graphite porous cups were employed with a goose-neck reservoir. The graphite porous cup cylinders were 5/8" long, 1/4" outside diameter, 5/32" inside diameter, and 0.025" bottom depth. The goose-neck reservoir was constructed of pyrex glass and designed to continuously supply the porous cup with a low level of sample

fluid. The wet sample program of analysis achieved sensitivities of 0.02 micrograms of iron, 0.01 micrograms of magnesium, and 0.06 micrograms of silicon. The values of precision obtained were: iron ±4%, magnesium ±3%, and silicon ±7%. Two internal control elements, molybdenum and indium, were employed. The internal control concentrations were: molybdenum 0.0003 mg./mg. of sample, indium 0.01 mg./mg. of sample. Thirty second exposures were required with a 5 ampere spark source. The sample consumed per exposure was approximately 1/4 milliliter, or 25 milligrams.

Best results for the dry sample methods were obtained when the sample was vacuum dried on flat graphite electrodes and then oven dried at 500° F. Vacuum drying furnished especially uniform sample coverage on the electrodes. The sample was excited with a 20 ampere spark source for a two second exposure. The electronic timer which timed the exposure interval is described. The dry sample program of analysis achieved sensitivities of 0.02 micrograms of iron, 0.01 micrograms of magnesium, and 0.01 micrograms of silicon. The values of precision obtained were: iron ±3%, magnesium ±5%, and silicon ±5%. Ammonium nitrate was used as a buffer to improve the reproducibility. Molybdenum was added as an internal control in concentrations of 0.0003 mg./mg. of sample. The sample consumed per exposure was approximately 1/10 milliliter, or 10 milligrams.

The best wet sample program and the best dry sample program compare favorably in sensitivity and precision. The dry sample program is recommended, however, because a smaller sample is required and the techniques involved are somewhat easier.

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INTRODUCTION

Many of the research problems of medical science involve the analysis of biological material for small amounts of metallic elements. The ultimate quality of such an analysis and the value of the resulting conclusions are inherently tied to both the method of analysis and the competence of the analyst. In evaluating the existing reports in the literature these two quantities must be given serious consideration. It is often true that in an information-starved border line field, such as medical physics, the conclusions drawn from an investigation are closely scrutinized while the quality of the investigation itself remains unchallenged. Most of the reported investigations in the domain of analytical medical physics have been carried out by physicians whose intense interest in a particular problem impelled them to employ tools from other scientific fields. It seems entirely proper to point out that the physician seldom has more proficiency or special training with the tools of physics than the physicist has with the tools of medicine.

This thesis is directed toward establishing a program of analysis of biological samples which will be beneficial to the trained and untrained experimenter alike.

The program incorporates the optimum operating conditions and should guide even the untrained experimenter to greater sensitivity and greater accuracy of analysis.

CHAPTER I

CHOICE OF METHOD OF ANALYSIS

In an investigation of the minute amounts of metallic elements in biological material, the method of analysis chosen should be such as to give high sensitivity and reproducibility. High sensitivity is imperative because the available sample is usually very small---of the order of ten milligrams---of which a considerable portion is carbohydrate structure.[1] High reproducibility is necessary so that the analysis can be depended upon.

In general there are three major methods of analysis which need to be considered. These are:

1) The flame photometer method
2) The chemical method
3) The spectrographic method

1. THE FLAME PHOTOMETER METHOD

The flame spectrometer[2,3] and the more recent flame photometer[4-9] are useful analytical tools, but two inherent properties make them unsatisfactory for the unrestricted analysis of small amounts of biological material.

a. Low Energy Excitation

Lundegårdh[3] and others[10-12] have demonstrated that it is possible to detect 34 metals employing an air-acetylene flame. However, the energies involved in the flame are so small that only elements with low excitation potentials can be detected in the lower concentrations. These elements are primarily the alkalies and the alkali earths. Because of this, most flame source reports in the literature pertaining to biological materials are limited to these elements.[13-24]

b. Extravagance of Sample

Although normally about 2 ml. of sample solution is actually consumed in the flame[10], more is required for loading. In fact Cholak and Hubbard[11] have used 10 ml. to load the sample chamber although the sample consumed was less than 1 ml. Thus, only a fraction of the available sample is actually being used. When the initial sample is so small, it must be made to yield as much information per cubic millimeter as possible.

It is noted that the method of Ramage[12], wherein the sample is dried on a tight paper spiral, does not suffer from the defect of extravagance of sample. However, the technique of advancing the filter paper roll into the flame is quite critical with respect to reproducibility. Furthermore, this method is still subject to the low energy excitation of the flame.

2. THE CHEMICAL METHOD

Chemical methods are most successful in the domain of large concentrations and few elements. The applicability of the chemical method decreases, however, as the number of elements under analysis becomes larger and their concentrations become smaller. Chemical methods are in general inadequate and unsatisfactory for the quantitative analysis of twenty elements with average concentrations of 0.001% of a 10 mg. sample. For this reason, most chemical methods in the literature pertaining to the analysis of biological material depend upon the chemical isolation and special treatment of a select number of elements.(25-37)

3. THE SPECTROGRAPHIC METHOD

Spectrographic methods of analysis of small amounts of biological material involve the following problems:

a) Molecular band structure masking emission lines in certain regions of the spectrum.

b) Radiation background masking emission lines of low concentration elements.

c) Inability to excite the element sulfur with normal spectrographic instrumentation.

d) The necessity of using carbon supports for the sample. Carbon electrodes enhance the cyanogen molecule band structure.

e) Photographic plate calibration.

The difficulties in the excitation of the sulfur atom arise from its high excitation potential and from its lack of sensitive lines in the visible or ultraviolet region of the spectrum. The sensitive lines of sulfur lie in the region of 1800Å. Radiation from this wavelength region is absorbed strongly by oxygen in the air, by the quartz of the quartz optics, and by the gelatin of the photographic emulsion. These difficulties can be overcome[38] by the use of a lithium fluoride optical train, by employing an atmosphere of nitrogen, and by using specially sensitized plates. These conditions, in addition to being troublesome, are not favorable for the excitation of the other elements. It is best, therefore, to preemptorily exclude sulfur from a spectrographic analysis. This, however, is not a serious exclusion since sulfur has been found to exist in concentrations of about 1% to 5% of biological sample.[39-46] It therefore easily lies within the domain of a good chemical analysis.[47]

All of the other defects in the spectrographic method, while troublesome, can be resolved satisfactorily. Furthermore, spectrographic methods, if properly used and evaluated, are capable of furnishing both the sensitivity and the accuracy required for the multi-element sample analysis. The spectrographic method was therefore selected as the method capable of providing the most fruitful quantitative analysis on small sample biological materials.

CHAPTER II

INDICATED STUDY OF PARAMETERS

The statement of selection of the spectrographic method does not at once specify the complete program of analysis. There are several distinct spectrographic methods which are available.[48-57] Consideration must be given to these individual methods to determine which is the most applicable to biological material.

Although many spectrographic analyses have been made upon biological material, no particular study has been reported which investigated the influence of such quantities as source variations, electrode shapes, and techniques of sample drying upon the analysis. It is evident, however, that to obtain an analysis of the highest quality such a study must be made. The fundamental parameters of the chosen method must be studied in order to select the conditions most favorable to the analysis.

A review of the literature indicates that previous spectrographic studies pertaining to biological materials were in general executed with special consideration for only one element or a few select elements as they applied to a particular body function or disease.[58-70] The few reports

which did attempt to deal with all of the elements in the biological material were not particularly concerned with the selection of the best conditions of analysis.[71-74] These reports were, in general, primarily interested in a survey of the particular biological material under analysis.

Iron, magnesium, and silicon are used in this study as representative elements. Iron represents the class of high excitation potential elements. Magnesium represents the class of low excitation potential elements. Silicon represents both the class of medium excitation potential elements and the analytically troublesome elements. The general techniques which have evolved are not prejudiced in favor of these three elements but are applicable to all other elements in the biological material.

CHAPTER III

PRELIMINARY CONSIDERATIONS

1. PREPARATION OF A STOCK SOLUTION OF SAMPLE

The ability to compare the sensitivity and reproducibility of various spectrographic methods upon biological material is contingent upon the use of an identical sample for each method. This requirement was satisfied by the preparation of a large uniform stock solution of biological material. The choice of human hair as the representative biological material was based largely upon the relative ease of obtaining large quantities of human hair as compared with human skin or nails. Previous study had shown the element content of skin, hair, and nails to be nearly identical, differing mainly in the ratio of the percentages of the metals.(75)

A salient study was made at this point to decide which acid would most satisfactorily digest the biological sample. The following acids were tried: nitric, sulfuric, hydrochloric, perchloric, acetic, and carbolic. All except nitric acid were found to be unsatisfactory because of inability to digest the sample or because of precipitate formation. Sulfuric acid, for example, is capable of causing

lead, barium, calcium, and strontium to precipitate from solution. Hydrochloric acid, in addition to non-digestion of sample, introduces the chloride ion which is capable of precipitating lead, silver, and mercury from solution. Perchloric acid is well known to be dangerously explosive when added to organic materials. Because of this the use of perchloric acid with biological material, whether alone or in combination with other acids, must be undertaken only with considerable caution. Neither acetic acid nor carbolic acid were capable of satisfactorily digesting the sample. Nitric acid was finally selected as the most satisfactory digesting agent on the basis of two factors:

a) Nitric acid does digest the sample.

b) All nitrates are water soluble.

Having chosen hair as the biological material and nitric acid as the digesting agent, the stock solution was prepared in the following manner. Three hundred and fifty grams of hair were collected from a variety of people and the large bulk of hair was thoroughly washed in (alconox) detergent three successive times to eliminate any extraneous oils and dirt. The sample was then washed profusely in doubly distilled water and divided into approximately three equal parts. Each 120 gram part could then fit comfortably into a 1000 ml. reflux condenser flask. A stock solution of nitric acid was prepared using one part of doubly distilled reagent grade nitric acid and one part of doubly

distilled water. Six hundred milliliters of this acid solution were added slowly to the flask by way of the reflux condenser tube. It was important that the acid be added in small quantities and slowly or else the gaseous reaction formed large bubbles which rapidly carried parts of the sample high in the reflux condenser. It was also important that a hood be provided for this operation to carry away the nitrogen dioxide (brown gas - disagreeable odor) that was formed. After most of the sample had been digested and the violent evolution of gases was no longer a problem, a sand bath was placed under the flask. The sand bath was maintained at about 200° Fahrenheit for twenty-four hours to insure complete digestion of the sample.

After all of the 350 grams of hair had been processed in the manner described above, the three individual solutions were mixed in one large container and diluted to a final volume of 3500 ml. This final solution then contained 350 grams of hair in 3500 ml. of solution for a concentration of 100 mg./ml. This concentration was chosen as representing a satisfactory dilution since it prevented a slight granular precipitate formation which was observed to occur at somewhat higher concentrations. This concentration also furnishes in one milliliter a weight of sample which is a rough average of the typical skin sample weight of 10 mg. and the typical hair and nail sample weights of 250 mg. It is therefore possible to normalize the weights of these dissimilar materials by establishing a uniform

concentration of 100 mg./ml. Such a standardization of concentration has specific advantages. For instance, in the dried precipitate method, the addition of a regular number of drops of diverse sample to the electrodes means that the intensity of the control element line falls repeatedly in the same locale on the H. & D. emulsion calibration curve. Maintainance of this condition leads to better reproducibility and better accuracy.

2. CHOICE OF AN INTERNAL CONTROL

Modern quantitative spectrographic analysis depends upon the comparative spectral line intensity of the investigated element relative to the spectral line intensity of a foreign element that has been added to the sample in a known concentration. The development of this method of analysis may be traced from Gerlach[76] through several investigators[77-81] to its present form.[82] The precision of the analysis is often highly dependent upon the selection of the foreign element which is termed the "internal control" or the "internal standard." The choice of the internal control is based fundamentally upon the desire to have the excitation of the control element in the discharge be as nearly identical as possible to the excitation of the investigated element. The inherent properties of elements which play an important role in determining intensity and reproducibility of spectral lines are (a) volatility,

(b) ionization potential, (c) excitation potential, (d) electronic configuration of the radiation level, (e) proximity of line pairs on the photographic plate, and (f) complexity of spectrum. In matching a control element to an investigated element, these then are the quantities which must be carefully considered.

When a single element is being investigated, one can readily find a control element with matching properties. When one is confronted with a multi-element analysis, as in biological material, one must compromise the demands to find a workable control element.

The element indium was selected as fulfilling the major requirements for an internal control. One operational difficulty developed, however, which led to the abandonment of indium as a precision control element. Indium proved to be more sensitive to heating than other elements in the biological matrix. For example, if hot plate dried samples were placed in a 500° F. oven for one hour and then excited in a spark discharge the intensity of the indium lines was increased <u>fourfold</u> over the intensity of indium lines from non-oven dried samples. In contrast the intensities of the other elements were approximately <u>doubled</u>. This hypersensitivity to heat made the indium intensities vary because of the inability to reproduce the drying and heating conditions exactly. Fluctuations in the indium intensities led to fluctuation in the element/control ratio. This of course, was undesirable. For this reason a new internal

control element molybdenum was chosen in concentrations sufficiently low to cause only the most sensitive lines of the spectrum to be detectable. Molybdenum proved to be satisfactory and good reproducibility has been obtained with biological materials using molybdenum as an internal control element.

3. PHOTOGRAPHIC PLATE CALIBRATION

The use of a photographic plate for the quantitative recording of light intensities requires a calibration of the sensitized emulsion.(83) This is necessary because manufacturing techniques are not sufficiently uniform to be able to declare that all plates produced are identical. This randomness is overcome by calibrating the emulsion of each individual plate used for quantitative measurement. There are many methods available for calibration. The particular method of plate calibration used for this study employed a rotating sector(84) driven by a 50 r.p.m. synchronous motor. This rotating sector (see Figure 1) was so designed as to divide the usable slit height into three equal portions of 4.5 mm. each. The perimeter was divided into octaves with angular openings in the ratio of 1:2:4. When the sector is rotated in front of the slit as shown in Figure 2, it divides the light received by the slit into three equal parts whose intensities are in the ratio of 1:2:4. The intensity 4 corresponds to the light falling

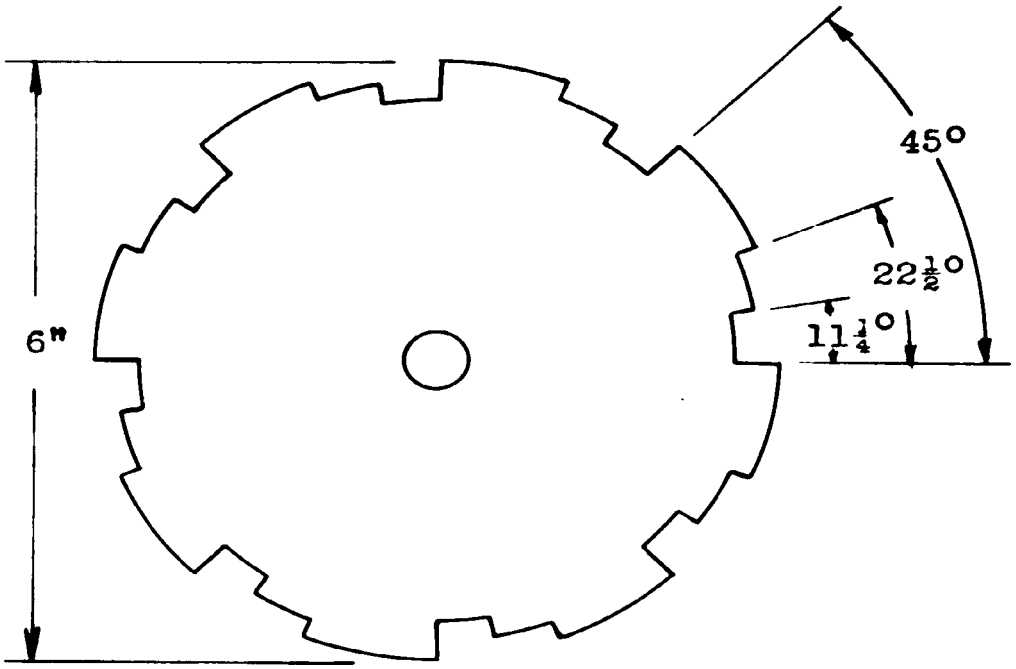


FIGURE 1

1:2:4 STEP SECTOR

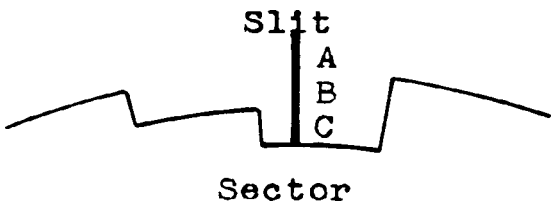


FIGURE 2

SECTOR IN FRONT OF SLIT

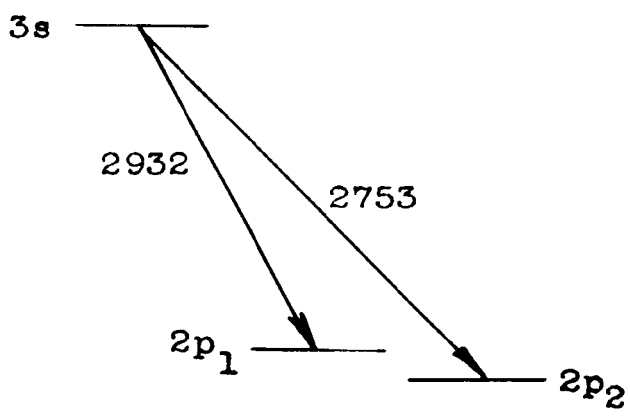


FIGURE 3

INDIUM ENERGY LEVEL DIAGRAM

on the slit which is not interrupted by the sector (slit position A). Intensity 2 corresponds to light through section B which receives one-half of the uninterrupted light. Intensity 1 corresponds to light through section C which receives one-quarter of the uninterrupted light. With the sector method any group of two or three lines in the spectrum can be used to calibrate the plate. It is preferable that the chosen lines be from the same element and bear a constant intensity ratio to each other, as is the case when the radiations originate from the same energy level. For example, In 2753Å and In 2932Å both originate from the same 3s level as indicated in Figure 3.(85) However, it is feasible to use lines from different elements and deal exclusively with the intensity ratio between the sector steps.

To obtain the H. & D. calibration curve the line density is plotted against the logarithm of the exposure. For step ratios of 1:2:4 and using a logarithmic base 1.5 the log ratios between the steps are $\log_{1.5} 2 = 1.7$ and $\log_{1.5} 4 = 3.4$. A line whose densities will obviously place it on the linear portion of the H. & D. curve is plotted first. Lighter lines and darker lines are then plotted to fill in the toe and knee of the curve, always using the postulate that the intensities of the steps are separated by a log ratio of 1.7.

It is noted that the use of a rotating sector is especially desirable with biological materials because of the wide range of intensities involved. Those elements

which exist in high concentrations may be read on the "low" step spectrum at 1/4 of their original intensities while the low concentration elements can be read on the "high" step spectrum at their full strength. This flexibility can be further increased, if desired, by changing the angular openings and thus the intensity ratios of the steps. For example, the angular openings of the sector may be changed so that the light intensities striking the slit are to each other as 1:3:9. This sequence is not as favorable for plate calibration, however, as 1:2:4.

Eastman Kodak 33 plates were used for this study because of the greater speed with low intensity radiation. Furthermore, they have a very desirable uniform gamma over a wide range of wavelengths and are sensitive from roughly 2200Å to 5500Å.[86] The plates were developed for 2-1/2 minutes in D-19 developer, stopped for 15 seconds in a 2% acetic acid stop bath, and fixed for 3 minutes in rapid (x-ray) fixer. The plates were then washed for 15 minutes and air dried. The intensity measurements were made on a Vincent-Sawyer type microphotometer.[87]

CHAPTER IV

WET SAMPLE METHODS

1. EVALUATION OF WET SAMPLE METHODS

Two major classes of methods are available for introducing the liquid sample into the analytical discharge gap. These are:

a) Rotating electrode methods

b) Porous cup methods

a. Rotating Electrode Methods (88, 89)

The rotating electrode is a porous carbon disk approximately 1/2 inch in diameter and 1/8 inch thick. The bottom of this electrode is permitted to dip into the biological material which is contained in a porcelain boat. The sample material is carried upward by the rotating electrode until it is consumed in the analytical discharge gap. This arrangement is indicated in Figure 4 where A represents the counter electrode, B the analytical gap, C the rotating electrode, and D the sample fluid.

The rotating electrode methods encounter the following problems. (a) The residue remaining after discharge which clings to the wheel is continuously polluting

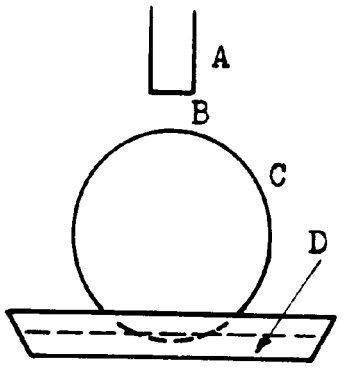


FIGURE 4

ROTATING ELECTRODE ENSEMBLE

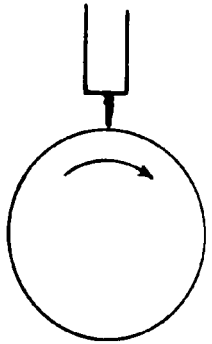


FIGURE 5

ROTATING ELECTRODE INITIAL ARC DISCHARGE

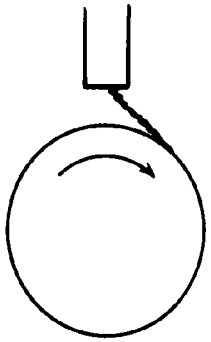


FIGURE 6

ROTATING ELECTRODE LATER ARC DISCHARGE

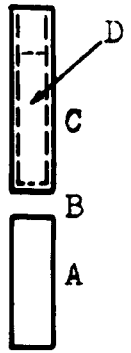


FIGURE 7

POROUS CUP ENSEMBLE

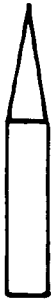


FIGURE 8

POINTED COUNTER ELECTRODE



FIGURE 9

FLAT COUNTER ELECTRODE



FIGURE 10

SHAPED COUNTER ELECTRODE

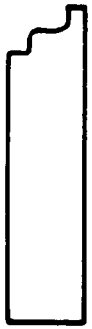


FIGURE 11

COUNTER ELECTRODE FORMING TOOL

the initially pure sample in the boat. (b) Special care must be taken to prevent foreign particles from falling into the open boat and contaminating the sample. (c) If the wheel turns too fast (say 40 r.p.m.), the periphery of the wheel is so wet that the discharge does not run smoothly and reproducibly. (d) The level of fluid sample supply to the wheel changes fairly rapidly during the discharge time. This means that the "wetness" of the wheel periphery may change considerably during an energetic discharge. (e) The ratio of sample excited to sample soaked up by the carbon wheel is small enough to be unfavorable for small sample analysis. (f) An arc discharge has difficulty striking to the wet wheel periphery. Once the discharge has struck, it maintains contact with the initial striking point for as long as possible. Thus the arc discharge strikes directly across from the upper electrode (see Figure 5) and clings to this point as the wheel rotates (see Figure 6) until the voltage phase changes.

b. Porous Cup Methods[90-92]

The most common type of porous cup is a hollow carbon cylinder with 1/4 inch outside diameter, 5/32 inch inside diameter, and 0.025 inches bottom thickness. The sample material is introduced into the top of the porous cup and it slowly seeps through the thin carbon bottom into the analytical gap. In Figure 7 A represents the counter electrode, B represents the discharge gap, C is

the porous cup, and D is the sample fluid.

The porous cup methods encounter the following problems. (a) Variation in the porosity of the thin carbon bottom causes a variation in the rate of flow of sample into the discharge gap. (b) Occasional irregularities in the thickness of the thin bottom due to the machining operation cause a variation in sample seepage. (c) Occasional pock marks exist on the thin wall bottom caused by air holes in the carbon rod from which they were machined. These may lead to variations in sample flow. (d) When an energetic source is used, there is a tendency for bubbles to form in the sample near the bottom of the porous cup. These bubbles expand rapidly ejecting the sample from the top of the porous cup. (e) The inability to dissipate large amounts of heat without sample ejection vitiates the use of porous cups with an arc discharge for all but the most feeble of arcs.

A short investigation on the relative merits of the rotating electrodes and the porous cups indicated that while no significant difference existed between the two, the porous cups promised slightly greater reproducibility than the rotating electrodes. It was also felt that the manipulations involved with the porous cups were somewhat easier than those with the rotating electrodes. The porous cups were therefore selected as providing the most promising method of introducing the liquid sample into the discharge gap.

2. SURVEY OF POROUS CUP PARAMETERS

a. Counter Electrode Shape

The shape of the counter electrode tip used with the porous cup is very influential on the excitation. For example, relatively high spectral intensity is achieved with a counter electrode sharpened in a pencil sharpener, but the discharge is so localized that a hole is burned in the porous cup bottom after a few seconds of operation. This pointed shape is shown in Figure 8.

In contrast to the pointed electrode behavior, a flat-top electrode causes preferential sparking at the edges of the electrode. This preferential sparking to the rim results in decreased spectral intensity and reduced precision. The flat-top electrode shape is shown in Figure 9.

A compromise counter electrode shape which was found to work very well is shown in Figure 10. This electrode shape was achieved by grinding a lathe tool bit to the reverse shape (see Figure 11) and using the lathe cross-feed to make the tool bit form the desired shape. This resultant shape tends to confine the discharge to the central region of the porous cup bottom -- giving better fluid flow and better sensitivity. The counter electrode, however, is sufficiently rounded that no burn-through and no preferential sparking occurs. Inspection of the porous cup after sparking shows a nice, uniformly shaped concave bottom which is satisfactorily moist with sample. Further evidence of the uniformity of the discharge is given by the even burning

which appears localized on the top of the counter electrode.

b. Porous Cup Bottom Depth

The porous cup bottom depth is nominally 0.025" thick. An investigation was made to determine whether a greater or a lesser thickness would furnish improved sample supply to the discharge gap. Six porous cups (type 202) were placed in a lathe and faced until three had 0.005 inches bottom depth and three had 0.015 inches bottom depth. These were run with Source No. 1 (see Appendix I) along with three regular 0.025 inches bottom depth porous cups and three porous cups with carbon tamped into the bottom to make a bottom depth of 0.050 inches.

The information gained from running these carbons is plotted in Figure 12. It may readily be seen that a variation in the bottom depth does not greatly affect the spectral intensity obtained. For smaller bottom depth thickness not only is the intensity somewhat less but also the burn-through time is less. Any intensity improvement for greater than the standard 0.025 inches bottom depth is insignificant. Furthermore, it is evident that slight variations in porous cup bottom depths due to the manufacturing process will not greatly affect the spectral intensities obtained.

c. Selection of the Optimum Source

Primary considerations for porous cup work deal with conflicting problems. The desire for suitable spectral sensitivity is normally satisfied by the dissipation of

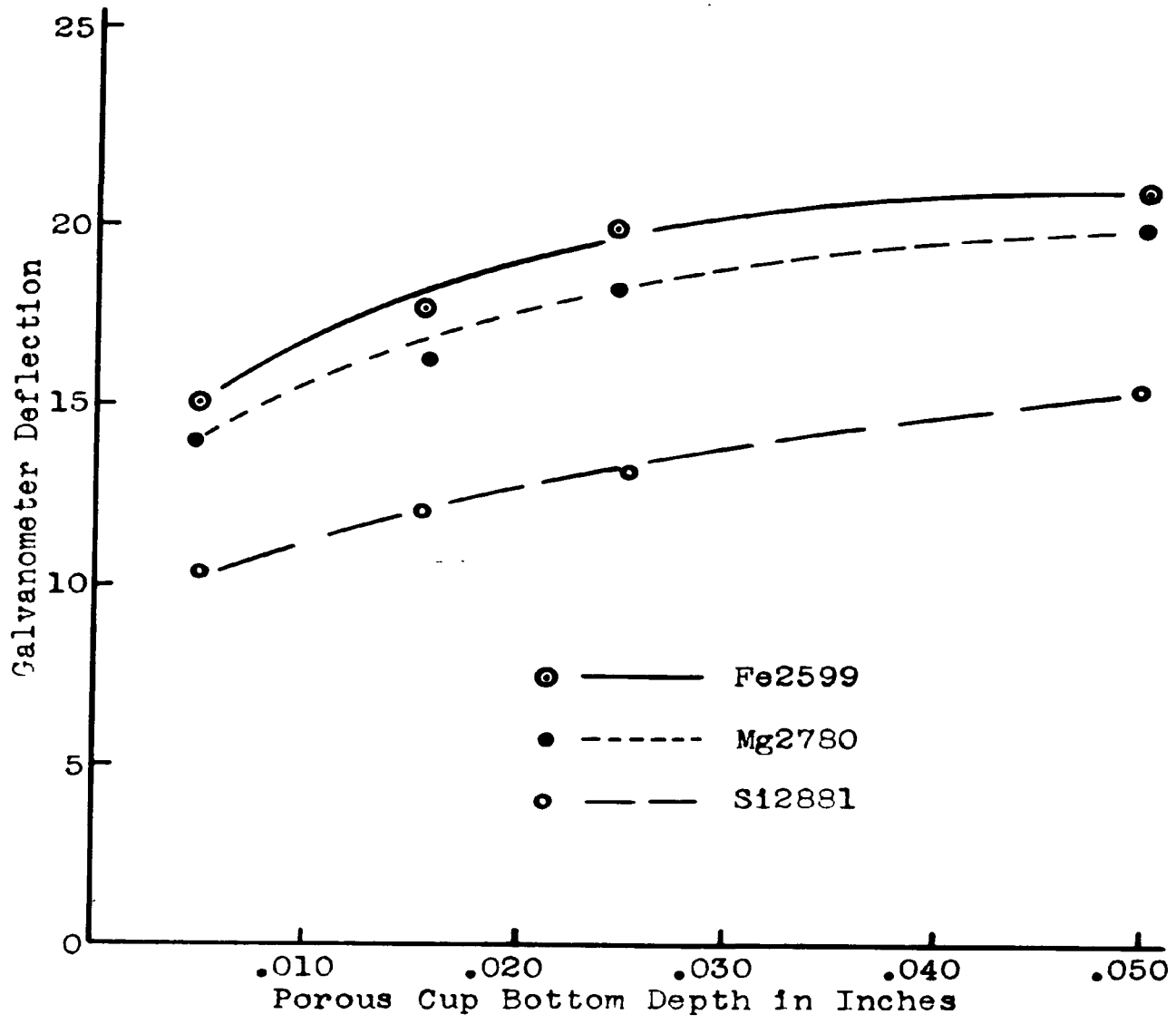


FIGURE 12

SPECTRAL BLACKENING VS POROUS CUP BOTTOM DEPTH

large amounts of energy in the discharge gap. This, however, is in conflict with the necessity of maintaining the electrode heating at a low level to prevent the formation of gas bubbles in the sample. The gas bubbles thus formed expand rapidly to blow the sample liquid out the top of the porous cup. Any compromise between sensitivity versus sample retention must pass the further test of being compatible with both good line-to-background ratio and good reproducibility. Thus the discharge source chosen for use with porous cups on small biological samples must satisfy four prime requirements. These are:

a) retention of sample

b) high sensitivity

c) good reproducibility

d) good line-to-background ratio

In a spark discharge circuit the most influential factors on these four items are the secondary capacitance and the secondary inductance. As the capacitance in the discharge circuit is increased, the gap current is increased and more heating takes place, yielding higher sensitivities. As the inductance in the discharge circuit is increased, the discharge becomes more arc-like in character. The duration of the discharge is increased and the general radiation background is decreased, giving better line-to-background ratios.

The secondary resistance, while variable, plays a subordinate role of dampening the r.f. oscillations. It is

desirable to keep this resistance small in order that the total gap energy will not be so dependent upon the gap spacing.(93)

The study of spark sources was begun with an investigation of the effect of the following sources on porous cups:

a) low capacity, low inductance

b) low capacity, high inductance

c) high capacity, low inductance

d) high capacity, high inductance

The first data of interest are displayed in Figure 13. The iso-current lines for different capacity and inductance emphasize that the highest currents and hence the highest spectral intensities are obtained with high secondary capacity and low secondary inductance. The spectral radiation background in Figure 13 is shown to increase markedly as the circuit inductance is decreased. Thus, there are two conflicting entities as featured below.

	High Capacity Low Inductance	Low Capacity High Inductance
Desirable Attributes	High Sensitivity	Low Background
Undesirable Attributes	High Background	Low Sensitivity

Some compromise must be achieved between a high-capacity-low-inductance source which furnishes desirable high sensitivity with undesirable high background and a low-capacity-high-inductance source which furnishes

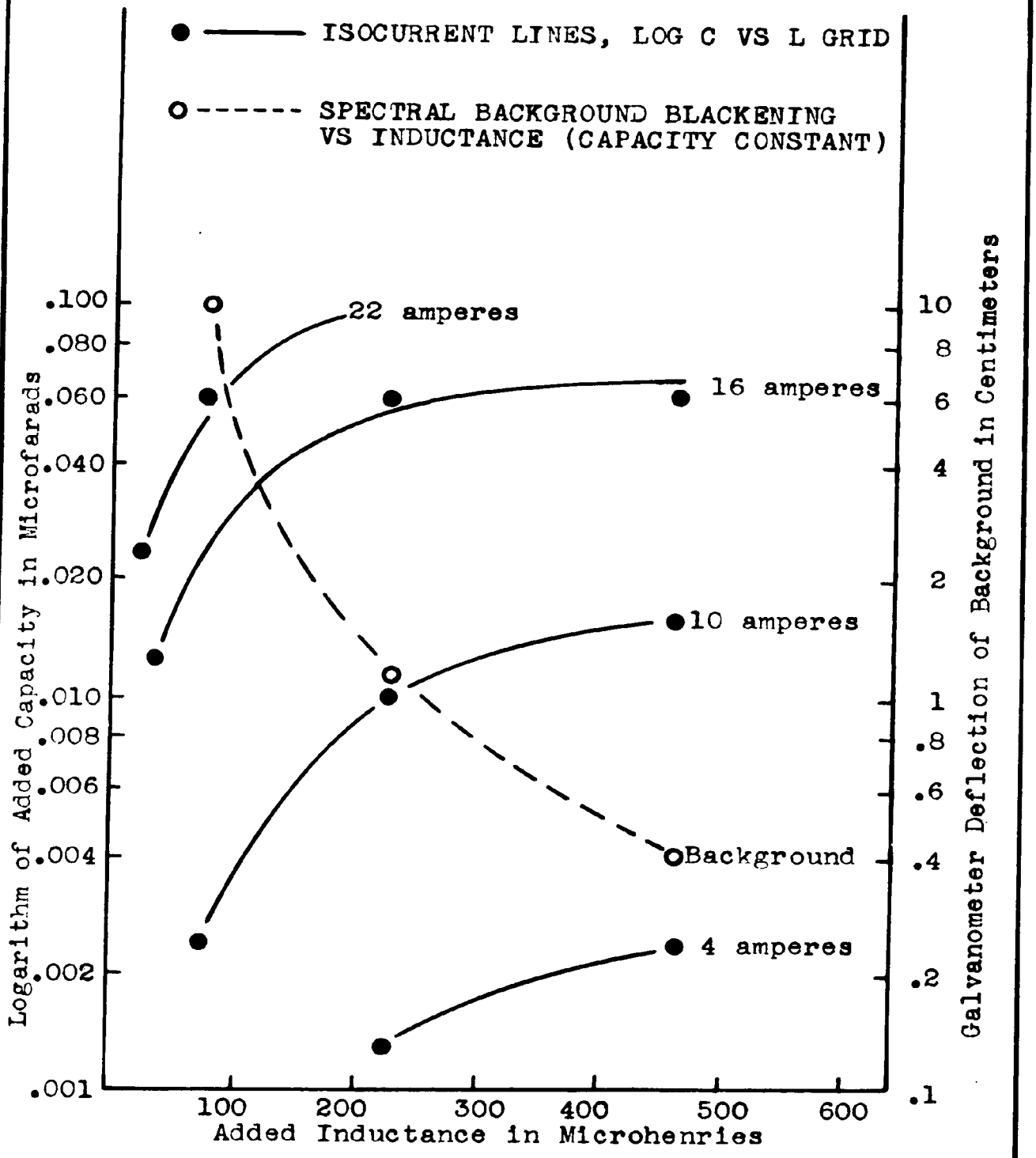


FIGURE 13

ISOCURRENT LINES ON A LOG C VS L GRID

undesirable low sensitivity and desirable low background. The fundamental problem here, then, is to find the values of inductance and capacitance which will furnish the highest line-to-background ratio which is compatible with high sensitivity and good reproducibility.

The domain of high capacity has an additional conflict in that the high currents (of the order of 20 amperes) dry the surface of the porous cup faster than the sample can be replenished. This tends to decrease the spectral intensity and increase the background intensity.

The area of high capacity (0.06 microfarads) and high inductance (250 microhenries) is difficult to investigate when using porous cups. A continuously running source generates heat so fast that the sample is rapidly ejected from the top of the porous cup. It was, however, possible to explore this region by pulsing the source with a one second exposure followed by a 10 second wait. This allowed time for some of the heat to dissipate and time for the sample to seep through and remoisten the end of the carbon. By making a summation of such pulsed intervals it was possible to build up exposures ranging from 15 seconds to 120 seconds. If high capacity and high inductance had proven especially desirable, a circuit could have been constructed to accomplish this process automatically. However, the line-to-background ratio achieved in this region was inferior to that obtained from other combinations of C and L so high capacity and high inductance were given no further consideration for

porous cup work. Although, usually, added inductance improves the line-to-background ratio, here it did not. The high inductance prolongs the pulse, thereby increasing the heating effects of the discharge. This heat dries the "wet" porous cup bottom so fast that the sample is consumed before the discharge pulse is over. This means that the final period of the discharge pulse is completed on the dry carbon. This greatly increases the background and hence decreases the line-to-background ratio.

It was anticipated that the high background found associated with the high capacities could be reduced with a reduction in the peak current density. The peak current density J is given by:

$$J = V\sqrt{\frac{C}{L}}$$

where V is the breakdown voltage, and C and L are the circuit capacitance and inductance respectively. This expression is valid only for relatively small ohmic resistance. From the equation it may be seen that to reduce the peak current density it is necessary to decrease the capacity and increase the inductance. Alteration of the breakdown voltage requires variation of the control gap spacing. The practical range of variation of the control gap spacing restricts the breakdown voltage to a subordinate role to C and L in influencing the peak current density.

In investigating the low capacity sources it soon

became evident that low capacity and low inductance could not furnish excitation sufficiently sensitive to be practical. A variation of the low-capacity-high-inductance source, however, was found to fulfill the prerequisites for a satisfactory source, viz., satisfactory sensitivity, reproducibility, and line-to-background ratio. It should be pointed out that in most cases the distinction among the various line-to-background ratios was sufficiently great that an evaluation could be made by visual inspection of the photographic plate.

With the problem of source selection narrowed to the low capacity - high inductance region, there were still two details which needed attention:

1) The energy pulse should not last so long nor be so energetic that the electrode surface is dried and devoid of sample before the pulse is completed. The existance of such a condition of sparking on bare carbon means lower intensity line spectra and higher intensity background. This effect can be diminished if conditions are established which favor a rapid replenishment of sample to the carbon tip. It is evident that several short low energy pulses are more favorable in this respect than are a few long high energy pulses. Specifically, in terms of the spark circuit, this means that several breaks per half cycle should be expected to give a better line-to-background ratio than two or three energetic breaks per half cycle. Furthermore, better sampling and better reproducibility should be associated with the larger number of pulses.

2) The energy that can be supplied to the discharge gap has for its upper limit that critical amount which causes ejection of the sample by heating. This is dependent not only upon the energy per pulse but also upon the amount of time provided for the energy of each pulse to be dissipated.

3. RETENTION OF SAMPLE

The solution to the problem of retention of sample when the carbon is subject to energetic spark pulses is the crux of porous cup analysis for small percentage sample constituents. The following work is a survey of the more fruitful methods which evolved to insure sample supply to the discharge.

a. Heating the Porous Cup

The possibility was considered that gases might be occluded in the carbon of the porous cup. With heat applied to the thin bottom these gases might expand, thereby ejecting the sample. To test this premise several porous cups were heated to a red heat. Part of these were allowed to cool in a vacuum and part of these were allowed to air cool. Only a slight improvement in the sample ejection characteristics of these carbons was noted. This indicates that the gas which expands to eject the sample does not originate in the porous cup carbon. Therefore pre-heating of the carbons, while slightly beneficial, is by no means a solution to the problem.

b. Heating the Liquid Sample

Inasmuch as sample ejection is caused by large amounts of heat applied over a short interval of time, one might expect that the bubble growth would be less rapid and less troublesome if the sample were initially heated. To test this possibility a porous cup was loaded with sample, heated to the boiling point and the discharge was started at once. It was found that by this method the sample would run for about 10% longer time before the sample was blown out the top of the porous cup. Although the results were in a favorable direction, the increase in permissible exposure time was not very great and, since the sample was finally ejected anyway, this method was not deemed worth the effort except in a very marginal analysis.

c. Closing the Porous Cup Top

The most satisfactory way of sealing off the top of a porous cup after loading was found to be insertion of a pyrex glass tube sealed at one end. (See Figure 14). Such a glass tube closed at one end does a very effective job of sealing the porous cup from sample ejection. It thereby prevents (a) permanent loss of sample and, (b) possible contamination of the discharge by material running down the outside of the electrodes over the electrode holders. It is noted that electrode holders made from carbon blocks minimize such contamination.

The fact that the sample material does not perma-

nently escape the porous cup by this method does not obviate the initial problem of uniform sample supply to the discharge gap. The intense heat of the discharge causes the sample to "percolate" within the confines of the glass tube and the porous cup. Although in the process of percolation some sample must presumably touch the bottom of the porous cup and introduce sample into the discharge, the amount introduced is so small after percolation begins that the carbon is effectively running dry. Frequently, too, a gas bubble forms in the bottom of the porous cup and holds the sample above it away from the floor of the porous cup and possible entry into the discharge gap.

As an instrument of furnishing prolonged uniform sample supply to the discharge gap, this closed glass rod insert was not satisfactory. The use of this cap, however, introduces no observed undesirable features and it does prevent possible overflow or ejection from contaminating the discharge.

d. Bubble Inhibitors

Various substances were inserted into porous cups to try to inhibit the growth of large gas bubbles which eject the sample. These substances were carbon, filter paper, and cotton.

The first material placed in the porous cup was loosely packed crushed carbon. No ejection of sample occurred with this filler, but the sample did not flow rapidly

enough from the loose filler carbon to the hard packed porous cup to keep the porous cup bottom from running dry. During the discharge time the loose carbon just above the bottom of the porous cup became dry and remained dry for the remainder of the exposure interval.

Next, a porous cup was filled with ashless filter paper. Here the sample fluid was reluctant to leave the filter paper and seep through the porous cup bottom. The line intensity was low with this method indicating negligible sample supply. When the filter paper plug was removed from the porous cup, it was found that the end nearest the discharge was dry while the rest of the plug was still moist.

When the porous cup was loosely packed with a wad of cotton, the result was somewhat better than that for either the carbon or the filter paper packing. The cotton tended to permit the escape of some large bubbles, breaking them up into smaller bubbles as they rose through the cotton. Two conflicting problems are associated with such a plug: (1) As more cotton is packed into the plug, it more effectively prevents ejection of the sample, but it also decreases the available volume remaining for the liquid sample. (2) Decreased amount of liquid sample loaded into the porous cup means decreased heat capacity. The smaller amount of material is vaporized in a shorter time interval and the bubble formation tends to be increased. As a result, too loose a cotton packing does not stop the sample ejection, while a tighter packed plug causes the porous cup to quickly

run dry and the cotton at the bottom of the porous cup chars. A moderately packed cotton plug, while beneficial in extending the exposure time slightly, is not considered a satisfactory solution to the problem.

e. Glass Well

A glass well with a capillary tube was inserted into a porous cup in an attempt to uniformly supply sample to the discharge for a long period of exposure. This particular method was conceived as a means of uniformly supplying sample fluid to the porous cup at roughly the same rate it was consumed by the discharge. The first type of well used is shown in Figure 15. This is a piece of pyrex tubing with an inside diameter of 8 mm. and a slender capillary stem. It was expected that the rate of drip through the capillary stem would be satisfactory to properly meet the consumption needs of the porous cup. In practice, however, it was found that if the stem rested on the porous cup bottom the spark prematurely burned a hole in the carbon directly beneath the contact point of the capillary tube. When the well was supported so that the capillary tube did not touch the bottom, sample bubbles formed which tended to obstruct satisfactory seepage through the porous cup bottom.

f. Glass Well with Pad

Although the glass well method was unsuccessful, it was felt that some variation of this method might prove fruitful. The first variation of the glass well method

involved the use of (a) a larger diameter capillary tube for faster fluid flow, and (b) a small tuft of cotton in the bottom of the porous cup. (See Figure 16). It was anticipated that the small cotton pad would overcome the objections of premature hole piercing by causing the sample to be more uniformly distributed over the bottom of the porous cup.

This new sample well was made from a piece of pyrex glass tubing with an inside diameter of 8 mm. and a slender stem of 1 mm. inside diameter. This stem filled about 1/3 of the available volume in the porous cup.

Initially it was thought that if a tuft of cotton were placed in the bottom of the porous cup, the pressure of contact of the well on the cotton would support a moderate head of fluid, metering it out at some slow rate to the bottom of the porous cup. In practice, however, the flow of sample was much greater than the rate of consumption in the discharge. Consequently, the sample soon filled and overflowed the top of the porous cup.

g. Glass Well with Pad and Plug

A successful variation of the previous method involves the use of a cotton pad and a cotton plug. Figure 17 shows the cotton pad A in the bottom of the porous cup and the cotton plug B near the top of the glass well. The loading technique for this method is as follows. First a plug of cotton is placed at B in the well, then an eye dropper

bulb is placed over the end at C (see Figure 17). The stem orifice is placed in the sample and the eye dropper bulb is squeezed and released to draw sample up into the well until it soaks the cotton plug at the top of the well. The stem of the well is then placed on the tuft of cotton in the bottom of the porous cup and the eye dropper bulb removed By this method the head of sample fluid is sustained at its original level for long periods of time so long as no discharge is taking place. When a discharge is taking place, this well meters out the fluid as requested by the drying of the cotton in the bottom of the porous cup.

In operation with a moderate source (see Source No. 1, Appendix I) tiny bubbles stream upward through the sample forming an air space just below the plug at B (see Figure 17). As the sample is consumed in the discharge, this air space increases. The plug at B retains some sample to form a liquid seal across the top. Occasionally, small bubbles stream upward through this plug, indicating that it is capable of adjustment by "breathing."

The performance of this well with Source No. 1 is shown in Figure 18. This is a direct reading record of a 10 minute exposure using a supply well. The intensity of over-all light from the source is recorded here along the horizontal and the time-of-run along the vertical. The great uniformity of intensity and hence uniformity of sample supply is at once evident. The introductory period for which a pre-exposure would be required is observed to



FIGURE 14

CAPPED POROUS CUP



FIGURE 15

GLASS WELL IN POROUS CUP



FIGURE 16

GLASS WELL WITH PAD

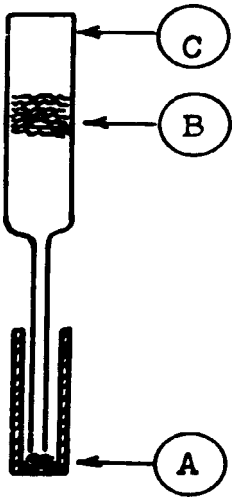
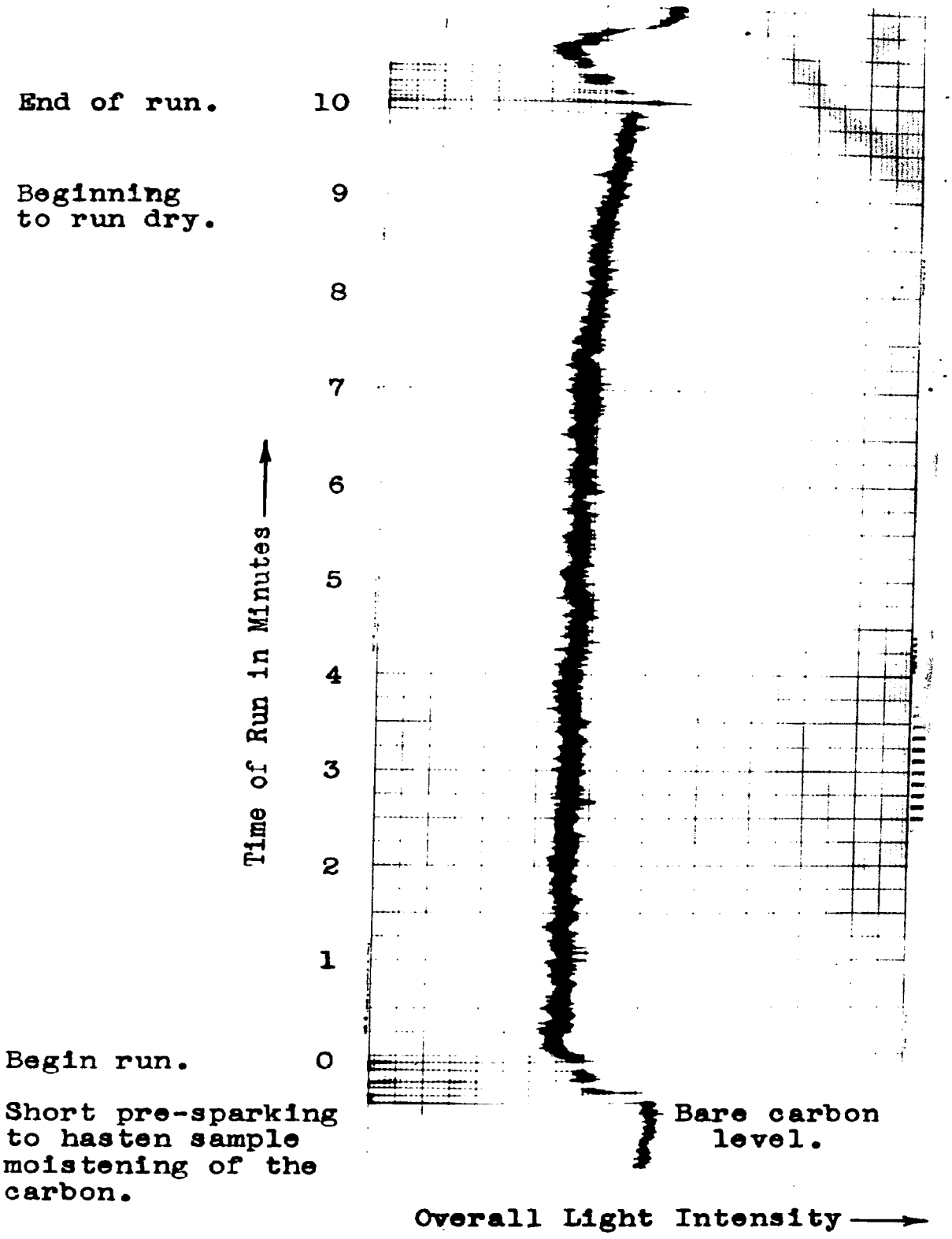


FIGURE 17

GLASS WELL WITH PAD AND PLUG

FIGURE 18

DIRECT READING RECORD



be small, of the order of 5 seconds. The deflection of the intensity at the end of the run is indicative that the sample is exhausted and the porous cup is beginning to run dry.

The influence of the shape of the well upon its performance was investigated. Some typical types of wells are shown in Figure 19. These are:

a) Small capillary, small reservoir

b) Large capillary, small reservoir

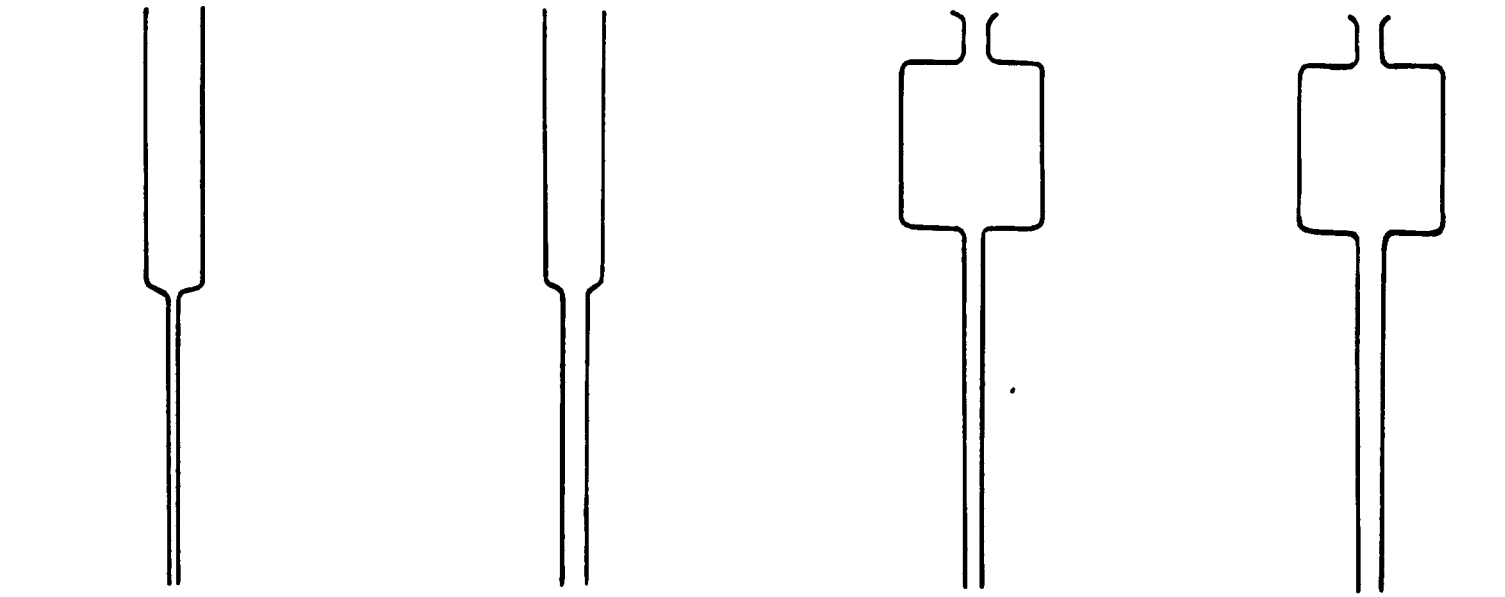
c) Small capillary, large reservoir

d) Large capillary, large reservoir

The various types are actually quite similar in performance. All are capable of furnishing exposure times which are increased at least four-fold over the porous cup filled with a normal sample load. It is of course possible to superimpose several regular porous cup exposures but most often a single continuous uniform exposure is preferred.

The cotton pad at A in Figure 17 was replaced by a tiny disk cut from a filtration accelerator pad. No change in performance was noted. Both the filtration accelerator disk and the cotton pad seemed to work equally well. The filtration accelerator pad was preferred, however, because the pads are approximately the same thickness so that when the disks are punched out they are nearly identical. In contrast it is difficult to load the porous cup with "tufts" of cotton which are nearly identical.

Although these various reservoirs worked very well with a moderately energetic source, they could not perform



SMALL CAPILLARY
SMALL RESERVOIR

LARGE CAPILLARY
SMALL RESERVOIR

SMALL CAPILLARY
LARGE RESERVOIR

LARGE CAPILLARY
LARGE RESERVOIR

FIGURE 19

VARIOUS GLASS WELLS

with a really energetic source. When large amounts of heat were developed in the gap, a large bubble of gas would force an entry into the bottom of the capillary tube and stay there, neither rising in the capillary tube nor permitting the liquid to flow downward (see Figure 20). The porous cup then began to run dry at once.

In summary then one can say that this method might prove valuable where the ultimate in sensitivity is not required. Satisfactory performance of these reservoirs can be obtained with a source similar to Source No. 1, Appendix I.

h. Inverted Porous Cups

Although the previous method was a promising development for moderate sources, it still did not possess the sensitivity it was felt porous cups could deliver. Yet in order to achieve higher sensitivities it was necessary to control the large amounts of heat generated in the discharge gap. Fundamentally, this heat makes the sample boil. That is to say, in a thin layer on the bottom the temperature of the liquid sample is raised until the saturated vapor pressure is equal to the external pressure on the surface of the liquid. Bubbles of saturated vapor are then produced in the liquid near the bottom of the porous cup which expand rapidly to eject the sample. It was anticipated that if the porous cup was inverted and used as a lower electrode, the following advantages might be realized:



FIGURE 20

CAPILLARY BUBBLE



FIGURE 22

PIERCED POROUS CUP

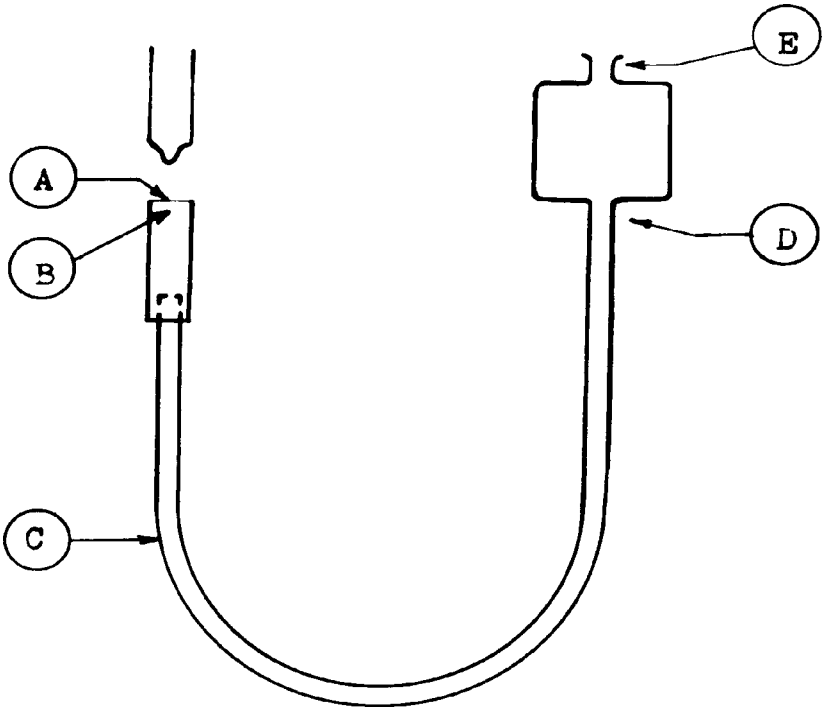


FIGURE 21

INVERTED POROUS CUP

a) Since heat from the discharge rises, the lower electrode should not get as hot.

b) More energy can be dissipated in the gap with a relatively lower temperature for the porous cup electrode.

c) Since heated vapors rise, it might be possible by puncturing a hole in the porous cup bottom to permit them to escape harmlessly into the discharge gap.

Figure 21 illustrates a typical set-up with this method. The lead-in tube may be either a one piece pyrex tube which fits snugly into the porous cup or it may be in two pieces with a piece of tygon tubing splice at some point such as C. With the tygon tubing union the unit is much more flexible and there exists less danger of breakage.

The loading technique for this method is as follows: First a sharpened graphite stick is used to pierce a tiny hole in the porous cup bottom at A in Figure 21. Then a cotton plug is inserted at B and the porous cup is loaded with sample. The lead-in tube is loaded with sample and inserted into the porous cup, making sure there are no trapped air bubbles. More sample is added to the lead-in tube to bring the level in the reservoir bowl to about 1 cm. above the discharge surface of the porous cup. This head of fluid helps to keep the fluid flowing and to push gas bubbles formed out through the escape hole A. The bowl of the well should be supported loosely at E.

By employing this method of introducing the sample into the discharge gap it is possible to use a much more energetic source. Source No. 2, Appendix I, represents the maximum practical source with this set-up. Although the r.f. secondary current is only 5 amperes, there is a large amount of inductance (225 microhenries) in the circuit which both prolongs the discharge pulse and improves the line-to-background ratio. This source is sufficiently energetic that 30 seconds represents the maximum usable exposure time. Beyond this there begin to occur failures due to vapor bubbles which form in the porous cup. These bubbles form faster than they can escape through the pierced hole A indicated in Figure 21. The sample is thus held away from the discharge surface and the carbon runs dry. In this sense about 25% of the exposures are failures when the exposure time is extended to 40 seconds.

It should be noted that it is possible to use Source No. 2 with the glass-well-and-pad arrangement of Figure 16 providing a small hole is pierced in the bottom of the porous cup. This method is indicated in Figure 22. The ability of this set-up to operate with energetic Source No. 2 is dependent upon a more rapid flow of liquid to the gap. This not only cools the electrode but somewhat quenches the discharge. In operation, this method is not as sensitive nor as reproducible as supplying the sample from below with an inverted porous cup.

In the investigation to this point the inverted porous cup method represented the most sensitive and reproducible method of analysis. The manipulations were certainly more difficult than for the regular porous cup technique, but the inverted porous cup method improved both the sensitivity and the line-to-background ratio.

When sample was permitted to flow into the discharge from both the upper and lower porous cup, the background was decreased. However, the overall gain of reproducibility and low background was too small to warrant the extra sample handling manipulation. Also, it was noted that when the sample was furnished from both upper and lower electrodes it was even more difficult to prevent sample ejection from the upper porous cup.

An asymmetry exists in porous cup analysis which might be exploited to improve the line-to-background ratio, the sensitivity, or the reproducibility; viz., the sample liquid is furnished to the discharge gap always from one side, the side of the porous cup. The dry counter electrode is situated on the other side of the gap. The possibility exists that this asymmetrical set of electrodes might show more favorable sensitivity, reproducibility, or line-to-background ratio for a rectified current.

To test the possible improvements associated with a rectified spark, a rectifying circuit was established similar to that suggested by Enns and Wolfe[94]. A comparison was then made between the behavior of the porous

cup as the cathode and its behavior as the anode. No particular distinction in sensitivity or line-to-background ratio was noted between the two polarities.

A comparison was then made between the reproducibility of rectified exposures versus non-rectified exposures. The data from 20 runs by each method are given in Table I.

To compensate for sparking intervals lost by rectification, the primary variac was turned to increase the secondary voltage and thus the number of breaks per half-cycle. By this method 5 amperes of r.f. current were achieved with both the rectified and the non-rectified source.

From the data of Table I it is evident that very little distinction exists between the reproducibility of the rectified and the non-rectified source. This is in agreement with oscillographic studies made by Dieke, Loh, and Crosswhite[95]. The rectified spark discharge is not truly unidirectional but is effectively an oscillatory discharge which has been initiated from one direction as indicated in Figure 23. Thus, although the breakdown of the discharge always occurs in the same sense, the energy of the circuit is dissipated largely in an oscillatory manner.

This conclusion is also in agreement with a calculation of the logarithmic decrement of the discharge circuit. From Appendix II it may be seen that the natural logarithm of the ratio of the amplitudes of successive oscillations is given by:

TABLE I

Rectified and Non-Rectified Spark Precision

Log $\frac{Fe3020}{In2753}$		Log $\frac{Mg2852}{In3039}$		Log $\frac{Si2881}{In2932}$	
Rectified	Non-Rectified	Rectified	Non-Rectified	Rectified	Non-Rectified
-.30	-.26	-.49	-.50	-.05	.00
-.28	-.35	-.51	-.39	-.03	.00
-.31	-.37	-.43	-.33	.04	-.01
-.28	-.43	-.48	-.40	.03	-.03
-.30	-.51	-.39	-.40	.03	-.05
-.17	-.39	-.49	-.55	.03	.01
-.28	-.22	-.58	-.43	.00	.03
-.20	-.42	-.50	-.30	-.03	-.01
-.10	-.25	-.59	-.43	.00	-.05
-.16	-.42	-.43	-.61	-.07	-.01
-.14	-.24	-.42	-.46	-.01	-.01
-.29	-.29	-.47	-.49	.03	-.03
-.34	-.37	-.58	-.40	.03	-.01
-.43	-.55	-.25	-.31	-.01	-.06
-.29	-.52	-.33	-.42	.00	.01
-.29	-.40	-.43	-.32	.05	.03
-.48	-.50	-.38	-.32	-.01	-.05
-.52	-.52	-.35	-.37	-.03	.05
-.41	-.51	-.40	-.28	-.03	-.05
-.44	-.55	-.36	-.39	.03	.09
Avg.-.30	-.40	-.44	-.41	.00	-.01
Std. Dev. .11	.11	.09	.08	.03	.04

$$\delta = \frac{R\pi}{\omega L}$$

Likewise, it is found that the number of oscillations n required for the amplitude to fall from an initial amplitude A_i to a final amplitude A_f is given by:

$$n = \frac{1}{\delta} \text{Log}_e \frac{A_i}{A_f}$$

For Source No. 2 $\delta \sim 10^{-1}$

Breakdown voltage $A_i \sim 18{,}000$ volts

Minimum sustaining voltage $A_f \sim 90$ volts

So, $n \sim 50$

Since the period of oscillation $T = \frac{2\pi}{\omega} \sim 4$ microseconds, the time for n oscillations is approximately,

$$n\,T = 200 \text{ microseconds}$$

Inasmuch as the discharge pulse for this source takes approximately 50 oscillations to be extinguished, most of the energy lies in the oscillatory portion of the spark train rather than in the initial pulse. In fact, it can be shown by calculation that less than one-tenth of the original condenser energy is dissipated in the first pulse. (See Appendix II).

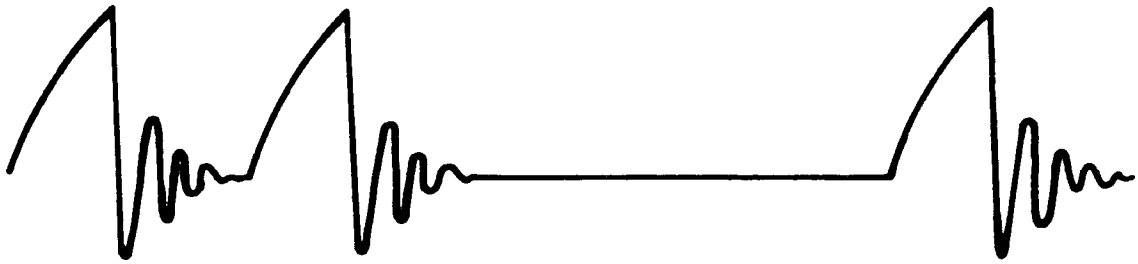


FIGURE 23

RECTIFIED CURRENT VERSUS TIME

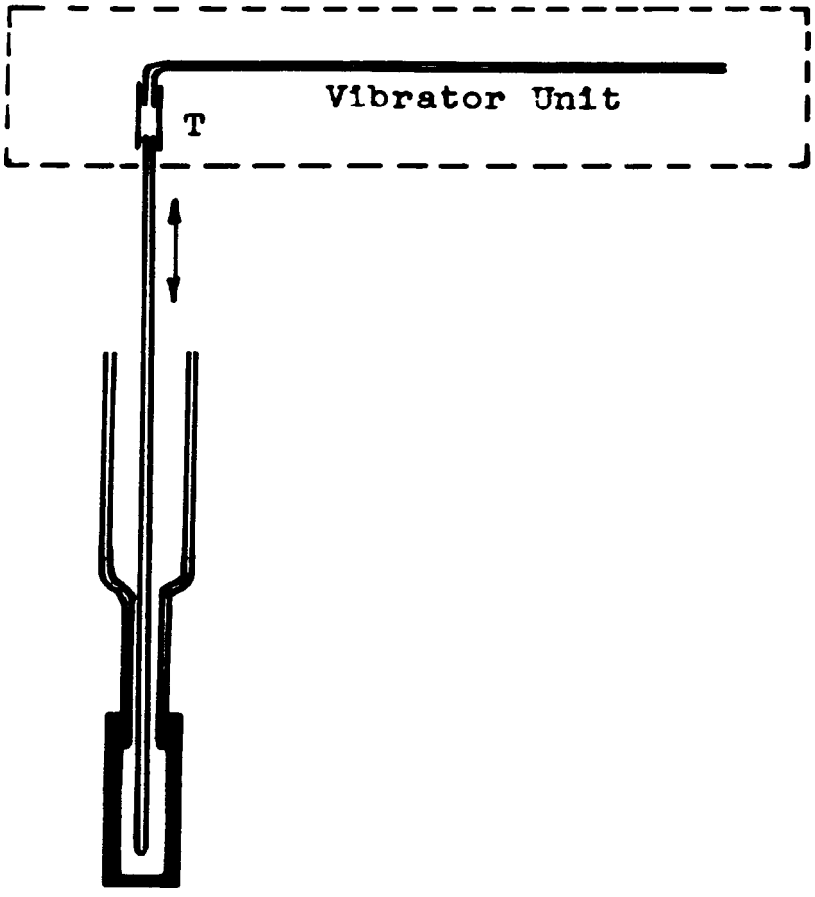


FIGURE 24

AGITATOR ROD ENSEMBLE

1. Use of an Agitator Rod

One may overcome the difficulty of sample ejection either by preventing the formation of the bubbles or by breaking the large bubbles up into smaller bubbles which will pass harmlessly to the surface of the liquid. Figure 24 illustrates a method of employing an agitator rod to stir the liquid sample. Sample ejection is thus reduced by both inhibiting the formation of bubbles and breaking up the ones that are formed. The agitator rod actually used was a piece of 1 mm. pyrex glass cane. This was coupled to the vibrator with a piece of small rubber tubing (electric wire insulation) to make the vibrating unit more flexible and less fragile. The vibrator (a bell ringer) drives the rod up and down like a piston, causing the hot liquid near the bottom to be mixed with the cooler layers of the liquid. This pile driver action tends also to fracture any large bubbles that are formed and assist them in reaching the surface without ejecting the sample. The use of a glass well whose stem fits the bore of the porous cup is desirable but not necessary. Use of the well permits more sample to be loaded. The larger amount of fluid provides more cool liquid to mix with the heated layer next to the porous cup bottom. This means that longer exposures are possible, or that a more energetic source can be used. For instance, by this method it is possible to increase the current to 8 amperes for a 30 second exposure. (Source No. 2, but with 0.014 microfarad capacity). The bubble formation and the background continue to increase

for currents above 5 amperes. However, even at 16 amperes (Source No. 2, but with .026 microfarads capacity) the violence of the bubble formation is controlled enough so that the sample stays within the confines of the porous cup and well.

It should be noted that although the 8 ampere source and the 16 ampere source are more sensitive in the sense of darker spectra, the 5 ampere source (regular Source No. 2) actually furnishes a better line-to-background ratio.

j. Goose-Neck Reservoir

The "goose-neck" wells used in this method are shown in Figure 25 and Figure 26. The first well employed was that of Figure 25. A 2 mm. inside diameter pyrex tube is used to fit inside the porous cup. This is beveled at the end to permit the sample to flow while it is resting on the bottom. To this tubing is affixed a piece of heavy capillary tubing. This furnishes the ensemble with greater rigidity so that it is not quite so fragile. This capillary tubing is then joined to the storage bowl. In operation an eye dropper bulb is placed over the bowl at B and squeezed and released to permit sample to be drawn into the bowl. The beveled end is then set down into the porous cup and the bowl is loosely supported at D. When the eye dropper bulb is removed, the sample flows into the porous cup until the level C' is equivalent to the level C in the bowl. When the discharge starts, sample is consumed but the level C' is maintained at approximately the same height by the

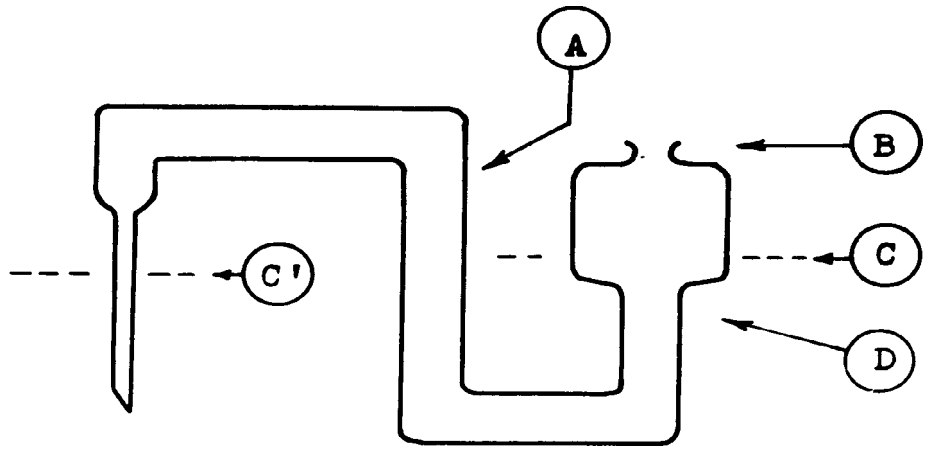


FIGURE 25

GOOSE-NECK RESERVOIR

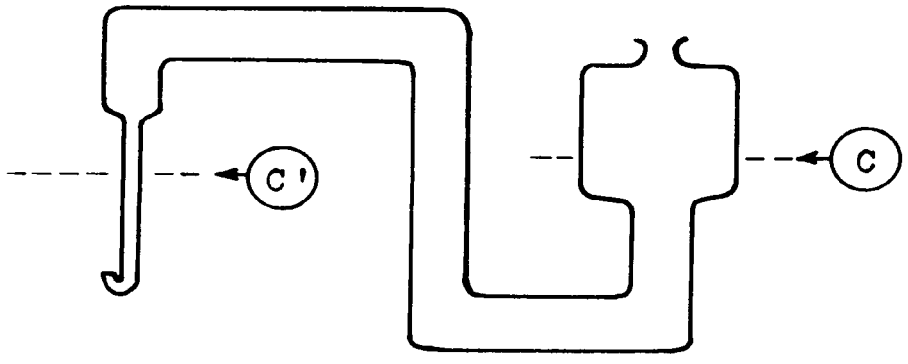


FIGURE 26

GOOSE-NECK RESERVOIR WITH A BENT TIP

inflow of sample from the bowl. Thus even if bubbles form and blow the sample higher in the porous cup, new sample rushes in at the bottom to replace it.

It sometimes happens, however, when using an energetic source, that a violent bubble will "bump" the liquid in the capillary tube over to the locale of A in Figure 25. The porous cup then rapidly runs dry. Although not a common occurrence, it certainly is undesirable. This particular feature is overcome by carefully bending over the tip of the lead-in tube as shown in Figure 26. None of the previous virtues are affected, but expanding bubbles traveling upward do not penetrate the capillary tube. The carbon then is always moist with sample and the uniform burning of the discharge is evident.

The arc-like character of the discharge manifests itself in the counter electrode which gets red hot during the 30 second exposure interval. The maximum running time is no longer limited by the sample ejection but by the time it takes for the discharge to burn a hole in the bottom of the porous cup. This time (with Source No. 2) is normally about 90 seconds ± 10 seconds.

To test the reproducibility of this method several repeat exposures were taken. The exposure procedure was as follows: First the porous cup and counter electrode were placed in a jig to align for proper gap spacing and then they were clamped in the electrode holders. The well was then loaded with sample and the "button-hook" tip was in-

serted into the porous cup and the eye dropper bulb withdrawn. Usually 1 ml. was loaded into the well even though only about 1/4 ml. is consumed per 30 second exposure. A few seconds wait at this point permitted the sample to seep through and moisten the bottom of the porous cup. The discharge was then begun. A pre-exposure of 5 seconds was taken before opening the shutter. A 30 second exposure was then taken. At the completion of this exposure the plate was racked down and another 5 second pre-exposure, 30 second exposure was taken without altering the sample electrodes in any way. No new sample was added to the well. This process was repeated a third time so that three exposure intervals were sampled. The standard deviations for these intervals are compiled in Table II under items 1, 2, 3, and 4. Item 4 consists of exposures for which the porous cup had had a hole burned in it near the end of the time interval.

To evaluate this table one should bear in mind the rough rule of thumb that a spectrographic process is considered to yield near optimum results when the standard deviation for the log ratios is $\sigma = \pm .10$ or less. One can then normally expect analysis variations of approximately $\pm$ 3% or less.

Item 5 is the data of 1C repeat, 60 second exposures with a 5 second pre-exposure. These show little variation from the 5" - 30" exposures.

Items 6 and 7 represent exposures taken with two different wells which were in all major respects identical.

TABLE II

Porous Cup Precision

Item	Number of Repeat Spectra	σ Summary for Indicated Line Pairs						Exposure Interval
		Fe2599 / Mo2816	Fe2599 / In2753	Mg2852 / Mo2816	Mg2852 / In3039	Si2881 / Mo2816	Si2881 / In2932	
1	30	.11	.17	.09	.06	.09	.08	First 5" - 30"
2	16	.08	.17	.08	.12	.15	.06	Second 5" - 30"
3	4	.07	.10	.05	.04	.06	.07	Third 5" - 30"
4	4	.08	.22	.12	.09	.24	.08	Third* 5" - 30"
5	10	.10	.13	.10	.08	.09	.08	First 5" - 60"
6	17	.11	.19	.10	.06	.10	.09	First 5" - 30"
7	13	.11	.13	.09	.05	.06	.08	First 5" - 30"

$$\sigma = \sqrt{\frac{\Sigma(\Delta x)^2}{n}}$$

* Carbon burned through near the end of the exposure interval.

It is evident that so long as the wells agree in basic construction the reproducibility values are not greatly affected.

Even though both molybdenum and indium are well suited for this biological matrix, there exists a distinction in how compatible they are with any given element. For instance, iron shows greater reproducibility with molybdenum internal control whereas slightly better values for magnesium are indicated when indium is the control element. For the analysis of such a large element matrix, it is deemed prudent to use both elements with porous cups where the ultimate in accuracy is desired. When one element is used for control, molybdenum would be a proper choice.

4. REPRODUCIBILITY MEASUREMENTS

The analytical curves of Figures 27, 28, and 29 represent data from four different types of solutions. These solutions were prepared as indicated in Table III. The actual log ratios of these curves are disregarded and the curves are presented side-by-side, slightly displaced from one another to facilitate a comparison of their slopes. Steeper slopes are to be associated with wider percentage variations of reproducibility. It is evident that there is no major distinction among the slopes of curves pertaining to any one element. That is, the addition of indium or ammonium nitrate to the hair-molybdenum base solution causes practically no change in slope of the analyti-

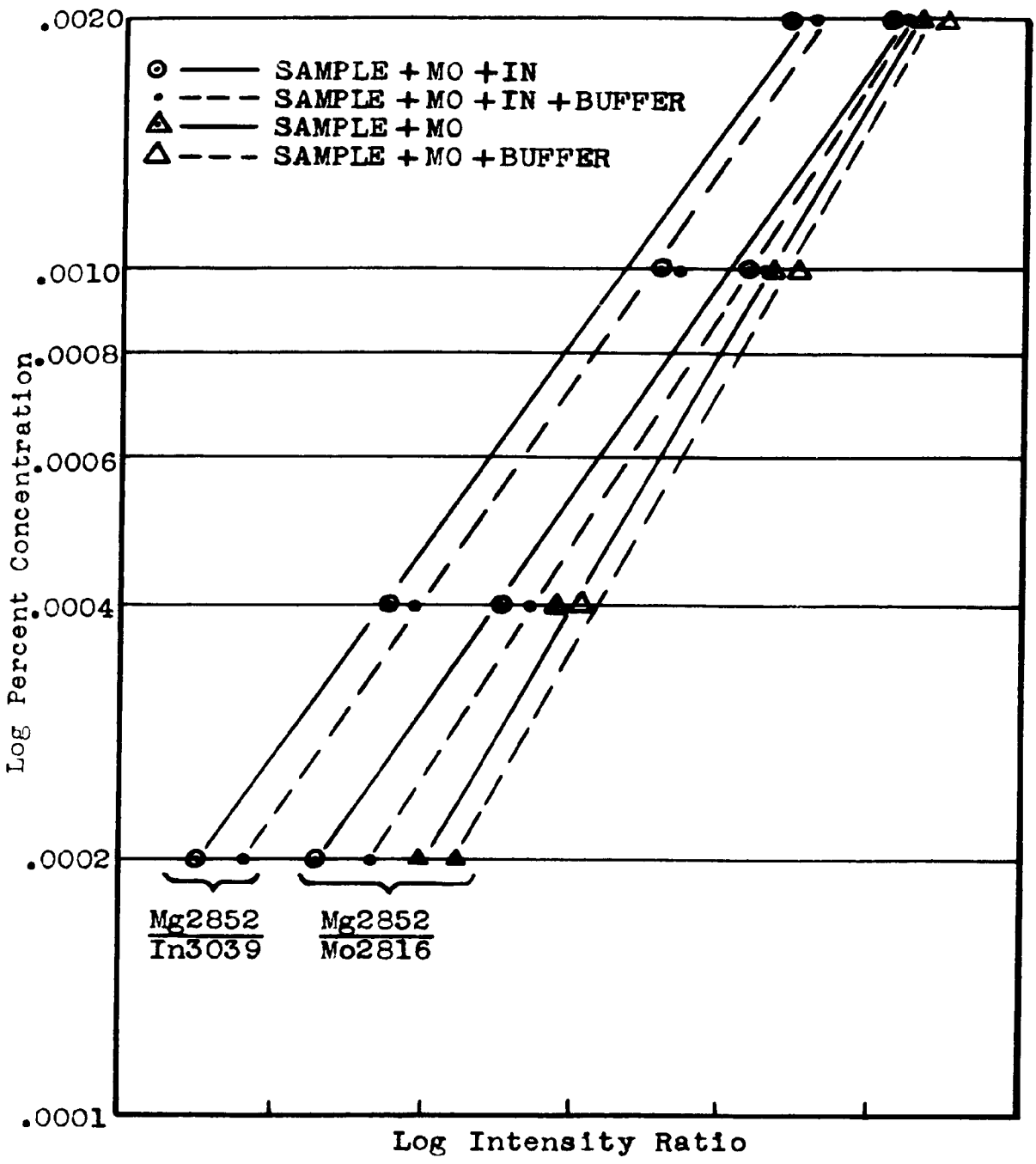


FIGURE 27

ANALYTICAL SLOPES FOR MAGNESIUM

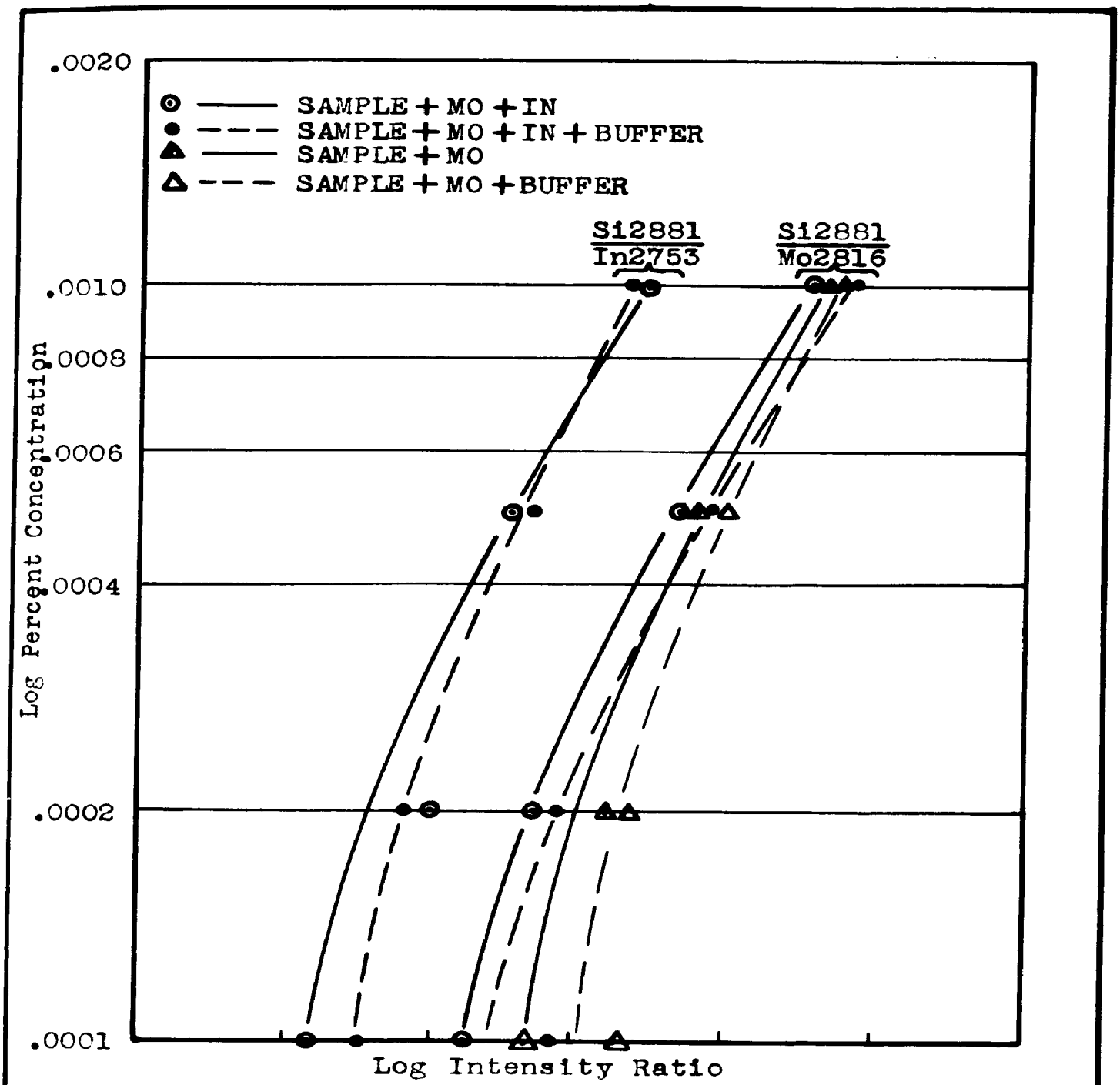


FIGURE 28

ANALYTICAL SLOPES FOR SILICON

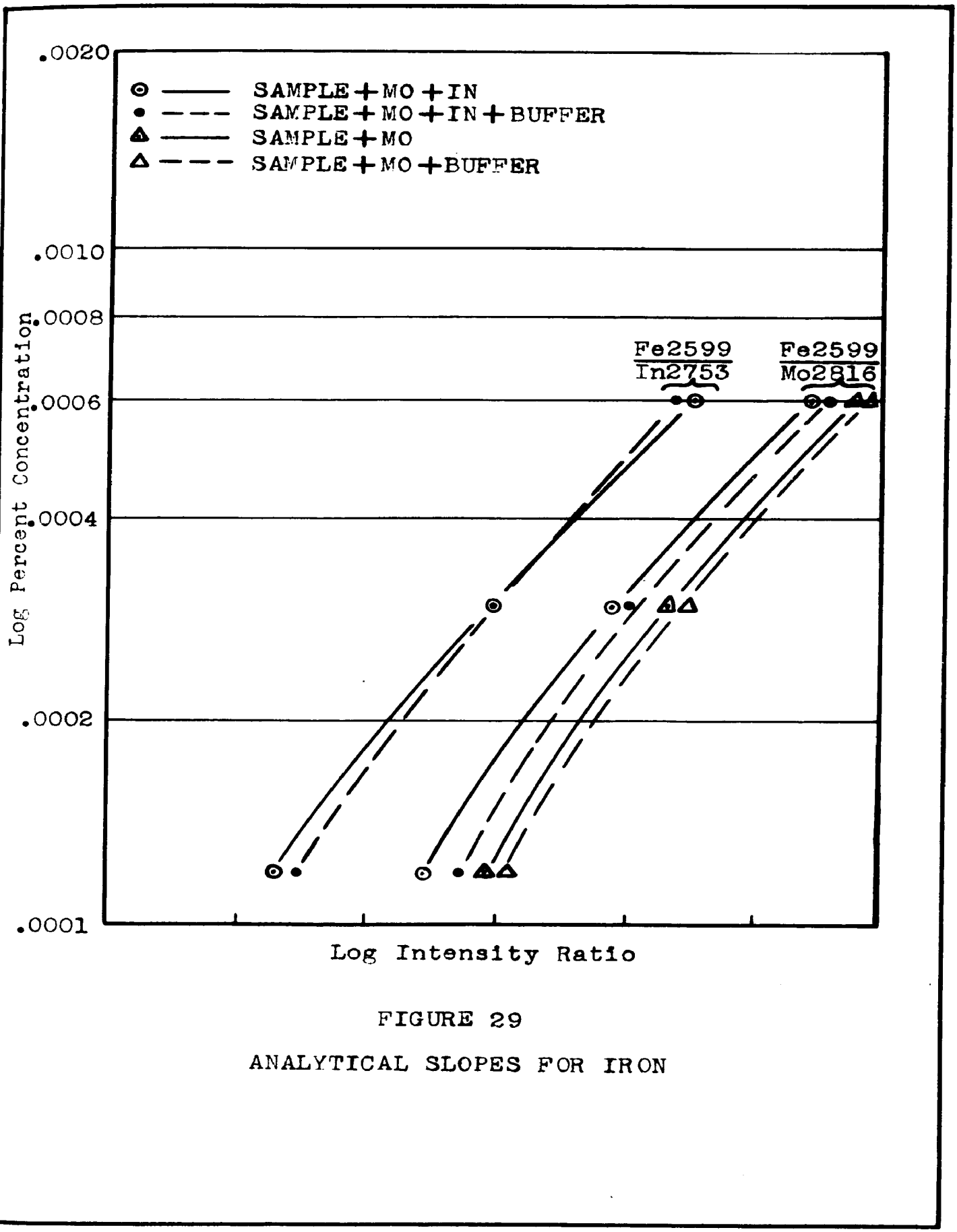


FIGURE 29

ANALYTICAL SLOPES FOR IRON

TABLE III

Composition of Various Test Solutions

	Solution Type 1	Solution Type 2	Solution Type 3	Solution Type 4
Hair 100, 50, 20 mg/ml	X	X	X	X
Molybdenum .0277 mg/ml (Internal Control No. 1)	X	X	X	X
Indium 1 mg/ml (Internal Control No. 2)		X		X
Ammonium Nitrate 50 mg/ml (buffer)			X	X

cal curves. This is equivalent to saying that the decision of whether or not to include indium and/or ammonium nitrate can be based upon factors other than the analytical curves.

It is recommended that indium be included in the matrix because: (1) The log ratios of the magnesium class repeat themselves with slightly better precision for indium than for molybdenum. (2) Indium has strong spectral lines in the visible region to supplement those of molybdenum for comparison with the strong spectrum lines of sodium, potassium, and calcium.

There is no reason to add a buffer to the hair-molybdenum solution unless it plays its intended role and brings about better reproducibility. Table IV lists a comparison of the reproducibility of 10 repeats of two similar solutions, one of which contains ammonium nitrate buffer.

No significant improvement is noted for the reproducibility of the buffered solution over the non-buffered solution. Likewise the spectral intensities of the buffered and non-buffered solutions are approximately the same. Since no particular virtue can be ascribed to the addition of the buffer, it is recommended that none be added.

In order to determine the element percentages in the hair stock solution, a set of synthetic standards was prepared. The analytical curves for these solutions are shown in Figure 30. It is readily verified that these curves possess the same slopes as the corresponding curves

TABLE IV

Buffered and Non-Buffered Solution Precision

	σ Summary for Indicated Line Pairs					
	$\frac{Fe2599}{Mo2816}$	$\frac{Fe2599}{In2753}$	$\frac{Mg2852}{Mo2816}$	$\frac{Mg2852}{In3039}$	$\frac{Si2881}{Mo2816}$	$\frac{Si2881}{In2932}$
Hair-Mo-In	.11	.17	.09	.06	.09	.08
Hair-Mo-In-Buffer	.11	.17	.11	.06	.09	.07

TABLE V

Stock Solution Concentrations

	Log Ratio	Standard Deviation	Element Percentage	Precision
Iron	Log $\frac{Fe2599}{Mo2816}$ = -.69	σ = .11	0.00064%	± 4%
Magnesium	Log $\frac{Mg2852}{In3039}$ = 1.54	σ = .06	0.00225%	± 3%
Silicon	Log $\frac{Si2881}{Mo2816}$ = -.53	σ = .08	0.00104%	± 7%

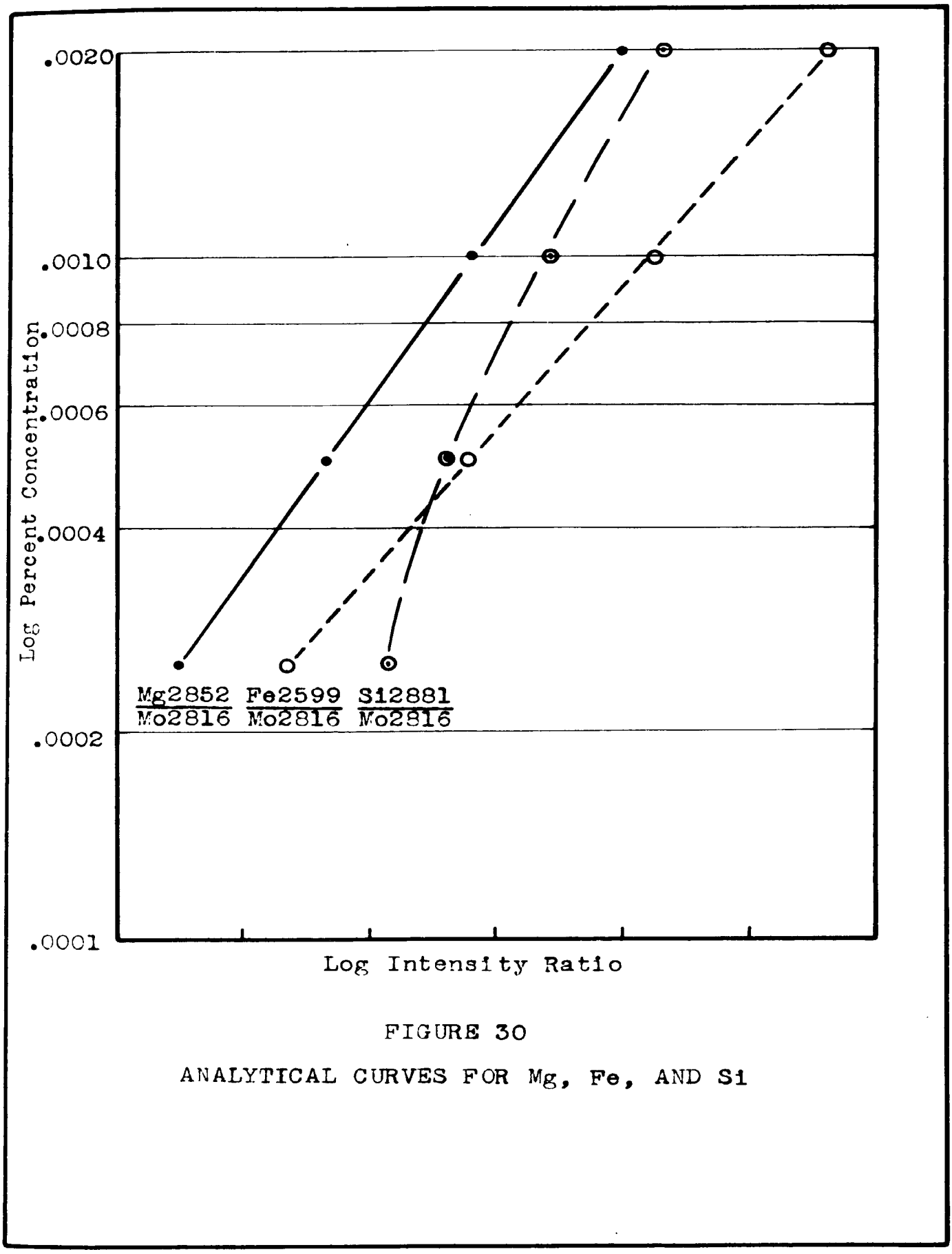


FIGURE 30

ANALYTICAL CURVES FOR Mg, Fe, AND Si

of Figures 27, 28, and 29.

Using the log ratios associated with Item 1, Table II, and the synthetic standard analytical curves, the element percentages and percentage variations were determined. These values are listed in Table V.

5. SENSITIVITY MEASUREMENTS

As the concentration of an element in the sample is continually decreased, a point is reached where the element is no longer detectable. This occurs when the spectral line blackening has been reduced to the point where it is indistinguishable from the surrounding background. The absolute sensitivity of a sample element is dependent primarily upon the type of source used for excitation but is also influenced by such factors as sample preparation, sample matrix, type of electrodes, and photographic plate response.

In establishing sensitivity limits for biological materials, it is desirable to establish values which represent not the absolute minimum which can be detected but rather the minimum detectable amount which may satisfactorily be used for quantitative analysis. To determine this level of detection, an arbitrary standard of measurement was established. It was decided that a satisfactory quantitative measurement could be made if the spectral line intensity exceeded a blackening density of $D = \log_{10}\frac{I_o}{I} = 0.01$ above fog or background. This is equivalent to a galvanometer

deflection of one centimeter or 1/50th of a full scale deflection on a Vincent-Sawyer type microphotometer.(87)

Table VI lists the sensitivities obtained according to this arbitrary density standard. These weights represent the minimum levels at which one can reasonably expect to make a quantitative measurement. The sensitivities in Table VI are based on a 30 second exposure of the biological material with an approximate consumption of 1/4 ml.

These values may be compared with those of Ells(10) with the Lundegårdh flame method. His values were: Fe 3859.9 absolute sensitivity 0.2 micrograms, and Mg 2852 absolute sensitivity 0.4 micrograms. Silicon was not reported. Ells' values were based on 60 second exposures consuming about 2 ml. of solution.

The absolute sensitivities of iron, magnesium, and silicon by the porous cup method on the recommended hair-Mo-In solution are: Fe 2599 -- 0.02 micrograms, Mg 2852 -- 0.008 micrograms, and Si 2881 -- 0.06 micrograms.

The superiority of the sensitivity of the spark-source porous cup method over the Lundegårdh flame method is impressive. Furthermore, it is evident that the porous cup method requires a smaller sample and a shorter time exposure than does the Lundegårdh flame method.

TABLE VI

Porous Cup Sensitivity

	Sensitivity in Micrograms*		
	Fe 2599	Mg 2852	Si 2881
Hair-Mo	0.14	0.02	0.03
Hair-Mo-Buffer	0.18	0.01	0.04
Hair-Mo-In	0.10	0.01	0.12
Hair-Mo-In-Buffer	0.14	0.01	0.12

* The number of micrograms of element necessary to produce a spectral blackening of 0.01 density.

6. RECOMMENDED WET SAMPLE PROGRAM

Minute amounts of biological material may best be analyzed in liquid form if the following program is adopted:

a. Instrument for Analysis

A spectrograph of moderate dispersion and resolution.

b. Type of Source

A controlled spark discharge.

c. Parameters of Source

The most desirable source parameters are those of Source No. 2, Appendix I.

d. Preparation of Sample

Dissolve the sample in dilute nitric acid. Use approximately 0.005 ml. of 34% doubly distilled nitric acid per mg. of biological sample. (Note: Ordinary concentrated nitric acid contains 68% HNO_3.)

e. Concentration of Internal Control

Two internal controls, molybdenum and indium, are preferred. The molybdenum may conveniently be obtained from ammonium paramolybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ which is water soluble. The indium stock solution may readily be prepared by allowing indium metal to react with dilute nitric acid to form indium nitrate. The desired final concentration of molybdenum and indium when they co-exist

with the sample are: Molybdenum concentration 0.0003 mg. Mo/mg sample, and Indium concentration 0.01 mg. In/mg sample.

f. Rotating Sector

The use of a rotating step sector in front of the slit is recommended. It is suggested that such a step sector possess eight sets of angular openings whose log ratios to the base 1.5 are 0, 1.7, and 3.4. Besides providing a means of plate calibration, the graded spectral densities facilitate a comparison of weak and strong lines.

g. Photographic Plate

A photographic plate of the Eastman Kodak 50 type is recommended as providing a satisfactory compromise of speed, resolution, and sensitivity to both the ultraviolet and the visible regions.

This plate should be developed 2 minutes in D-19 developer. The development stage should be followed by a 15 second stop in a 2% acetic acid stop-bath. This plate may then be transferred to the rapid fixer for 2 minutes and then washed thoroughly in running water.

h. Exposure Time

An exposure time of 30 seconds is recommended, preceded by a 5 second pre-exposure. The exposure time may readily be extended to 60 seconds if increased spectral density is desired.

i. Type of Electrodes

A porous cup 1/4 inch in diameter with a 5/32 inch bore and a 0.025 inch bottom depth provides satisfactory seepage of the sample to the discharge gap.

A counter electrode shaped as in Figure 10 is found to furnish desirable burning characteristics to the discharge.

j. Method of Supplying Sample to the Discharge Gap

The use of a carbon porous cup upper electrode with a goose-neck reservoir similar to that of Figure 26 is recommended. This system provides a continuous and uniform supply of sample to the discharge gap.

If the preceding program of analysis is adopted, the following magnitudes of sensitivities may be expected:

a) High excitation potential elements such as iron 0.1 μg
b) Low excitation potential elements such as magnesium 0.01μg
c) Silicon 0.1 μg

The reproducibility of this program of analysis is suggested by the following values of precision:

a) High excitation potential elements such as iron ±4%
b) Low excitation potential elements such as magnesium ±3%
c) Analytically troublesome elements such as silicon ±7%

CHAPTER V

DRY SAMPLE METHODS

1. EVALUATION OF DRY METHODS

Biological samples may be handled directly as extracted solids by placing them in a cavity of a hollowed carbon (see Figure 31) and exciting them with an arc or a spark discharge. If the cavity is shallow, the discharge causes the sample to be knocked from the cavity and lost. If the cavity is deep, the discharge tends to play on the rim of the carbon and does not do much exciting of the sample. One additional fundamental objection to this method is that no provision is possible for the uniform introduction of an internal control.

A method of overcoming a portion of the above problems is to pulverize the sample with some loose carbon and an internal control. This mixture is then packed into the carbon cavity of Figure 31. The major difficulty associated with this method is the inability to make the mixture sufficiently homogeneous for good reproducibility.

A preferable method involves the digestion of the sample in acid to form a uniform liquid, the mixing of this liquid with an internal control, and the drying of the mixed solution on filter paper. The filter paper is

then pulverized and packed into a carbon cup as in the previous method. This method also suffers from the inhomogeneous mixing of the sample and the carbon packing, but not as seriously as in the previous method. Here the sample and the internal control have become closely integrated by sample mixing. There is the tacit assumption, however, that the discharge employed will be an arc. Without the intense heat of the arc the sample is not evolved from the lower layers of the carbon packing into the discharge gap. The spark discharge just plays upon the surface of the packed electrode and the sample material which lies below the surface is never excited. This is a negligent waste of sample. When such a small sample is involved, one must always think in terms of economy of sample. However, inasmuch as a controlled spark discharge can always be depended upon to furnish better reproducibility than an arc discharge, it is desirable to use a spark whenever possible.

One method which is applicable to either an arc or a spark discharge is that of drying the liquid sample on the surface of a flat electrode. An investigation by this method demonstrated that the sensitivity of a 5 ampere spark is approximately equal to that of a 5 ampere high voltage arc. Since no major distinction in sensitivity existed between the arc and the spark by this method, the spark was the source selected.

The method of drying the sample on the end of a flat electrode has virtue, not only because it permits the

employment of a spark discharge but also because it permits the sample to be supplied from both the upper and the lower electrode. This is not usually feasible with other methods. When the sample is dried on the end of an electrode, it adheres to it strongly and there is no danger of losing the sample when the carbon is inverted. It is desirable to divide the sample and dry it on two electrodes because (a) better line-to-background ratio is achieved when the discharge is not permitted to strike directly upon a bare carbon, and (b) cyanogen band structure is reduced.

2. SURVEY OF DRIED PRECIPITATE PARAMETERS

a. Electrode Shape

The simplest electrode shape is that shown in Figure 32, where a carbon has been cut off squarely. When a sample drop is added to the top of the electrode, it assumes a somewhat hemispherical shape on the carbon. When the carbon is heated, the sample slowly dries, leaving the main portion of the residue as a deposit around the rim. Certainly better reproducibility and perhaps better line-to-background ratio would result from a more uniform distribution of sample over the surface of the electrode.

The following electrode shapes were investigated in an effort to improve the sensitivity and/or reproducibility: (1) pointed and flat, (2) serrated, (3) dome, and (4) chamfered.

The pointed and flat electrode pair is shown in Figure 33. This particular electrode combination was characterized by high background and relatively low spectral intensity. This is primarily because the sample can be dried only on the flat electrode and can, therefore, only enter the discharge from one side. Also, the major portion of the sample dries so that it lies near the rim and not at the center of the electrode where most of the discharge is taking place.

An end view and a side view of a pair of serrated electrodes are shown in Figure 34. The concentric indentations on the top of a flat electrode help to retain the sample near the center of the electrode. Although the sample is more equally distributed over the surface of the electrode, the line-to-background ratio is impaired by this method. The sample tends to lie unexcited in the troughs while the discharge plays on the crests.

Figure 35 represents a pair of dome shaped electrodes. This style electrode is very difficult to load with sample drops. The sample drops tend to roll off before they have a chance to dry. Inasmuch as the resultant spectral intensity is only mediocre, there is no particular advantage in employing this pair.

The most desirable electrode pair combination from the standpoint of intensity and line-to-background ratio is represented in Figure 36. Basically this is a pair of flat electrodes. However, reproducibility is improved if the

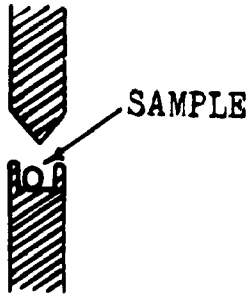


FIGURE 31

SAMPLE IN CARBON CRATER



FIGURE 32

SAMPLE DROP ON FLAT ELECTRODE



FIGURE 33

POINTED AND FLAT ELECTRODES



FIGURE 34

SERRATED ELECTRODE



FIGURE 35

DOME ELECTRODES



FIGURE 36

CHAMFERED ELECTRODES



FIGURE 37

CENTER POST ELECTRODE

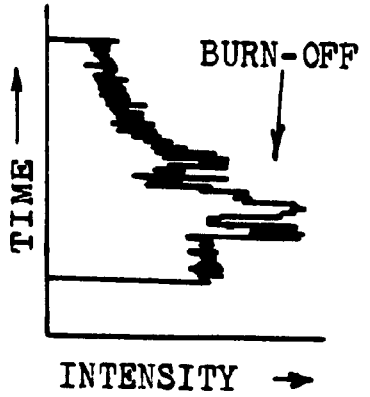


FIGURE 38

DIRECT READING BURN-OFF RECORD

rim of the flat electrode is beveled to prevent preferential sparking to the edge. This tends to localize both the sample and the discharge in the central region of the electrode. In general, anything which inhibits the discharge from striking on the bare carbon also reduces the background and the cyanogen bands.

b. Prevention of Sample Penetration

When a liquid sample drop is placed on a carbon electrode to be dried, there always exists the problem of penetration. With minute amounts of material it is critical that this penetration be reduced to as small a distance as possible. Ideally the material should deposit only on the surface of the carbon so that it may be consumed in the discharge with no loss. In practice, however, the carbon is so porous that normally only a fraction of the sample stays on the surface. It is possible to reduce this penetration considerably by adding various materials to the carbon which decrease its porosity. Since the element concentration of the small biological material is not large, the material which is used for a sealer must possess a reasonably high purity.

The first material employed to decrease the carbon porosity was celluloid dissolved in amyl acetate. This material is quite pure and it is capable of preventing sample penetration. A survey was made, therefore, to investigate the best conditions of adding the celluloid-amyl-acetate

sealer to the carbon. The first comparison was made between the soaking time and the drying time of the celluloid-amyl-acetate. The relative superiority of the various combinations of soaking time and drying time was evaluated on the basis of the spectral intensity of the sample which was subsequently dried on the test electrode. The soaking of the electrodes in the celluloid-amyl-acetate solution was carried out in a large test tube. The carbons were then dried in a carbon block on a hot plate. By this means a comparison was made among:

a) short soaking time, short drying time
b) short soaking time, long drying time
c) long soaking time, short drying time
d) long soaking time, long drying time

It was concluded that the spectral intensity associated with the long soak ($\sim$ 4 days) and long dry ($\sim$ 1 hr.) was best. However, the drying process is the most influential factor. The spectral intensity from a 4 day soak is not significantly greater than that from a 4 minute soak.

By use of electrodes soaked in celluloid dissolved in amyl acetate, it is possible to effectively prevent penetration and keep the sample on the surface of the carbon. However, there exist traces of magnesium in this sealer material. It is possible, therefore, to improve the sensitivity by decreasing the carbon porosity with celluloid and amyl acetate, but this addition introduces an error into the

magnesium concentration percentage. Since normal concentration of magnesium in biological material is relatively high, the use of this sealer to prevent penetration need not arbitrarily be excluded. Unless the biological samples are very small, it may be feasible to use celluloid and amyl acetate as a sealer.

In a further attempt to evolve a satisfactory method of retaining the sample on the surface of the electrode, the following method was investigated. A carbon electrode was cut so that it had a center post approximately 1 mm. high and approximately 1 mm. in diameter. (See Figure 37). Some celluloid-amyl-acetate solution was added to the carbon to make it non-porous. A small doughnut-shaped piece was punched from a filtration accelerator pad. This doughnut piece was then fitted over the center post of the carbon. Sample drops were then added to this highly absorptive doughnut and dried. The spectral intensity by this method was subdued until the filtration accelerator doughnut burned off the carbon. Then there was a sudden increase in intensity as indicated in Figure 38, which is a facsimile of a direct reading record of one of the burn-off periods. At first it was thought that the intensity flash was due to excitation of sample when the doughnut flipped off the carbon into the discharge gap. Further investigation, however, suggested that the intensity flash was really due to the sample deposited on the carbon which was suddenly revealed when the doughnut flipped off in the burning. Although a good line-

to-background ratio was obtained, the main excitation seemed to come in the burn-off flash and this was quite unpredictable. The burn-off flash varied randomly from two to seven seconds after the start of the exposure. The non-reproducibility of this method excluded it from further consideration.

A satisfactory method of overcoming the porosity of the carbon electrode was established by using paraffin as a sealer. This substance is available in satisfactorily high purity. One drop of paraffin when added to the top of a flat electrode is sufficient to retain the sample drops on the surface of the carbon. Besides adequate purity, paraffin has the added virtue of being acid resistant. Since the biological material solution is properly kept somewhat acid to prevent possible precipitation of such elements as aluminum, copper, gold, iron, magnesium, mercury, tin, and zinc, the feature of paraffin of imperviousness to acid is desirable.

A comparison of the sealing abilities of celluloid-in-amyl-acetate and paraffin was made by adding equal numbers of sample drops to carbons prepared with each sealer. The paraffin sealed carbons yielded the more intense sample spectra. Furthermore, the sample drops took longer to dry on the paraffin sealed carbons, indicating that the sample was really drying on the surface instead of soaking into the carbon. For these reasons paraffin was considered to be a satisfactory and desirable sealer for the carbon electrodes.

Figure 39 represents a plot of the spectral line

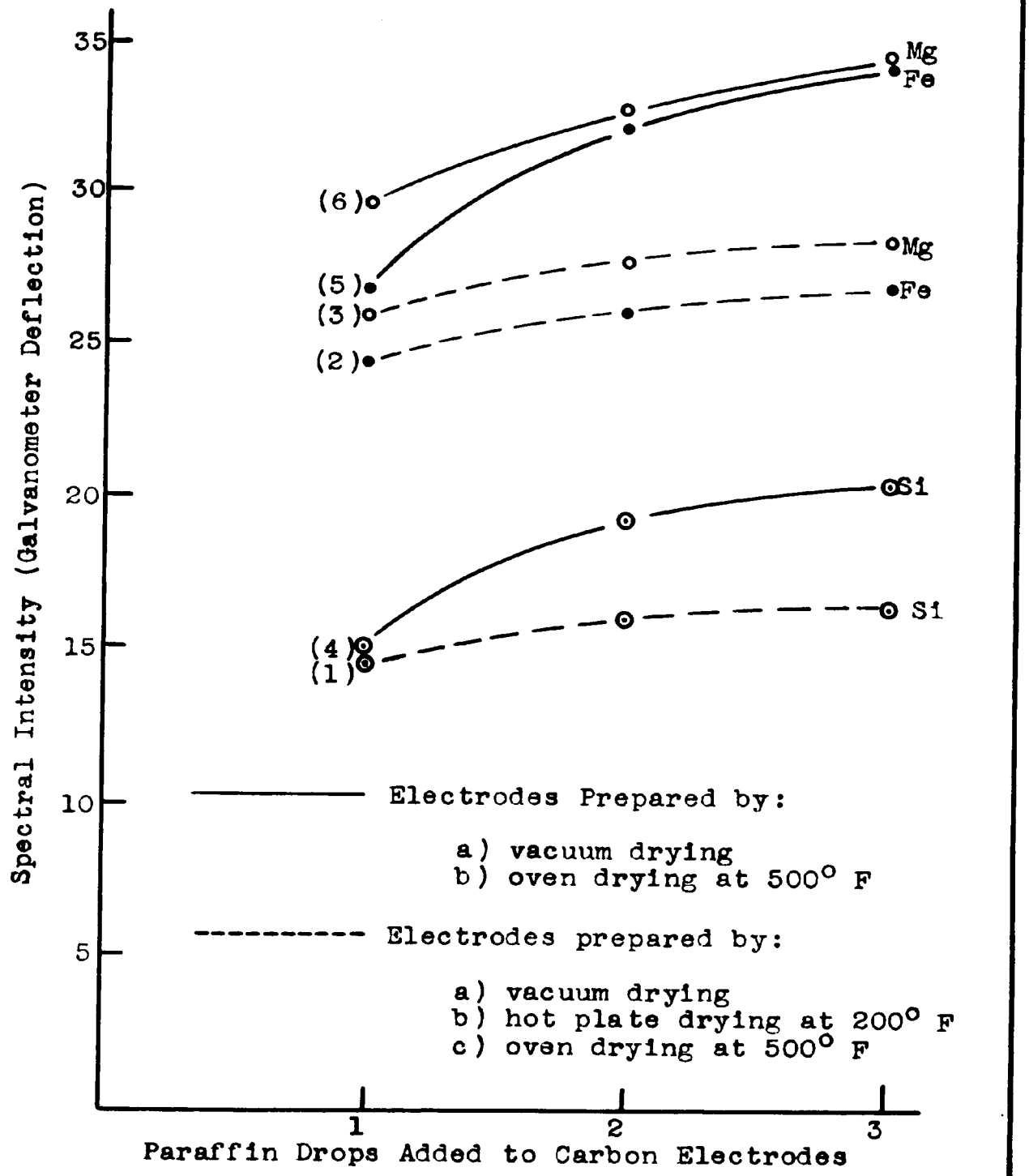


FIGURE 39

SPECTRAL BLACKENING VS PARAFFIN DROPS

intensities of iron, magnesium, and silicon versus the number of paraffin drops added to the carbon electrode to prevent sample penetration. The dashed curves 1, 2, and 3, represent data from electrodes prepared by (a) vacuum drying, followed by (b) hot plate drying at 200° F, followed by (c) oven drying at 500° F. The solid curves 4, 5, and 6, represent data from electrodes prepared by (a) vacuum drying, followed by (b) oven drying at 500° F. Two things are of primary interest in Figure 39. These are:

1) The increase in spectral intensity after two paraffin drops have been added is not large.

2) The sample drying program represented by the solid curves yields greater spectral intensities than the drying program represented by the dashed curves.

All future work in dried precipitate techniques is therefore based upon electrodes which have been sealed with two paraffin drops. This amount is considered enough to provide adequate sealing of the carbon with no significant sacrifice of spectral intensities.

c. Optimum Sample Load

Figures 40, 41, 42, and 43 are plots of data representing the variation of spectral intensity with drops of sample added to the electrode. Successive time intervals are plotted showing the spectral response in the 0-2 second interval, in the following 2-4 second interval, and in the final 4-6 second interval. After each two second duration exposure the plate was racked down and the succeeding ex-

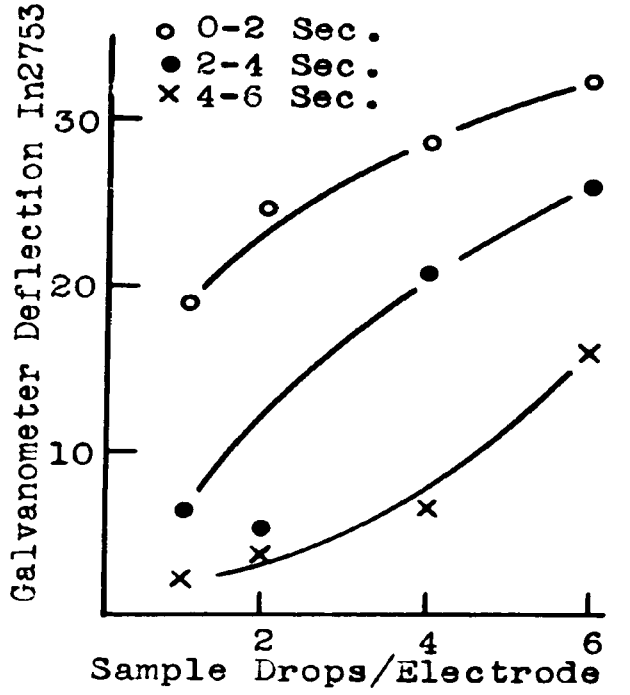


FIGURE 40

In2753 INTENSITY VS
SAMPLE DROPS/ELECTRODE

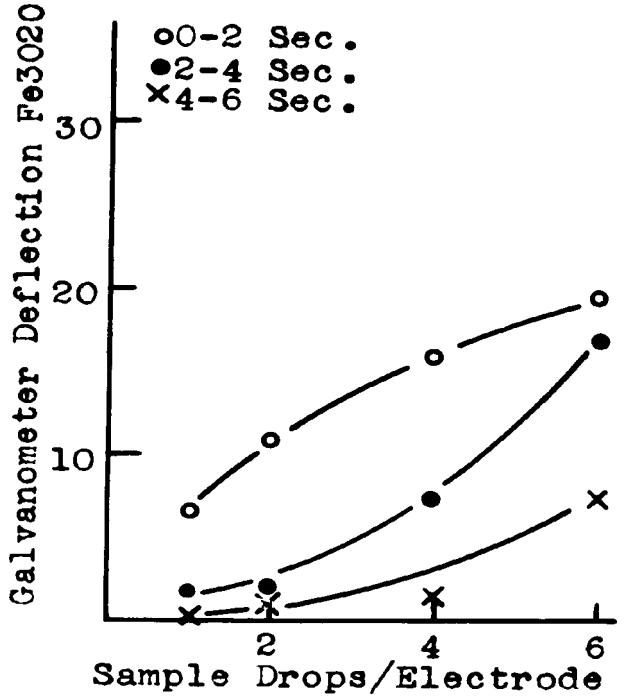


FIGURE 41

Fe3020 INTENSITY VS
SAMPLE DROPS/ELECTRODE

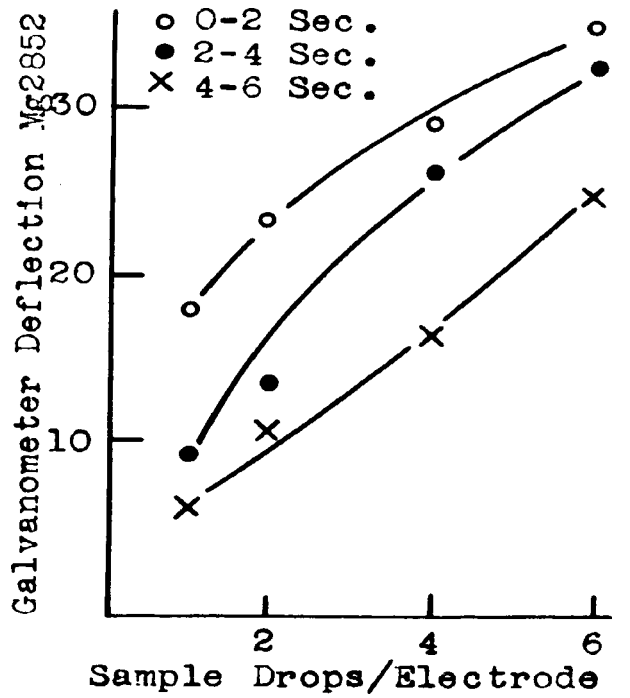


FIGURE 42

Mg2852 INTENSITY VS
SAMPLE DROPS/ELECTRODE

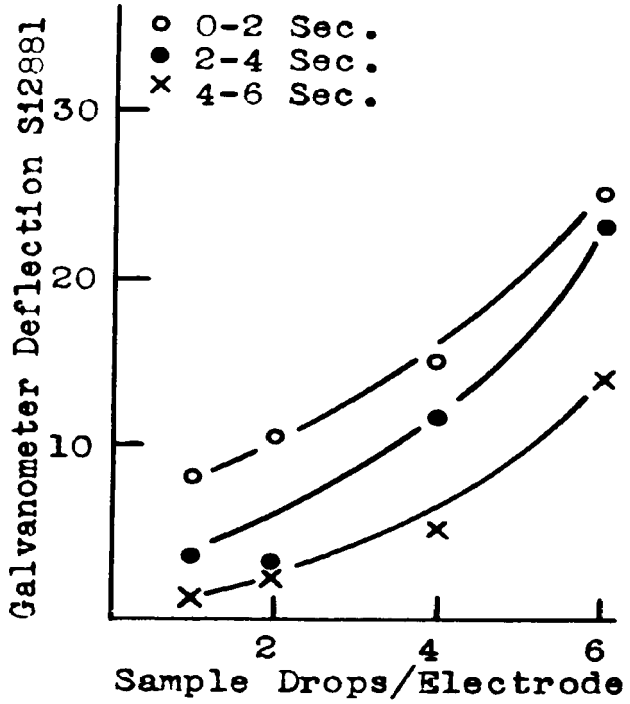


FIGURE 43

Si2881 INTENSITY VS
SAMPLE DROPS/ELECTRODE

posure taken with no change in electrodes. In interpreting these curves it should be borne in mind that a compromise is sought which will best satisfy two divergent desires. These are:

1) The desire to obtain the most spectral intensity for the least sample expended. It is noted that any sample which remains on the electrode after the exposure is wasted.

2) The desire to obtain good reproducibility. Good reproducibility requires that enough sample be provided to prevent any "edge effect" at the end of the exposure interval. It is not desirable, for instance, to burn-to-completion before the exposure interval has ended.

The curves of Figures 40, 41, 42, and 43, therefore, have to be evaluated in terms of these two requirements. With one sample drop added, most of the sample is consumed in the first two second interval. This is economical of sample but does not favor reproducibility. With six drops added, the amount of material left on the electrodes is abundant but the spectral intensities have not increased in proportion to the sample material added. Consequently, six drops of added sample represents a condition favoring reproducibility but not economy of sample. On this basis, the addition of three sample drops to the electrode was considered to be the optimum load for favoring both economy and reproducibility. This loading corresponds to approximately 10 milligrams of sample.

d. Exposure Time and Electronic Timer

A survey of the possible source parameters,

1) low capacity, low inductance

2) low capacity, high inductance

3) high capacity, low inductance

4) high capacity, high inductance

at once indicated that the maximum spectral intensities were associated with high capacities and therefore high currents. The problem of the proper amounts of inductance for optimum line-to-background ratio could not be solved by visual inspection of the photographic plates. However, from visual observations of the discharge flame and the resultant spectra, it was readily evident that with a high current source the major portion of the sample had burned from the carbon within three to four seconds. From then on the discharge ran mainly on the bare carbon and emitted primarily background radiation. Long exposures ($\sim$ 30 seconds) yielded spectra which contained a more unfavorable line-to-background ratio than did the spectra from short exposures ($\sim$ 2 seconds). The short exposures included most of the spectral radiation and excluded the subsequent high background period which occurred after the sample was expended and the carbon was bare. It became evident at this point that the general optimum conditions of analysis were akin to those of the exploded wire technique of Anderson[96] and Sawyer and Becker[97]. Fundamentally, this method involves the dissipation of a very large amount of energy in a short time interval.

Since the line-to-background ratio is favored by the use of higher energy discharges and shorter exposures, some accurate method of timing such short time exposures is required. A stop watch is not satisfactory. The average error in exposure when timed with a stop watch is at best ±0.1 second. For a two second exposure this is a timing error of ±5%. This error might be tolerated were it not for the difficulty that the discharge often fails to make its initial strike coincident with the closing of the starting switch. Occasionally, several cycles may elapse before the discharge is initiated. The timing error inherent in this failure is then an appreciable portion of the two second interval.

To overcome the problem of variable exposure interval, an automatic electronic timer was designed and constructed. The circuit of this timer (see Figure 44) is an amalgamation of basic designs suggested by Dr. Lyman Orr and Mr. Glenn Edict of the Engineering Research Institute. It contains four basic parts. These are:

1) Triggering unit
2) Pulse shaping circuit
3) Time delay circuit
4) Shutter release circuit

In brief, the principles of operation of the timer are: (a) The light striking the phototube applies a triggering voltage pulse to the pulse shaping circuit. (b) The pulse shaping circuit controls the height of the

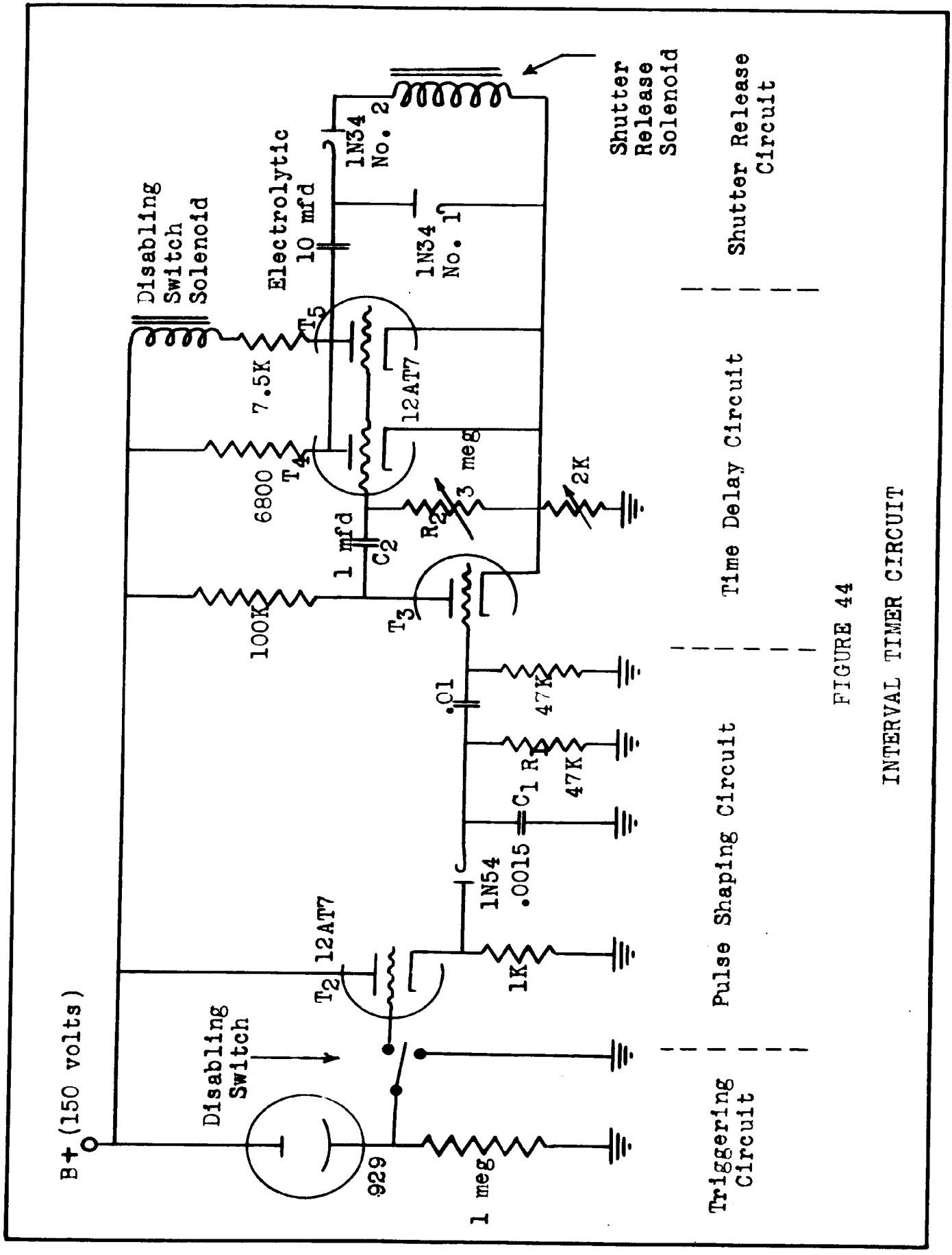


FIGURE 44

INTERVAL TIMER CIRCUIT

triggering pulse and passes this controlled pulse along to the timing circuit. (c) The controlled pulse drives tube T_4 beyond cut-off. Tube T_4 remains non-conducting for a period determined by the R_2C_2 time constant. (d) When T_4 goes into conduction, a slug of charge is pulsed from the 10 mfd electrolytic condenser through the shutter release solenoid, thus ending the exposure interval.

The triggering unit consists of a one megohm resistor in series with a 929 phototube. The 929 tube is a high vacuum tube with a spectral response as indicated in Figure 45. When the first flash of light from the discharge gap strikes the cathode of the phototube, a current of the order of a few microamperes flows through the one megohm resistor. The voltage pulse developed over the resistor is fed to the grid of tube T_2 which is one-half of a 12AT7 tube. The 12AT7 tube is a twin triode tube.

The increase in grid voltage of tube T_2 causes a pulse of current to flow through the 1000 ohm cathode resistor. This current pulse through the cathode resistor elevates the cathode voltage with respect to ground. By means of the 1N54 crystal diode the 0.0015 mfd condenser C_1 is effectively subjected to this charging voltage of approximately 5 volts. As the fast light pulse begins to decrease in intensity, the voltage supplied to the T_2 grid becomes less. This in turn causes less current to flow through the cathode resistor and results in a lower cathode voltage. With a lowering of the cathode potential, the 0.0015 mfd capacitor C_1 begins

to discharge. The low resistance discharge path through the cathode resistor is blocked by the 1N54 crystal diode. The discharge must therefore take place through the 47,000 ohm high resistance. This tends to prolong the condenser discharge so that the voltage across C_1, and thus the voltage pulse applied to the grid of T_3, has the form of Figure 46. The pulse shaping circuit therefore provides two primary features:

1) Since the tube T_1 is normally conducting, the triggering pulse merely throws it into full conduction. The tube current and the resulting cathode voltage reproduce their respective values almost independent of the intensity of the triggering light pulse. The height of the voltage pulse applied to the grid of tube T_3 is therefore consistently uniform.

2) The 1N54 crystal diode acts to prolong the voltage pulse to the grid of tube T_3. This makes the timing cycle independent of the time of duration of the triggering light pulse.

The voltage applied to the grid of T_3 which activates the timing cycle is therefore effectively independent of either the intensity of the triggering light pulse or the duration of the triggering light pulse.

The electronic timer, as originally constructed, did not contain the pulse shaping unit. Under these conditions the voltage developed across the phototube 10 megohm resistor was impressed directly upon the grid of the 12AT7

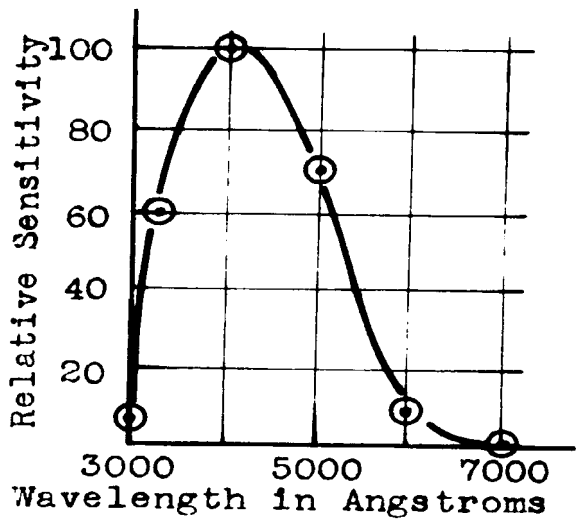


FIGURE 45

SPECTRAL RESPONSE OF 929 PHOTOTUBE

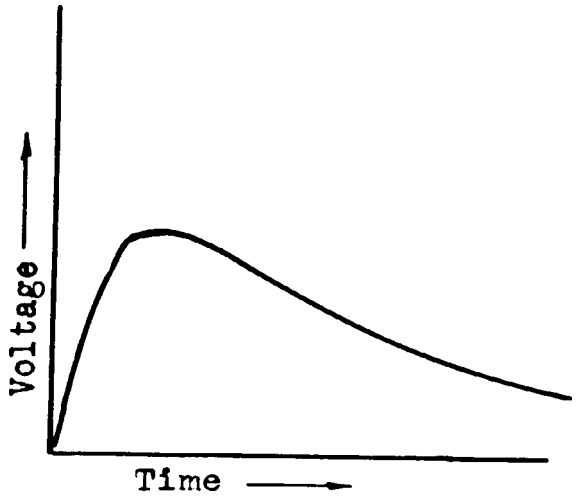


FIGURE 46

SHAPED TRIGGERING PULSE

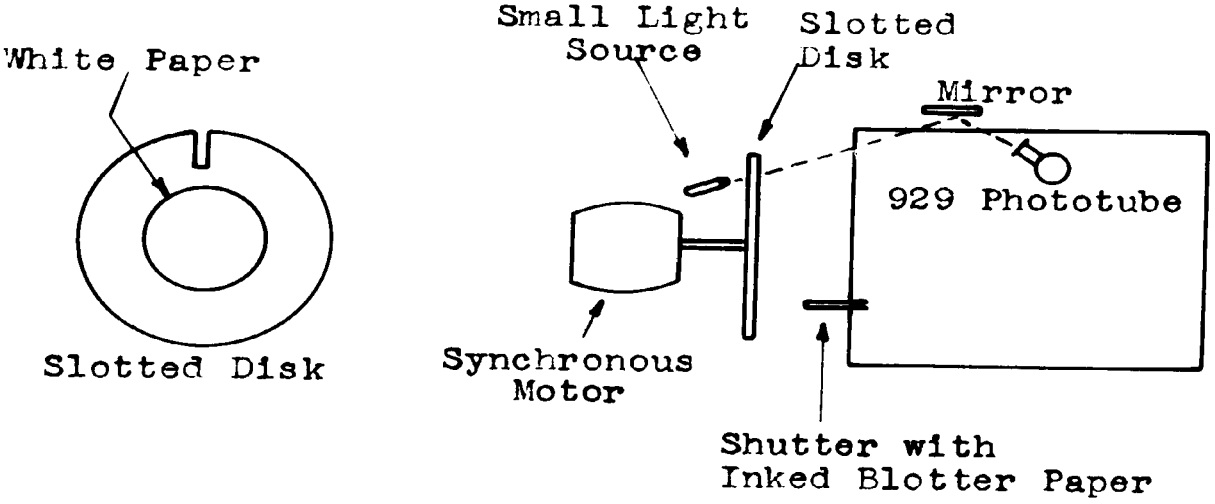


FIGURE 47

APPARATUS FOR CHECKING TIMER PRECISION

tube T_3. With the pulse shaping circuit absent, the timing interval was found to be dependent upon whether an arc-like discharge or a spark-like discharge triggered the timing circuit. That is, when there was a large amount of inductance ($\sim$ 200 microhenries) in the discharge circuit, the timing interval was two seconds. When the discharge circuit inductance was small ($\sim$ 10 microhenries), the timing interval was of the order of 1.5 seconds. For a particular value of inductance in the discharge circuit, the timing interval was constant and reproducible. This effect is attributed to the decrease in cyanogen band radiation with a more spark-like discharge. With a minute sample supply on carbon electrodes, cyanogen bands form a high percentage of the discharge radiation. Moreover, the cyanogen bands dominate the 3500 Å to 4500 Å region, the region of maximum sensitivity for the 929 phototube. (See Figure 45).

Without the pulse shaping circuit, therefore, anything which tends to decrease the cyanogen band intensity will decrease the height of the triggering voltage pulse and decrease the duration of the timing interval. Such a circuit would probably be satisfactory for routine analyses where the discharge conditions would not be changed. For flexibility, however, the time interval measured by the timer should certainly be independent of the discharge source parameters. This condition is brought about by the addition of the pulse shaping circuit. The disabling switch was added to the circuit of Figure 44 to assure that once the

timing interval was initiated, no further light pulses could have any effect upon the duration of the timing interval. With these added features the timer was found to furnish reproducible time intervals which were independent of the discharge source parameters.

In Figure 44, the timing circuit is effectively a one-shot multivibrator. Initially tube T_4 is conducting and T_3 is not conducting. When the shaped triggering voltage pulse is applied to the T_3 grid, T_3 goes into conduction. The resultant lowering of the T_3 plate voltage sends a negative voltage pulse to the grids of tubes T_4 and T_5, biasing them beyond cut-off. When the tube T_5 is quenched, the T_5 plate disabling switch solenoid releases, thereby grounding the phototube cathode. Succeeding light pulses, therefore, can have no effect upon the timing interval.

The quenching of the tube T_4 causes the T_4 plate voltage to rise. The 10 mfd electrolytic condenser is therefore charged through the 1N34 crystal diode No. 1 to approximately B+ voltage (150 volts). Tube T_4 remains in a state of non-conduction until the capacitor C_2 has discharged sufficiently through the resistor R_2 to bring the biasing voltage on the T_4 grid above cut-off. The timing interval is thus determined by the RC time constant of R_2 and C_2.

When tube T_4 goes into conduction, its plate voltage drops sharply from approximately 150 volts to approximately 80 volts. The 10 mfd electrolytic condenser sends a pulse

of current through 1N34 crystal diode No. 2 and activates the shutter release solenoid. The shutter release solenoid trips the shutter latch, permitting the shutter to close. This ends the exposure interval. Once closed, the shutter stays closed until reset by hand.

The following method was devised to check the reproducibility of the timing interval. A beam of light from a pen-light was permitted to fall upon the photocell once during each revolution of a slotted disk, as indicated in Figure 47. The incident light on the photocell activates the timing cycle. At the end of each timing cycle the shutter closes and drives a freshly inked blotter paper against the white paper of the rotating disk. The angle turned through is a measure of the time elapsed since the light passed through the slot in the disk. In this particular case the wheel made almost exactly two revolutions. Since the spread of eight repeat trials was confined to 15° of wheel turning, the angular spread corresponds to $(15°/720°) \times 100 \cong 2\%$. Therefore the shutter accuracy is approximately $\pm$ 1%.

e. Determining the Optimum Source

In determining the proper values of inductance and capacitance for dried precipitate analysis, the guiding factors were:

1) high spectral intensity

2) low background intensity

3) good reproducibility,

The first variable to be investigated in detail was that of capacitance. Large circuit capacity is associated with large discharge currents. Large discharge currents, in turn, mean high spectral intensity and high background. Choosing the proper capacitance value then consists primarily of selecting the largest value of capacitance possible for which the background is not prohibitively high. To identify this value of capacitance the inductance was kept constant at 100 microhenries and the capacitance was varied. One-hundred microhenries of inductance was selected on the basis of a rough survey made where the capacity was kept constant at a representative 0.024 microfarads and the inductance varied from 0 to 225 microhenries.

Using a secondary inductance of 100 microhenries, the secondary capacitance was varied from 0.005 mfd to 0.078 mfd, the maximum available capacitance. By means of a variac in the primary circuit, the secondary voltage was limited until the discharge pattern was one break per half-cycle. The data as plotted in Figure 48 represent the response of the spectral line intensities with varying capacity. The considerable increase in spectral line blackening with increased capacity is at once evident. Increases in capacity beyond 0.045 mfd do not substantially contribute to the line density, while the background is beginning to rise. Therefore, 0.045 mfd was chosen as the proper value of capacity to use for dried precipitate analysis.

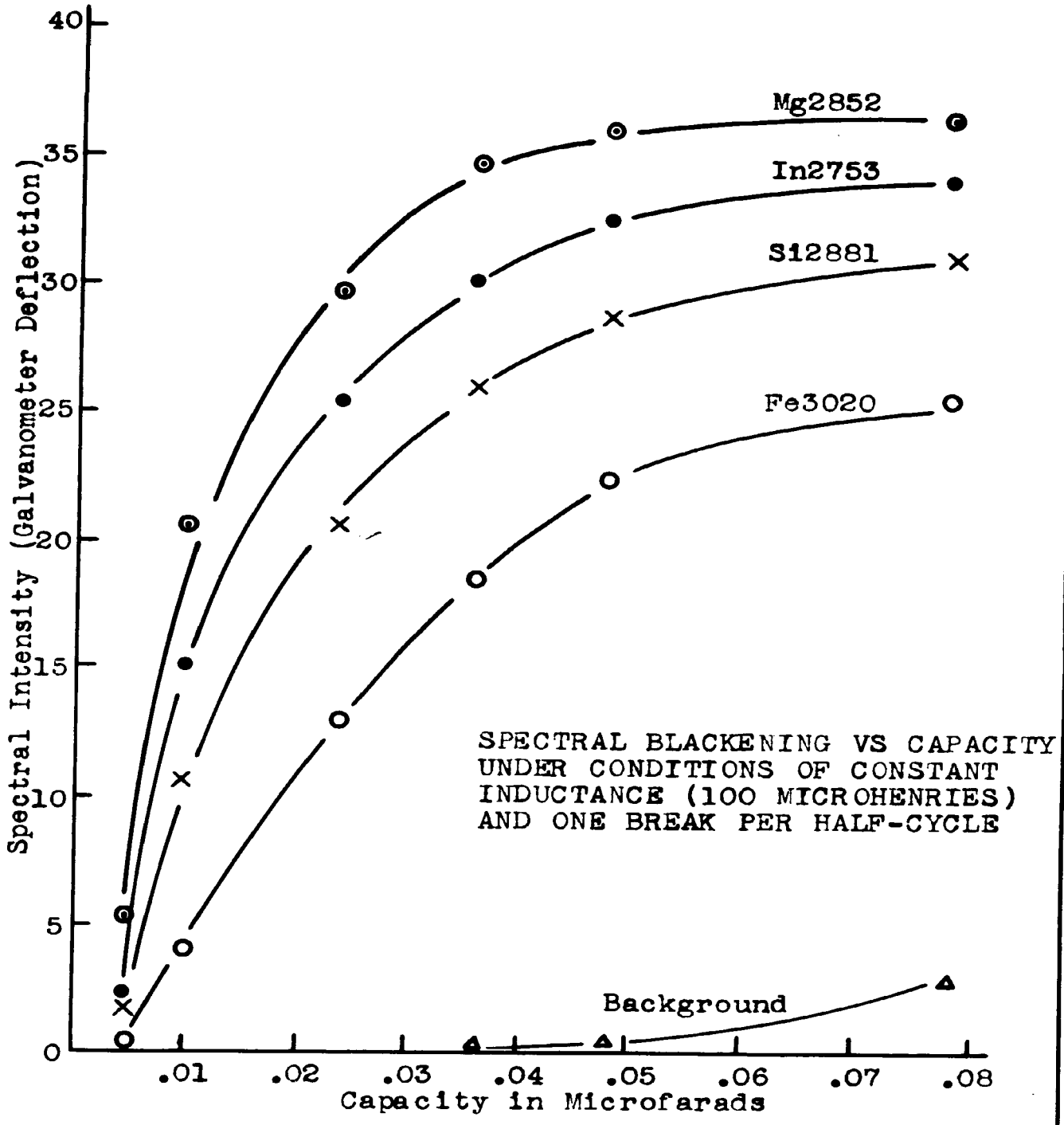


FIGURE 48

SPECTRAL BLACKENING VS CAPACITY, CONSTANT BREAKS

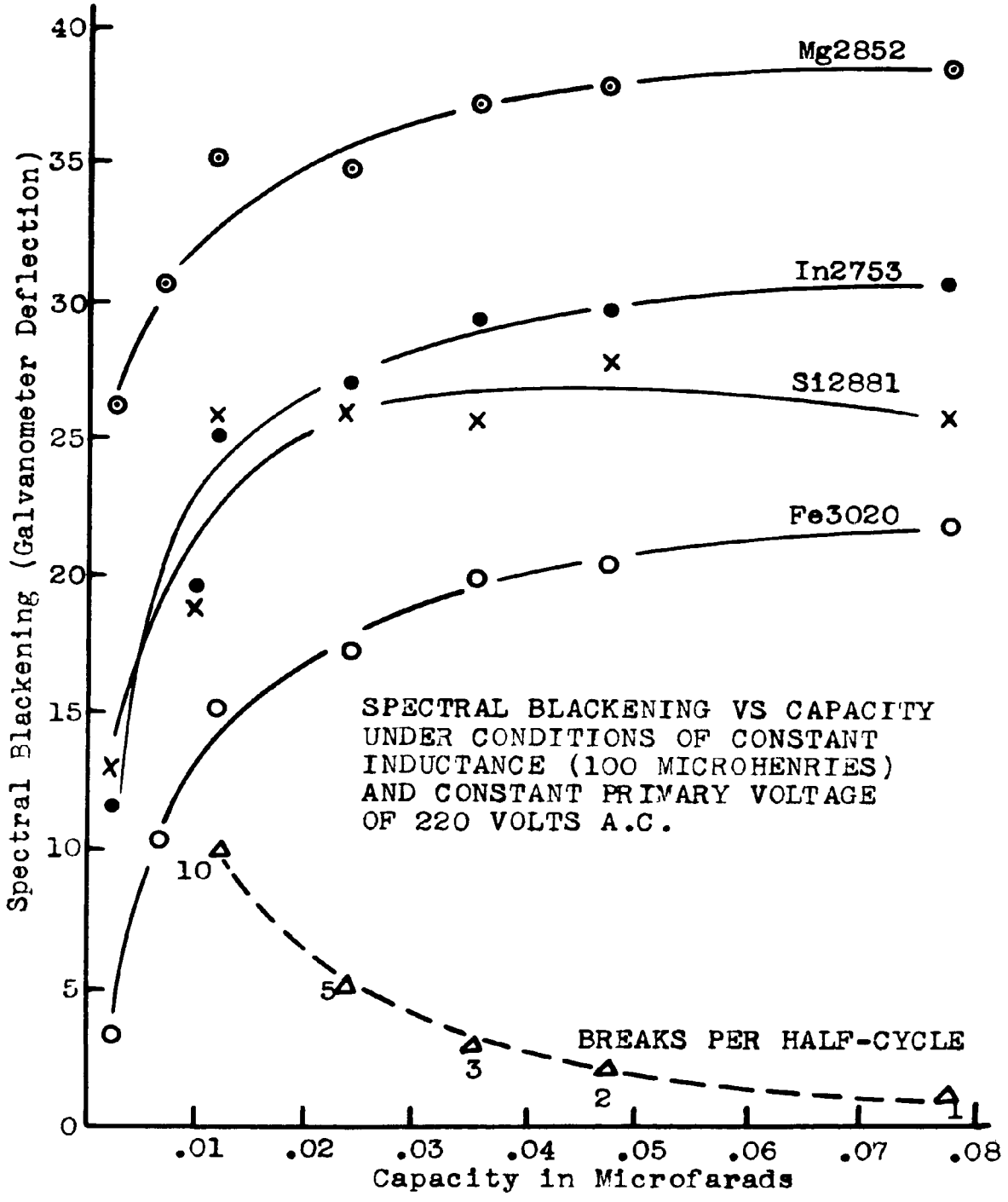


FIGURE 49

SPECTRAL BLACKENING VS CAPACITY, VARIABLE BREAKS

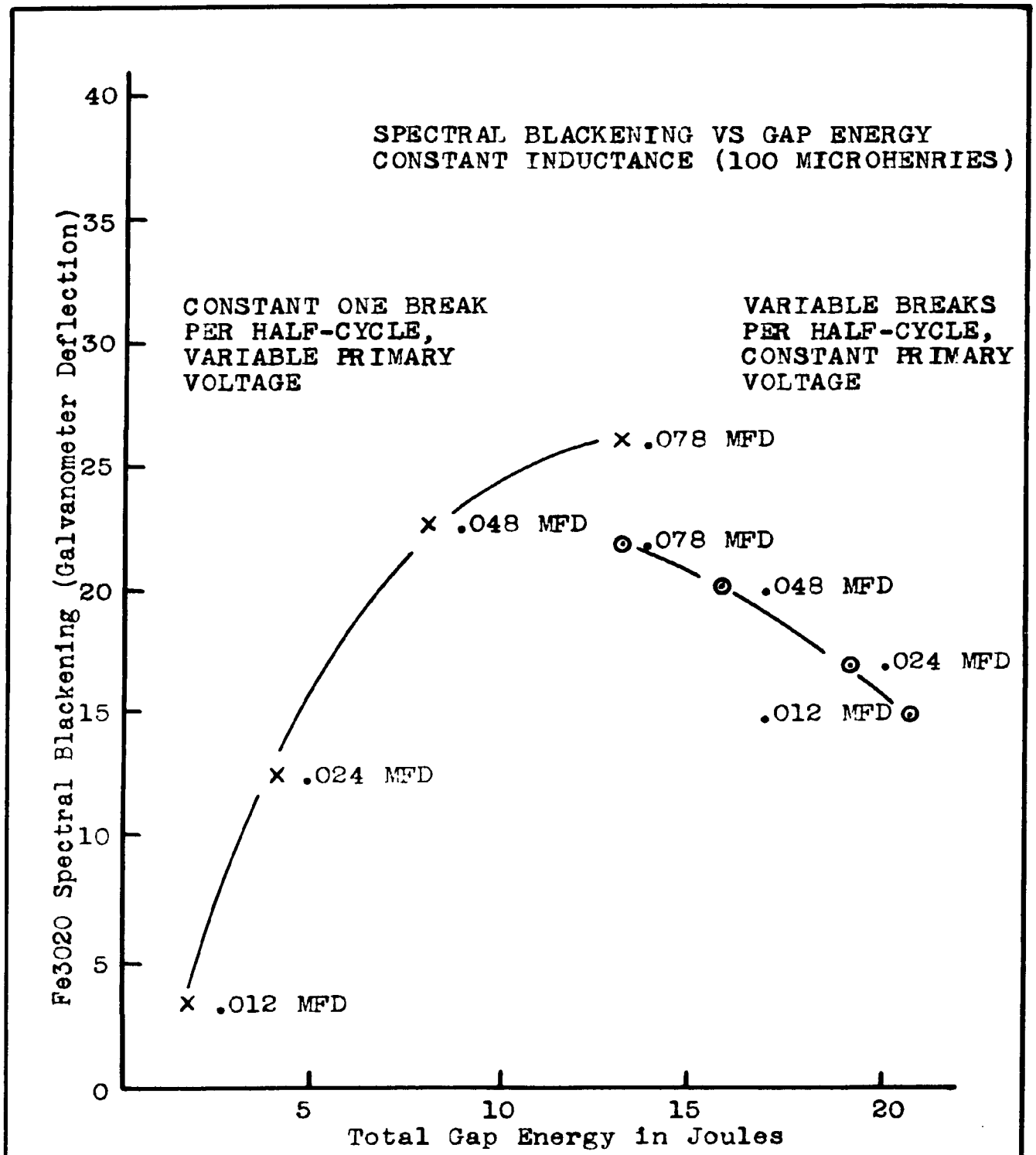


FIGURE 50

SPECTRAL BLACKENING VS GAP ENERGY

Figure 49 represents the same general type of plot as Figure 48 except that for these data the primary variac was turned to give an applied 220 volts for all values of capacitance. The number of breaks per half cycle vary with the capacity as follows:

Capacity	Breaks per half cycle
0.078	1
0.048	2
0.024	5
0.012	11

As is to be expected, the spectral intensity rises much more rapidly in Figure 49 for low capacity than in Figure 48 where only one break per half cycle is employed.

It is also interesting to plot the gap energy against the spectral line blackening. These data are given in Figure 50. When the sparking frequency is held constant at one break per half cycle, the spectral intensity at high capacity does not keep pace with the energy dissipated in the gap. When the height of the voltage wave applied to the secondary is kept constant, the gap energy decreases rapidly with increasing capacity.

Having selected 0.045 mfd as the proper value of capacity for the discharge circuit, the next step was to determine a satisfactory inductance. With the secondary capacity set at 0.045 mfd the secondary inductance was varied in steps of 25 microhenries to observe the effect of

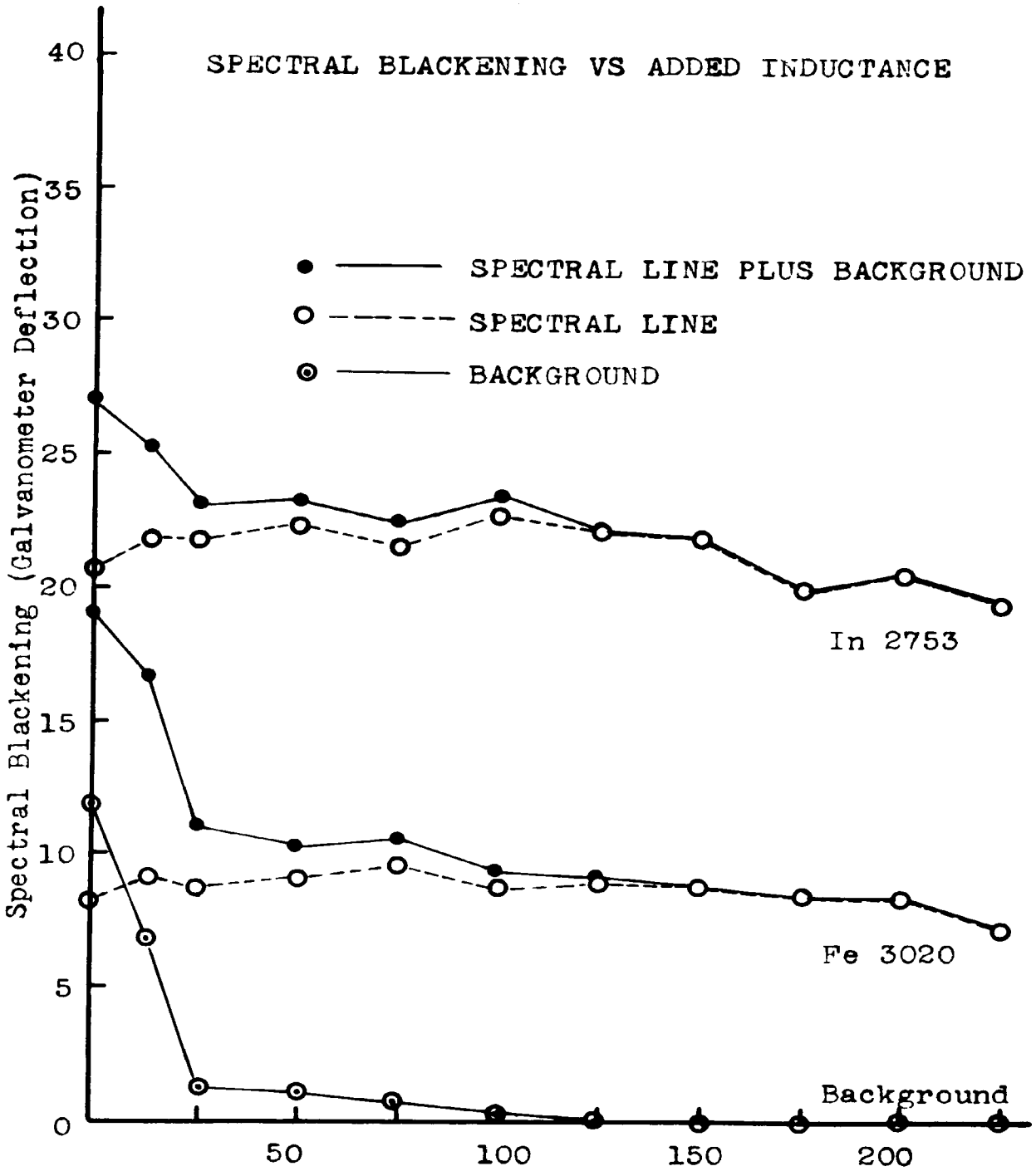


FIGURE 51

SPECTRAL BLACKENING VS ADDED INDUCTANCE

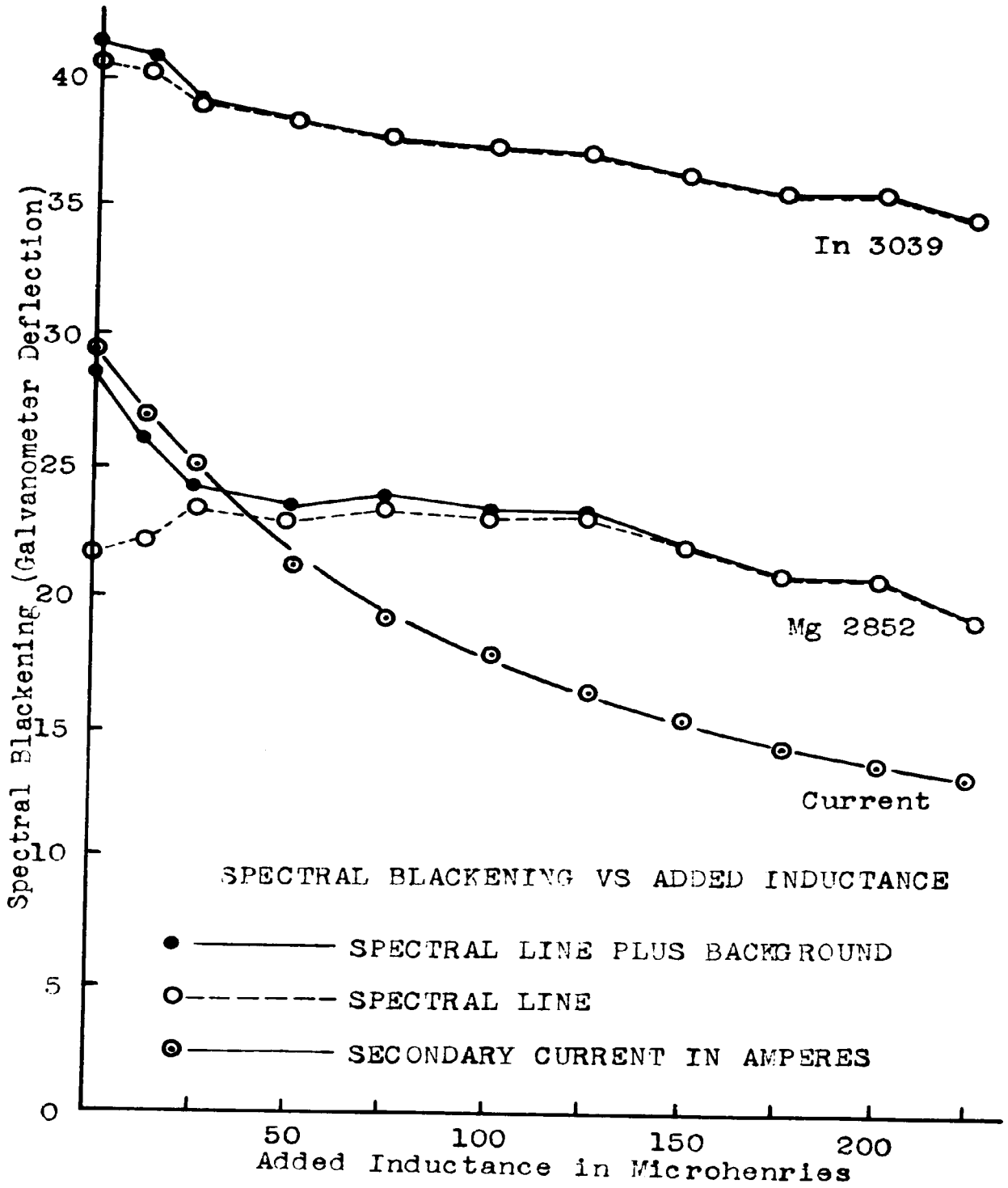


FIGURE 52

SPECTRAL BLACKENING VS ADDED INDUCTANCE

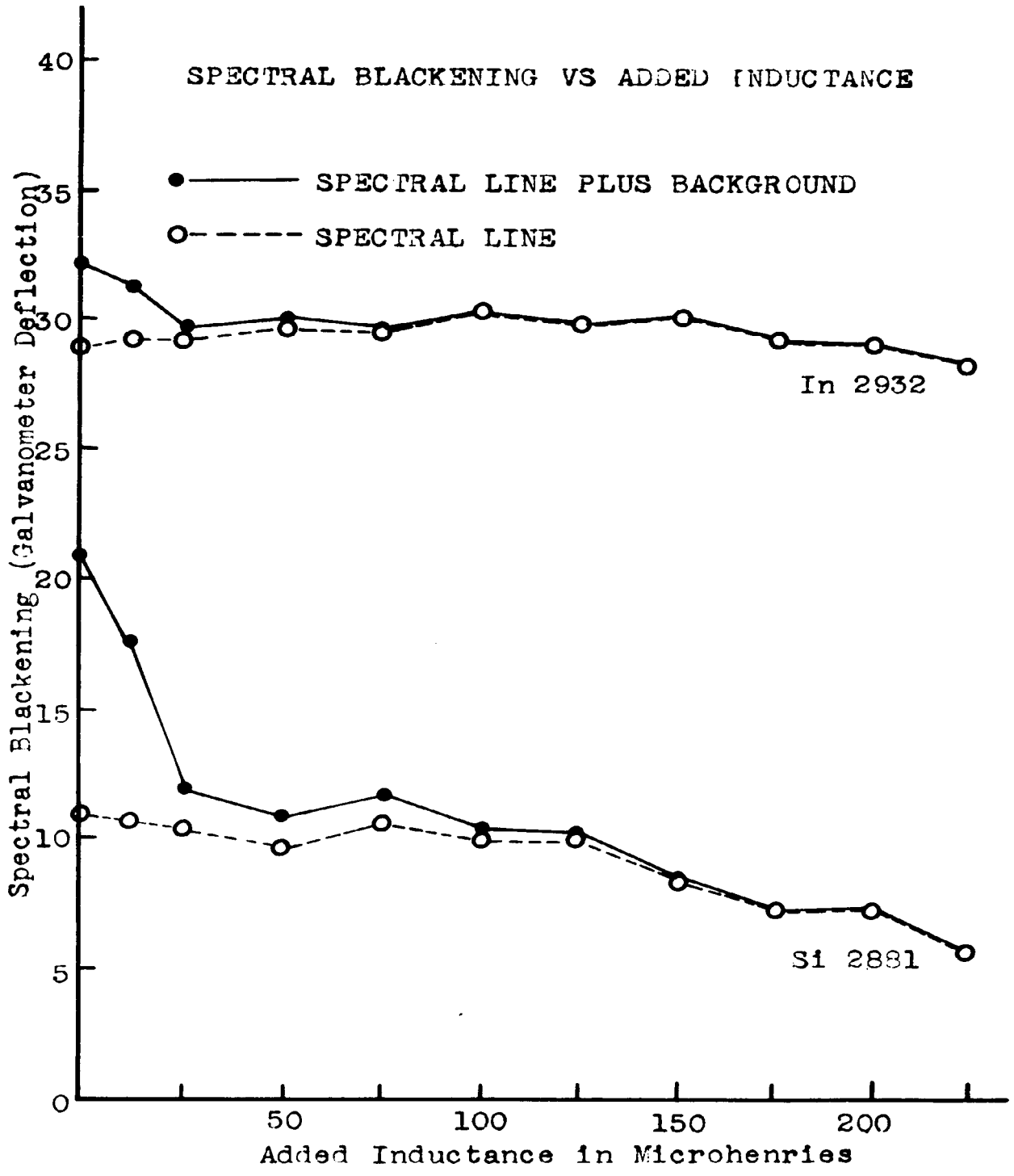


FIGURE 53

SPECTRAL BLACKENING VS ADDED INDUCTANCE

each increment upon the background. A plot of these data appears in Figures 51, 52, and 53. Since the background rises so sharply for small values of inductance, it is necessary to make a correction for the background of all datum points to enable a better evaluation of the variation of spectral intensity with inductance. The method of correction is illustrated in Figure 54, which is an H and D plot of photographic plate blackening versus the logarithm of light intensity to the base 1.5. A separate equivalent intensity scale is also furnished along the abscissa. I_o represents the intensity of light corresponding to the fog level of the plate. This will generally be very small and quite uniform over the whole plate. I_B represents the relative intensity of the radiation background. I_L represents the relative intensity of the spectral line. It is the blackening due to $(I_L + I_o)$ which needs to be evaluated in terms of added inductance. This blackening is the value that would be read on a microphotometer if there were no background. When a spectral line is read on a microphotometer, the blackening is really due to the sum of the intensities $I_L + I_B + I_o$. Likewise, blackening of the background region is really due to the sum of $I_B + I_o$. To make a background correction it is first necessary to subtract the value $(I_B + I_o)$ from the value $(I_L + I_B + I_o)$. The intensity represented by this difference is I_L. This value is then added to $I_o = 1$ and the blackening obtained either from $(I_L + I_o)$ on the intensity scale or from $\log(I_L + I_o)$ on the log intensity scale. These

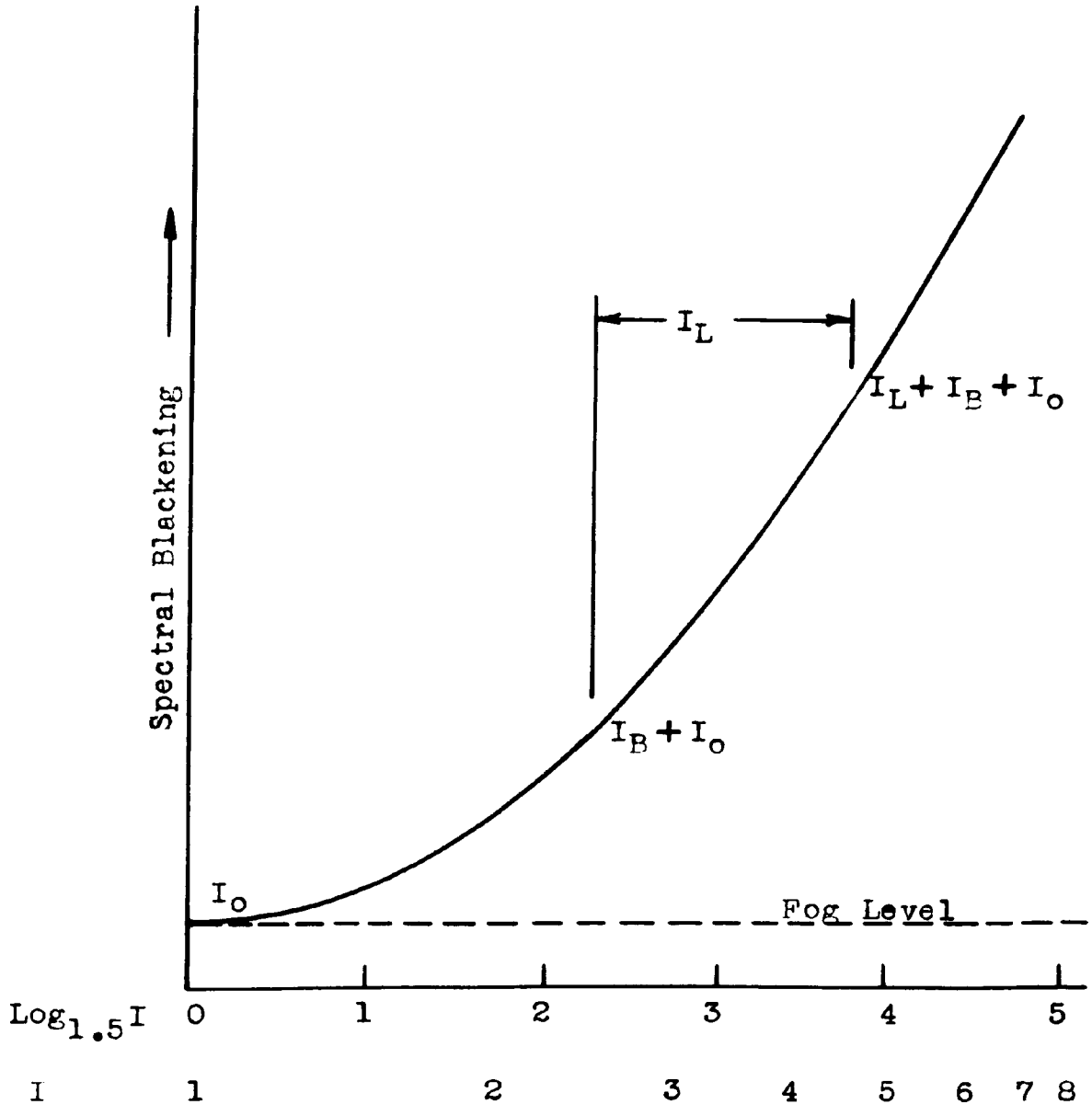


FIGURE 54

BACKGROUND CORRECTION

are the values of corrected blackening plotted in Figures 51, 52, and 53. These values represent the blackenings that would be read from the spectral lines if no radiation background were present.

With the background correction made, most of the curves of Figures 51, 52, and 53 look similar with a broad spectral intensity maximum in the 90 microhenry to 120 microhenry range. In reviewing the curves it was decided that 90 microhenries was the smallest inductance value which was compatible with low background. Reproducibility precision is favored by decreased inductance which makes the discharge more spark-like in character. Hence 90 microhenries was chosen as the inductance value which should furnish intense, reproducible spectra with low background intensity.

f. Desirability of a Buffer

When preparing samples in either liquid form or in dried precipitate form, it is often desirable to include a buffer. The purpose of such an additive is to stabilize the discharge by creating conditions which favor uniform volatilization and excitation of the sample over the exposure interval. The principal mechanism by which a buffer additive imparts to the discharge these desirable features is by cooling the discharge. This cooling action normally is characterized by (1) stabilization of the discharge, which is desirable, and (2) reduction in spectral excitation, which is undesirable. The decision of whether or not to use a

buffer is then largely determined by an evaluation of whether the gain in reproducibility is sufficient to offset the loss in spectral sensitivity.

In selecting a buffer which is satisfactory for analysis of biological materials, it is necessary to exclude chemicals which contain elements known to exist in the sample. Since biological materials contain so many elements, and since the nitrate radical is preferred over other radicals, ammonium nitrate is a very logical substance to use.

The representative data given in Table VII are indicative of the desirability of a buffer and the satisfactory performance of ammonium nitrate. Three things should be noted. These are:

1) When no buffer is present, the log ratio values are more dependent upon the drying conditions.

2) When ammonium nitrate is added, the spectral intensity is higher. This unusual enhancement is not due to contamination and will be discussed later.

3) The spectral intensities (microphotometer readings) reproduce themselves better when a buffer is added.

From consideration of these three items it can be concluded that reproducibility is favored by the addition of the ammonium nitrate buffer. Furthermore, there is indication that the addition of the buffer will bring about an unexpected but desirable spectral enhancement.

TABLE VII

Dried Precipitate Buffer Data

	Microphotometer Deflection		Log Ratio	Drying Condition
	Fe 2599	Mo 2816		
No buffer	6.0	12.4	-1.38	Vacuum dried then hot plate dried.
	5.9	11.8	-1.30	
	4.6	10.0	-1.20	
	12.6	18.4	-1.00	Hot plate dried.
	8.5	16.6	-1.34	
	7.7	13.5	-1.10	
With ammonium nitrate buffer	18.9	23.3	- .77	Vacuum dried then hot plate dried.
	17.5	21.3	- .64	
	18.8	22.3	- .61	
	13.3	17.4	- .70	Hot plate dried.
	16.7	19.8	- .53	
	13.3	17.7	- .74	

3. TECHNIQUES OF SAMPLE DRYING

The carbon electrodes are prepared for sample drying by chamfering the flat top perimeter approximately 1/32 of an inch. This tends to prevent preferential sparking of the discharge to the edges. Any excess carbon can then be removed by rubbing the face of the electrode with a clean paper towel. If this loose carbon is not removed, it gets heated to incandescence in the discharge and contributes to the background radiation.

The next step preparatory to sample drying is to reduce the porosity of the carbon by adding two drops of melted paraffin to the heated electrode tip. This is satisfactorily accomplished by placing the electrodes in a carbon block multi-electrode jig to hold them in an upright position. This carbon block is placed on a hot plate at approximately 160° F. One drop of melted paraffin at 160° F is then added to the chamfered electrode top and allowed to sink into the carbon. The second drop is then added. It is desirable that the paraffin be retained close to the electrode surface to prevent sample penetration. Yet, equally well, no excess paraffin should be left on the carbon surface because this leads to non-uniform sample distribution and therefore inferior reproducibility. In practice the electrodes were removed from the hot plate about five minutes after the electrode surfaces lost their "wet" appearance. Although the wet-dry transition is quite sharp, permitting an accurate measurement of the succeeding penetration interval, an

accurate measurement is unnecessary because the interval is not at all critical.

a. Infrared Lamp

The infrared lamp is a very versatile source of heat for drying electrode samples because (1) application of the inverse square law permits a great flexibility of intensity and (2) the heat can readily be directed at the samples from any angle.

For the drying of a single pair of electrodes this is a method well worth considering. However, when the applied heat must cover a large area, as when several electrodes are prepared, difficulty is encountered in establishing uniformity of illumination. Visual inspection of the sample deposits left on a set of electrodes after drying under a heat lamp shows a variety of drying patterns. Since nonuniformity of reproducibility is associated with nonuniformity of drying, the heat lamp method of drying is not considered satisfactory for group drying.

b. Inverted Electrodes

Although the possibility of drying sample material on an inverted electrode at first appears ludicrous, it is, however, worthy of serious consideration. When a sample drop is applied to an electrode in an upright position, there is a dominant tendency for most of the material to deposit around the perimeter of the electrode. This is indicated

in an exaggerated form in Figure 55. When a drop of sample is applied to an inverted electrode (as shown in Figure 56), the material dries in a satisfactorily uniform deposit over the whole of the sparking surface.

The actual application of the drop to the bottom of the electrode is not as difficult as it might seem. If a glass rod carrying the sample drop is touched to the electrode in the region of A in Figure 56, the sample drop will adhere to the carbon and the glass rod may be pulled away, allowing the sample drop to run down underneath the electrode in the drying position. If desired, successive drops may be added after the previous drop is dry or nearly dry.

Although the uniformity of drying achieved by this method is very desirable, it does not lend itself well to group drying because of the physical difficulties of the application of the sample drops to a large block of inverted electrodes.

c. Vacuum Drying

The difficulties of achieving uniform sample distribution by heat drying were finally resolved when a partial vacuum was employed to bring about the drying. Not only is this method desirable from the standpoint of uniform drying but this uniformity is independent of the number of electrodes being dried. It is, therefore, equally applicable to large or to small groups of electrodes. Apparently,



FIGURE 55

DRYING DROP ON
ERECT ELECTRODE

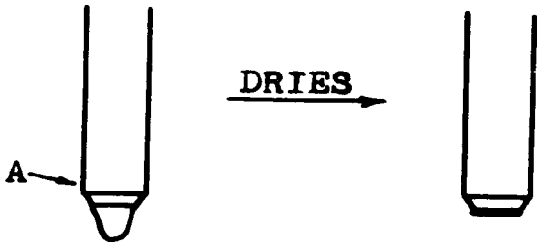


FIGURE 56

DRYING DROP ON
INVERTED ELECTRODE

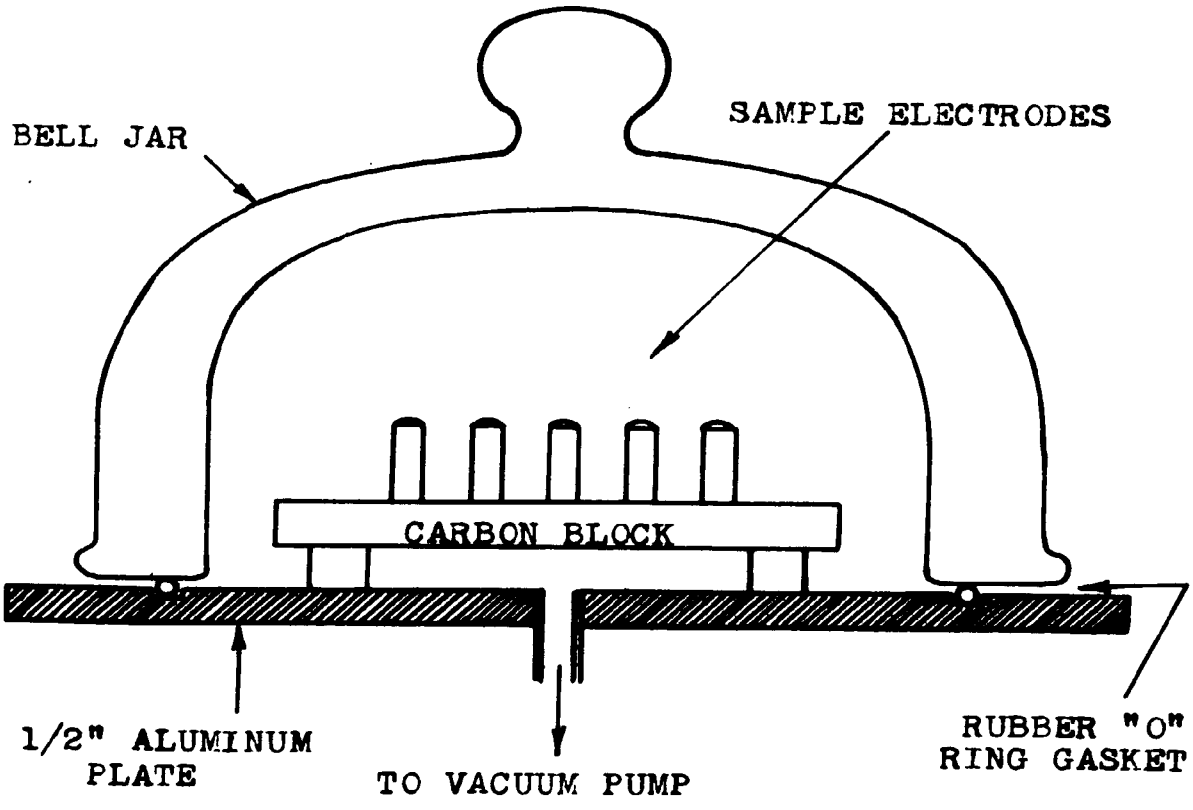


FIGURE 57

BELL JAR ENSEMBLE

the number of sample electrodes which can be uniformly dried by this method is limited only by the size of the vacuum bell jar. The particular bell jar vacuum system employed for the following work is shown in Figure 57.

In practice the vacuum must be applied slowly enough for gas bubbles trapped in the sample to expand and escape without bursting the sample drop. With this proviso the liquid drop may readily be dried under a vacuum of the order of one millimeter of mercury. The resultant deposit at this pressure is uniform in coverage over the surface of the electrode but does not appear completely dry in appearance. That it is not completely dry is manifest in the fact that the spectral intensity by this method is inferior to the spectral intensity from hot plate dried samples. An obvious possibility exists, therefore, that if the samples are first vacuum dried and then dried on a hot plate, they may combine uniform coverage with high spectral intensity. It is readily confirmed that electrodes prepared in this manner do indeed yield both uniform coverage and high spectral intensity.

Where high sensitivity is not required, the vacuum drying method can be the last preparatory step before excitation in the discharge. Where high sensitivity is required, an additional drying step is required.

d. Vacuum Drying Plus Hot Plate Drying

When the samples are vacuum dried, the sample uniformly covers the surface of the electrode. As the water is gradually removed from the sample, the sample becomes increasingly tacky until finally a solid glaze is formed. When the electrode is then transferred to the hot plate, this drying process continues. The appearance of the material on the surface of the electrode varies with the hot plate temperature. At temperatures of the order of 150° F the deposit is white-ish in appearance, whereas at temperatures around 500° F the residue is more black-ish in appearance. It is reasonable to expect that this distinction in appearance is associated with a distinction in spectral intensity and perhaps reproducibility.

To a lesser degree this variation in appearance is apparent within any set of hot plate dried samples. When a set is placed in a carbon block jig, as in Figure 58, the surface temperatures of the electrodes around the outside of the set (indicated by ■) are cooler than those in the central region (indicated by O). Although this variation is only of the order of a few degrees, the difference manifests itself in (1) a difference in drying time and (2) a small distinction in appearance. This situation can be much improved by the addition of a dummy row of electrodes around the set as indicated in Figure 59 by ●. These additional electrodes tend to distribute the heat more evenly and improve the drying uniformity of the sample set.

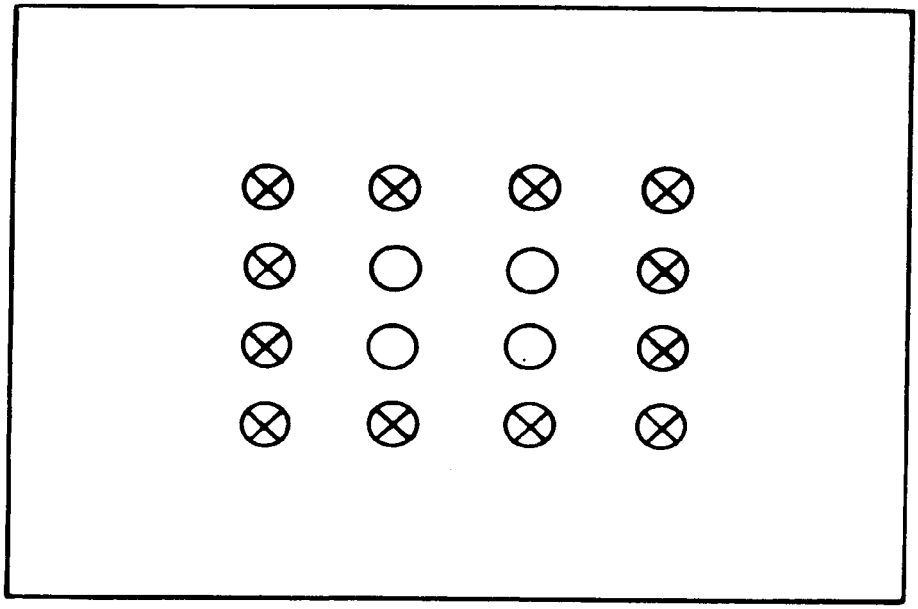


FIGURE 58

LOADED CARBON BLOCK

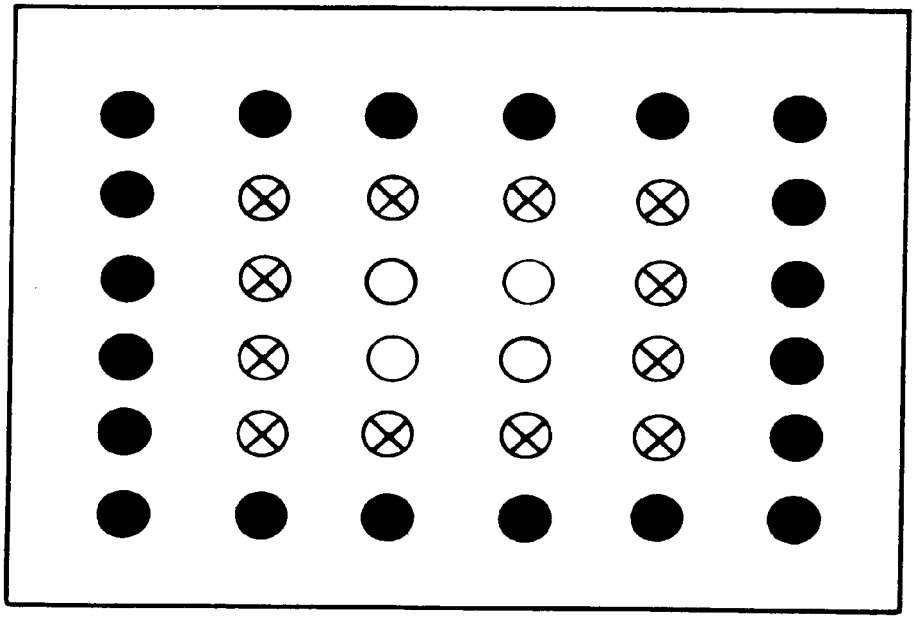


FIGURE 59

LOADED CARBON BLOCK WITH DUMMIES

Inasmuch as a non-uniformity of heating yields a non-uniformity of sample drying, oven dried samples can be expected to furnish better uniformity of drying than hot plate dried samples. This is confirmed by visual inspection of the resultant electrodes.

e. Vacuum Drying Plus Oven Drying

The results of an investigation of the variation of spectral intensity with oven drying temperature are listed in Table VIII and plotted in Figures 60, 61, and 62. The large circles around the plotted points of Figures 60, 61, and 62 are measures of the spread of repeat values at the indicated temperatures. It is evident that the most favorable oven temperature from the standpoint of reproducibility lies in the range of 500° F. It is also evident that the samples which were not pre-dried at 125° F on a hot plate are more reproducible than those which were pre-dried. All further work, therefore, is based upon samples which have first been vacuum dried and then oven dried at 500° F.

A further study of the effect of moisture in the sample before drying at 500° F was made. The results of this investigation are listed in Table IX. This table indicates clearly that the reproducibility of samples does not vary appreciably among the various stopping points of the vacuum drying. It is interesting to note that samples removed from the vacuum jar while still wet consistently yield higher spectral intensities than those which were

TABLE VIII

Influence of Temperature on Precision

Predried at 125° F			Oven Temperature °F	Not Predried		
Fe	Mg	Si		Fe	Mg	Si
σ=.16	σ=.18	σ=.10	230°F	σ=.13	σ=.21	σ=.13
σ=.07	σ=.18	σ=.10	400°F	σ=.10	σ=.16	σ=.17
σ=.15	σ=.12	σ=.13	500°F	σ=.05	σ=.06	σ=.07
σ=.11	σ=.13	σ=.13	700°F	σ=.08	σ=.08	σ=.13

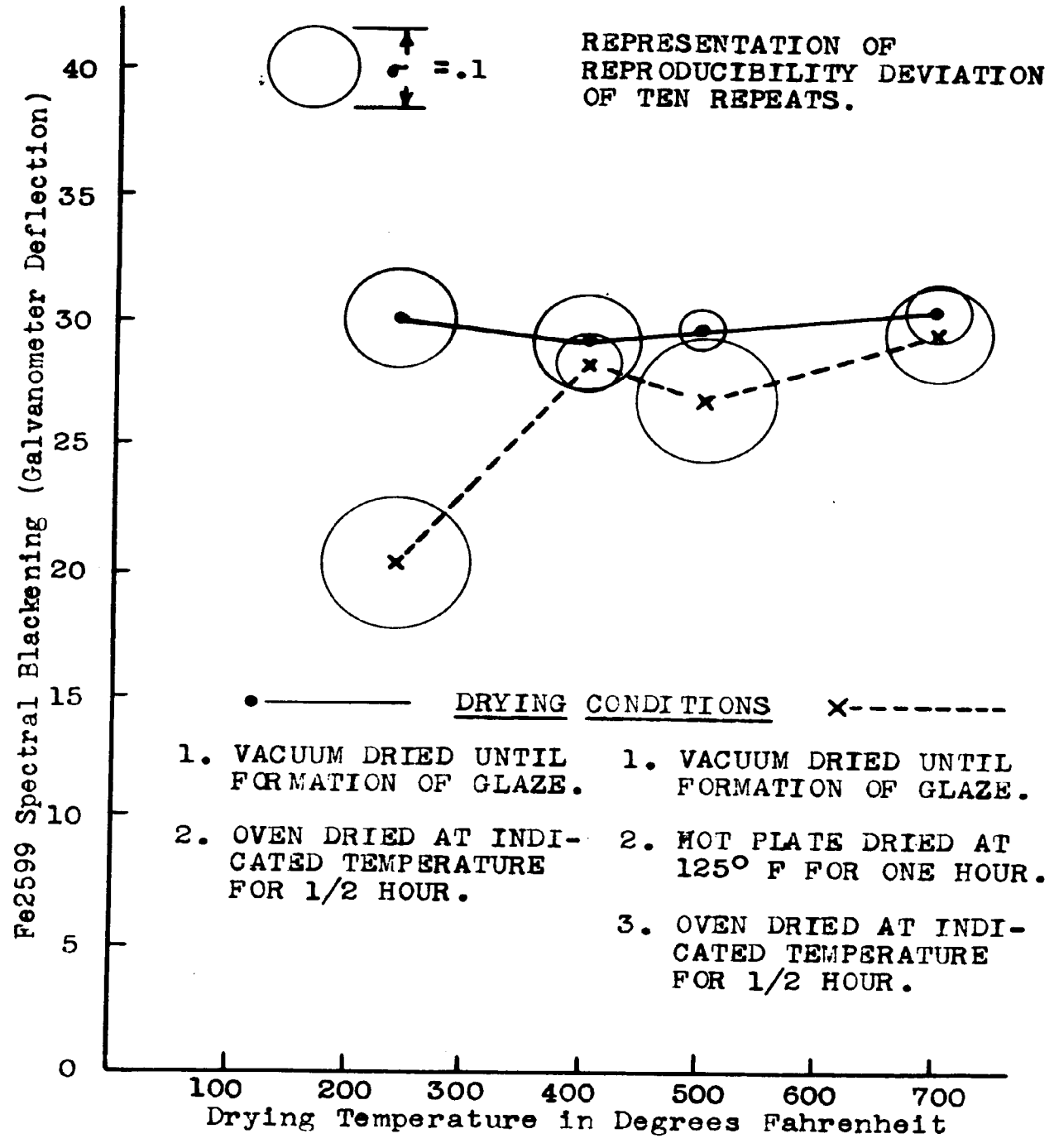


FIGURE 60

IRON SPECTRAL BLACKENING VS DRYING TEMPERATURE

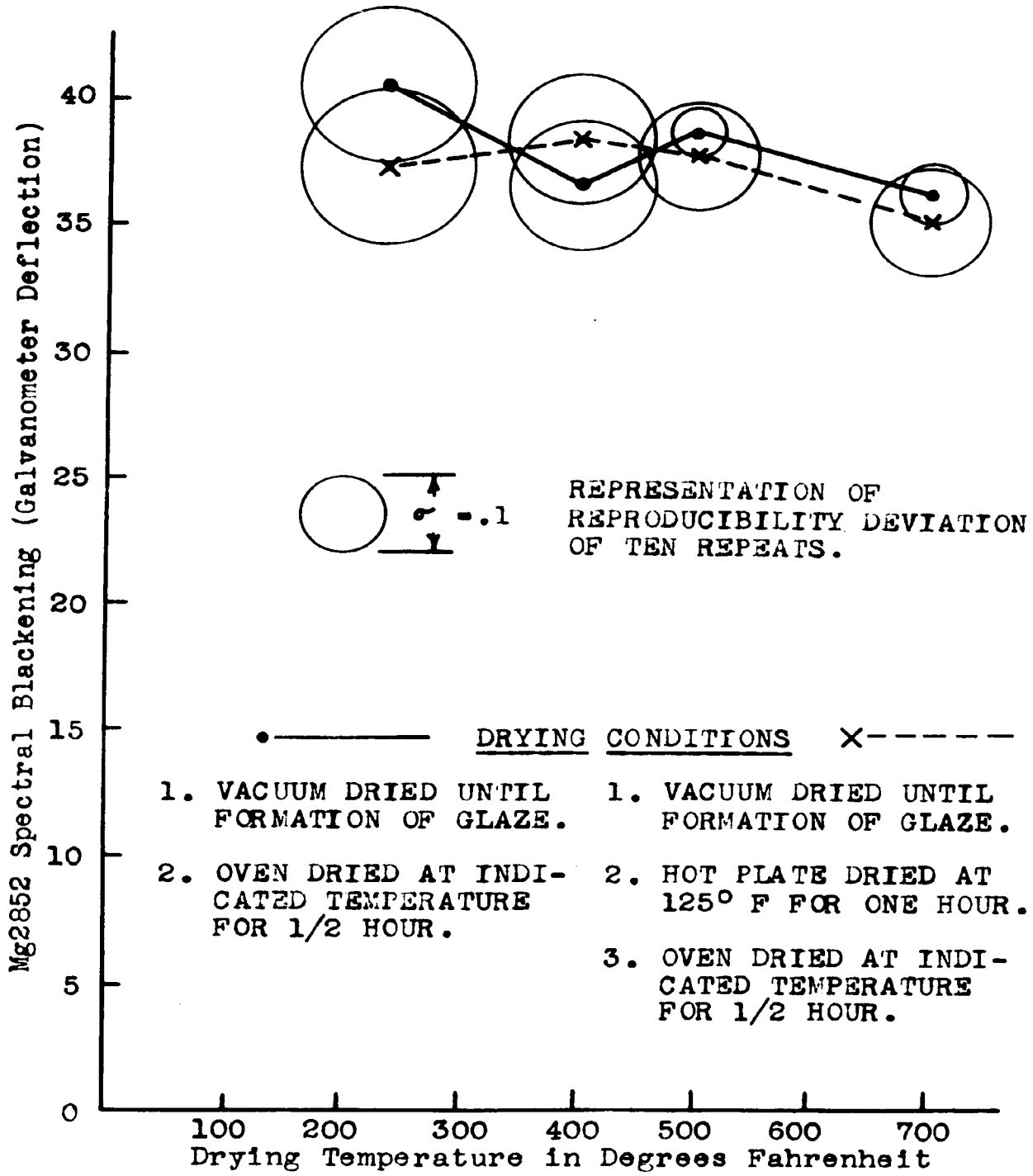


FIGURE 61

MAGNESIUM SPECTRAL BLACKENING VS DRYING TEMPERATURE

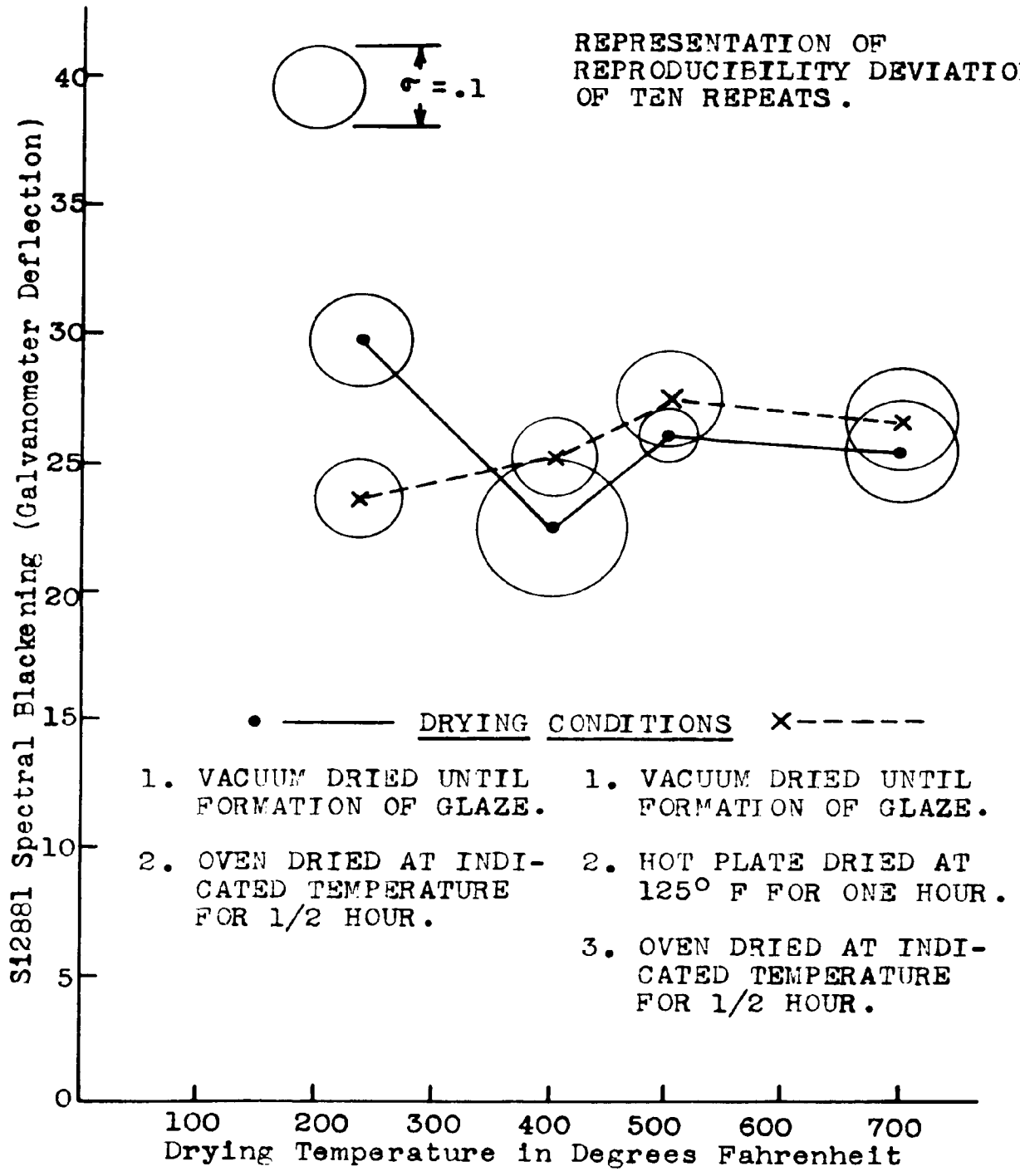


FIGURE 62

SILICON SPECTRAL BLACKENING VS DRYING TEMPERATURE

TABLE IX

Comparison of Drying Conditions

Treatment	Element	Spectral Intensity (Galvanometer deflection)	Reproducibility (Standard deviation of twenty repeats)
Treatment A	Fe	22.0	.09
Vacuum dried until a full glaze exists	Mg	34.5	.09
	Si	20.0	.09
Treatment B	Fe	21.5	.08
Vacuum dried until a glaze just starts to form	Mg	35.0	.11
	Si	20.0	.10
Treatment C	Fe	26.0	.08
Vacuum dried but removed while still moist before glaze formed	Mg	37.5	.09
	Si	24.0	.08

Note: After vacuum drying, all samples are dried in a 500° F oven for one-half hour.

permitted to glaze over. Since the standard deviations of these representative elements all lie below $\sigma = .10$, the reproducibility of this "Treatment C" is considered satisfactory. It is evident, however, that there is no large distinction among the treatments from the standpoint of either reproducibility or spectral intensity.

4. SENSITIVITY AND SPECTRAL ENHANCEMENT

In the investigation which led to the determination of the ultimate sensitivity of the dried precipitate method, a very interesting feature of spectral enhancement was encountered. This spectral enhancement is found associated with the addition of ammonium nitrate and/or the addition of indium.

Such an enhancement is directly contrary to expectations. The addition of ammonium nitrate should cool the discharge, giving greater stability but also lower spectral intensities. The addition of a volatile, low work function metal such as indium should cause all other spectra to be subdued and reduced in intensity.

This enhancement is not due to any contamination in the additives. This was verified by analysis. Rather, the spectra are enhanced merely by virtue of the presence of the additive. In effect the additives behave as catalysts. The actual mechanism by which this enhancement is accomplished is thought to be approximately as follows: As the sample material is dried, relatively large volatile crystals of

ammonium nitrate or indium are formed. The analytical elements tend to deposit out on the surface of, or perhaps become inclusions in, the volatile crystals. When the total dried precipitate is excited in the discharge, the volatile indium or ammonium nitrate is vaporized so rapidly into the discharge gap that it carries along with it the analytical elements. In effect the ammonium nitrate or indium additive imparts to the analytical elements a volatility comparable to its own.

The data representing this enhancement are plotted in Figures 63, 64, 65, 66, and 67. Spectral intensities are plotted as ordinates and sample concentration as abscissae. The range in concentrations was achieved by successive dilutions of the 100 mg./ml. sample solution. The amount of additive per ml. of solution, however, remains constant for each concentration. These constant values are: 0.0277 mg. of Mo per ml., 1 mg. of In per ml., and 50 mg. of NH_4NO_3 per ml. In every case the spectral enhancement caused by the indium or the ammonium nitrate (buffer) is clearly evident. It is noted that at higher sample concentrations the "carrier" role of the ammonium nitrate is becoming subordinate to its intended "buffering" role. In some cases at high concentrations the spectral intensities from sample solutions with ammonium nitrate have fallen below those spectral intensities of their non-ammonium nitrate counterpart.

Table X lists the sensitivities obtained on the basis of the arbitrary standard of spectral density equal

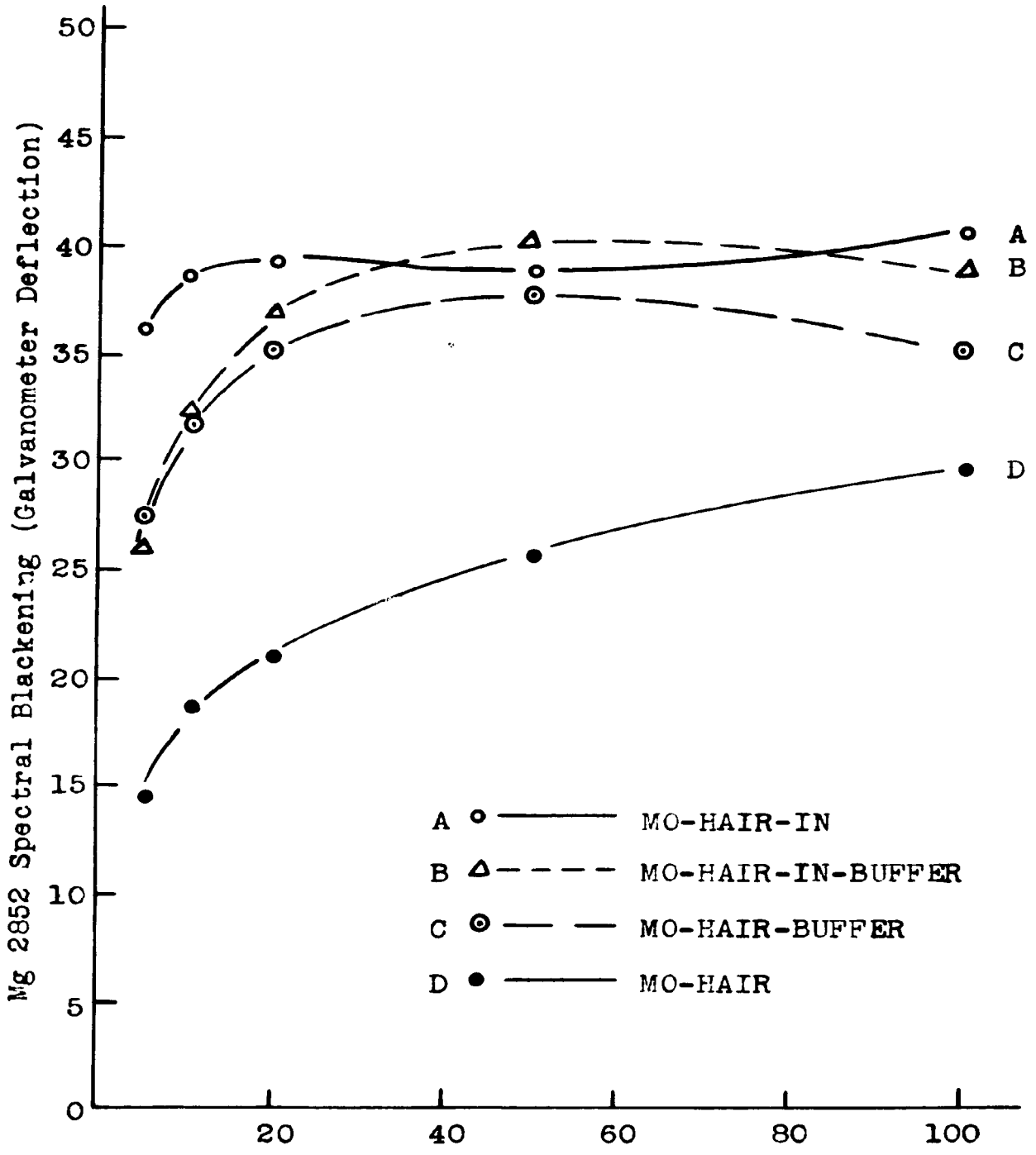


FIGURE 63

SPECTRAL RESPONSE OF MAGNESIUM TO SAMPLE DILUTION

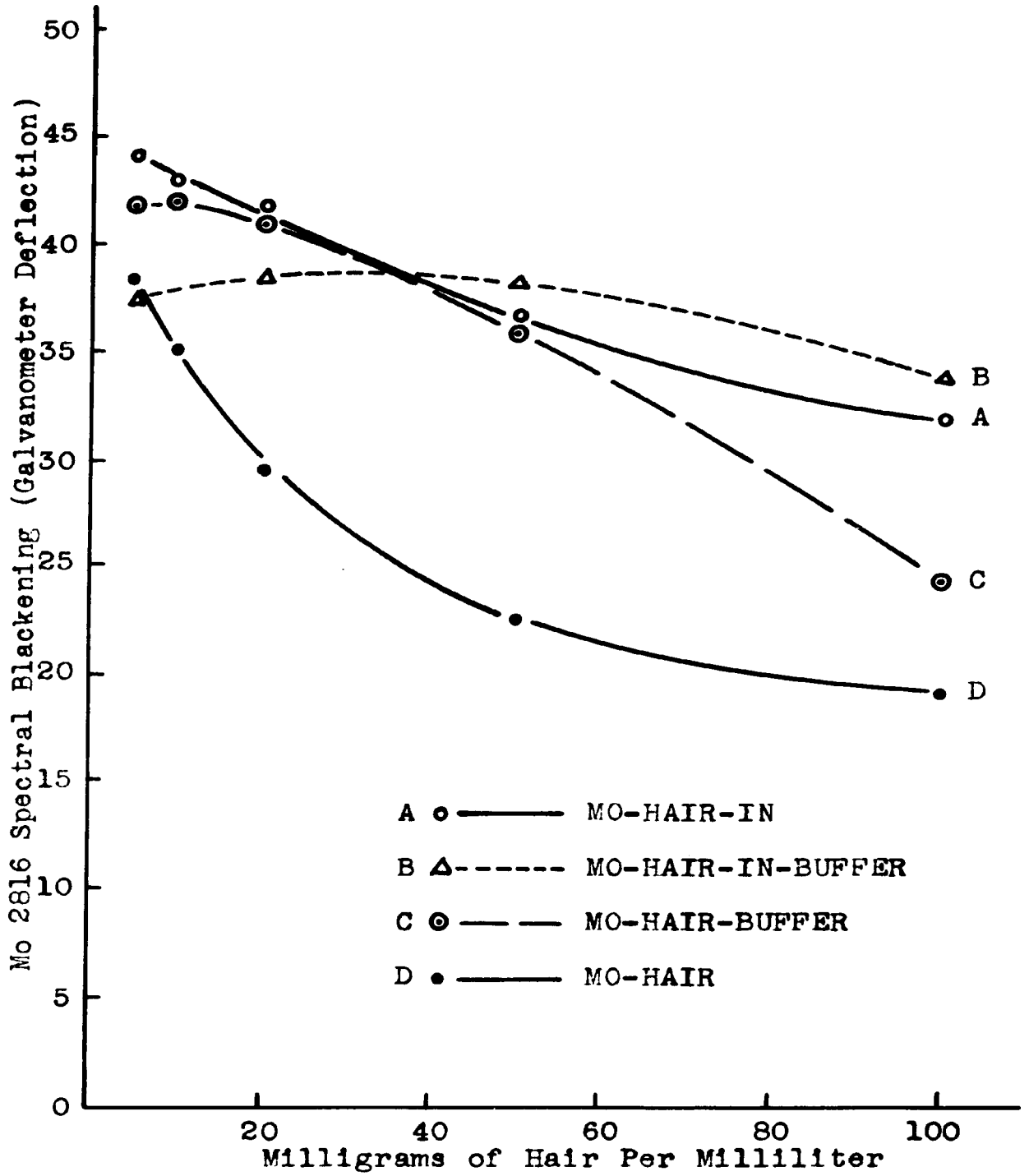


FIGURE 64

SPECTRAL RESPONSE OF MOLYBDENUM TO SAMPLE DILUTION

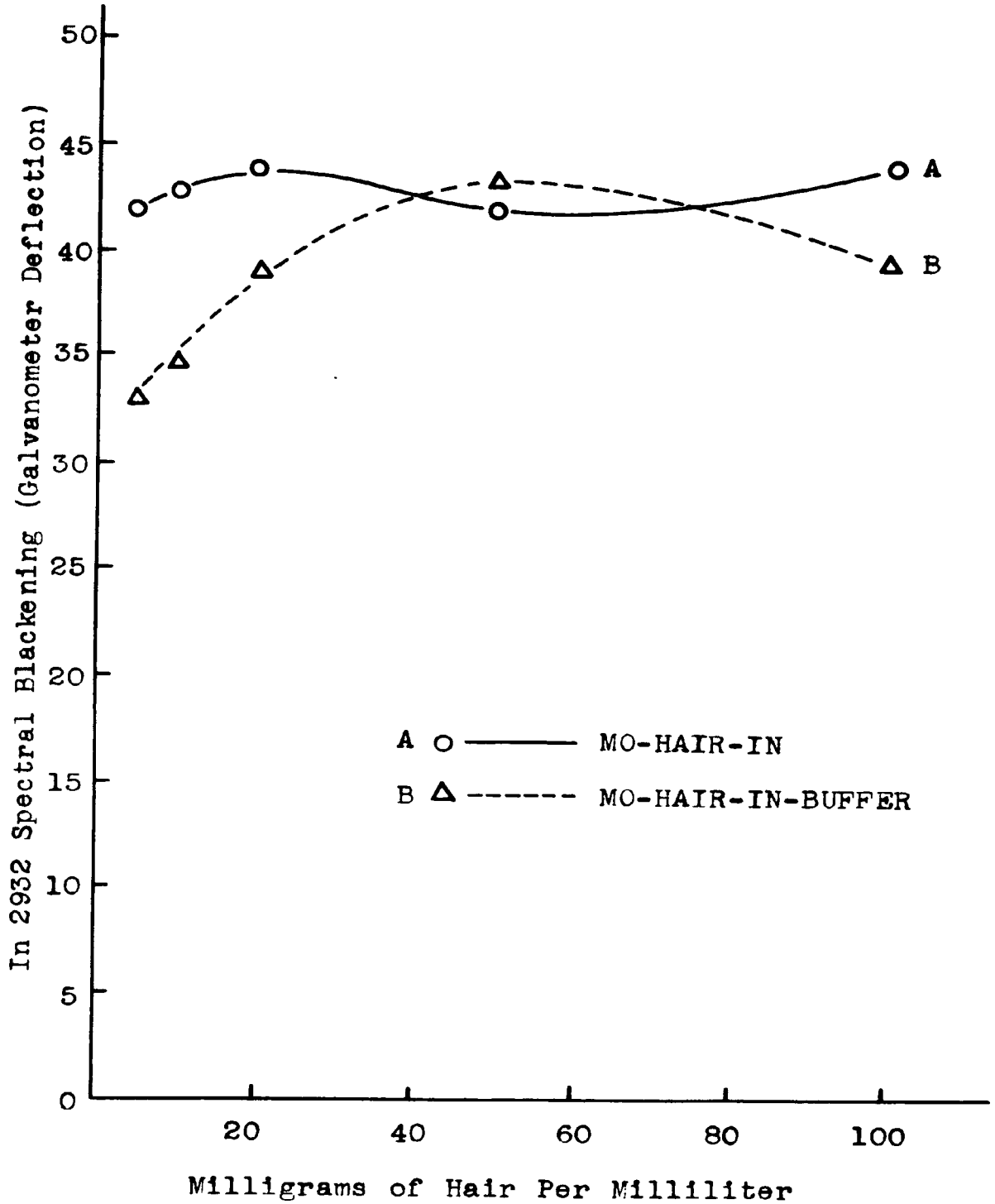


FIGURE 65

SPECTRAL RESPONSE OF INDIUM TO SAMPLE DILUTION

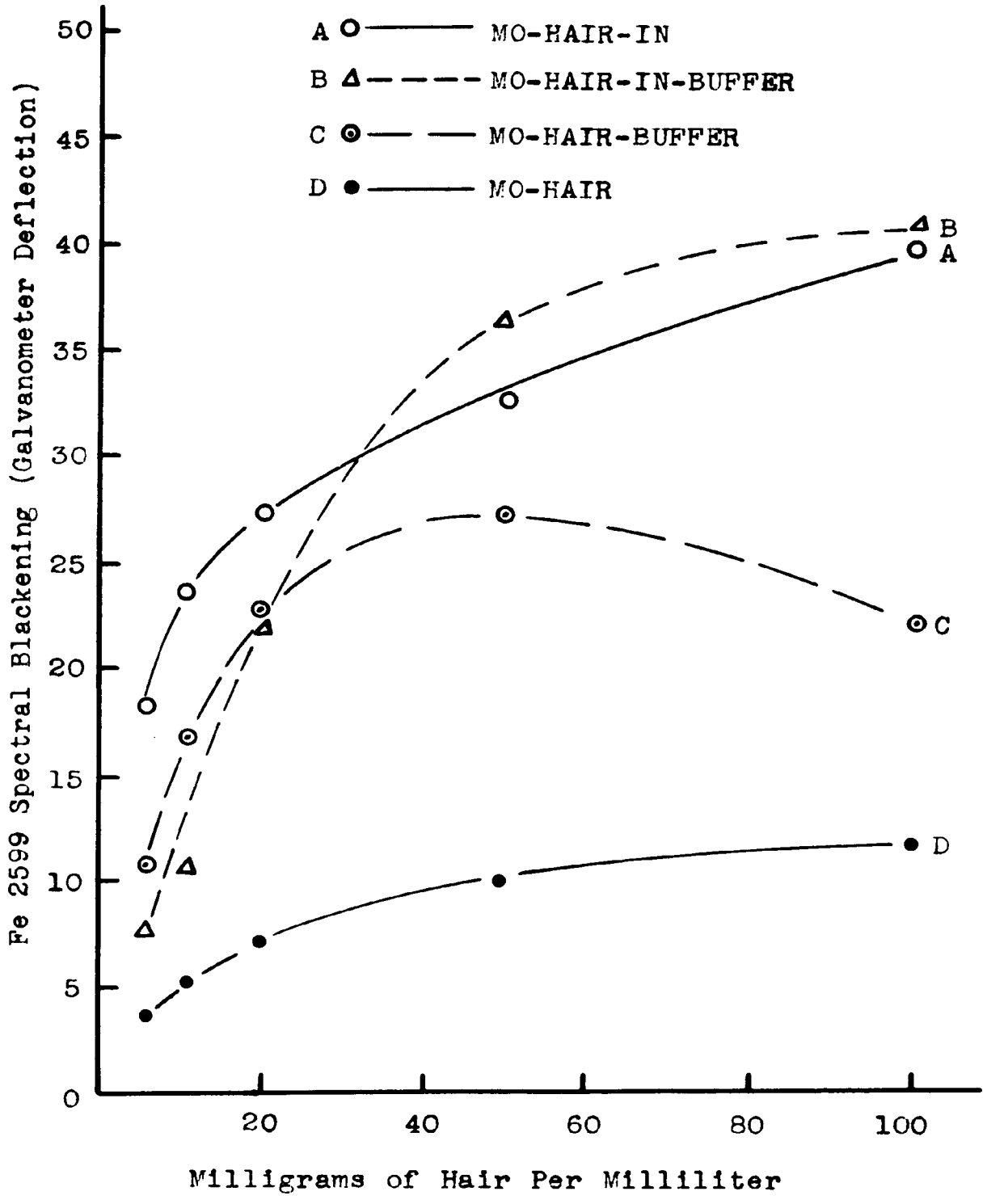


FIGURE 66

SPECTRAL RESPONSE OF IRON TO SAMPLE DILUTION

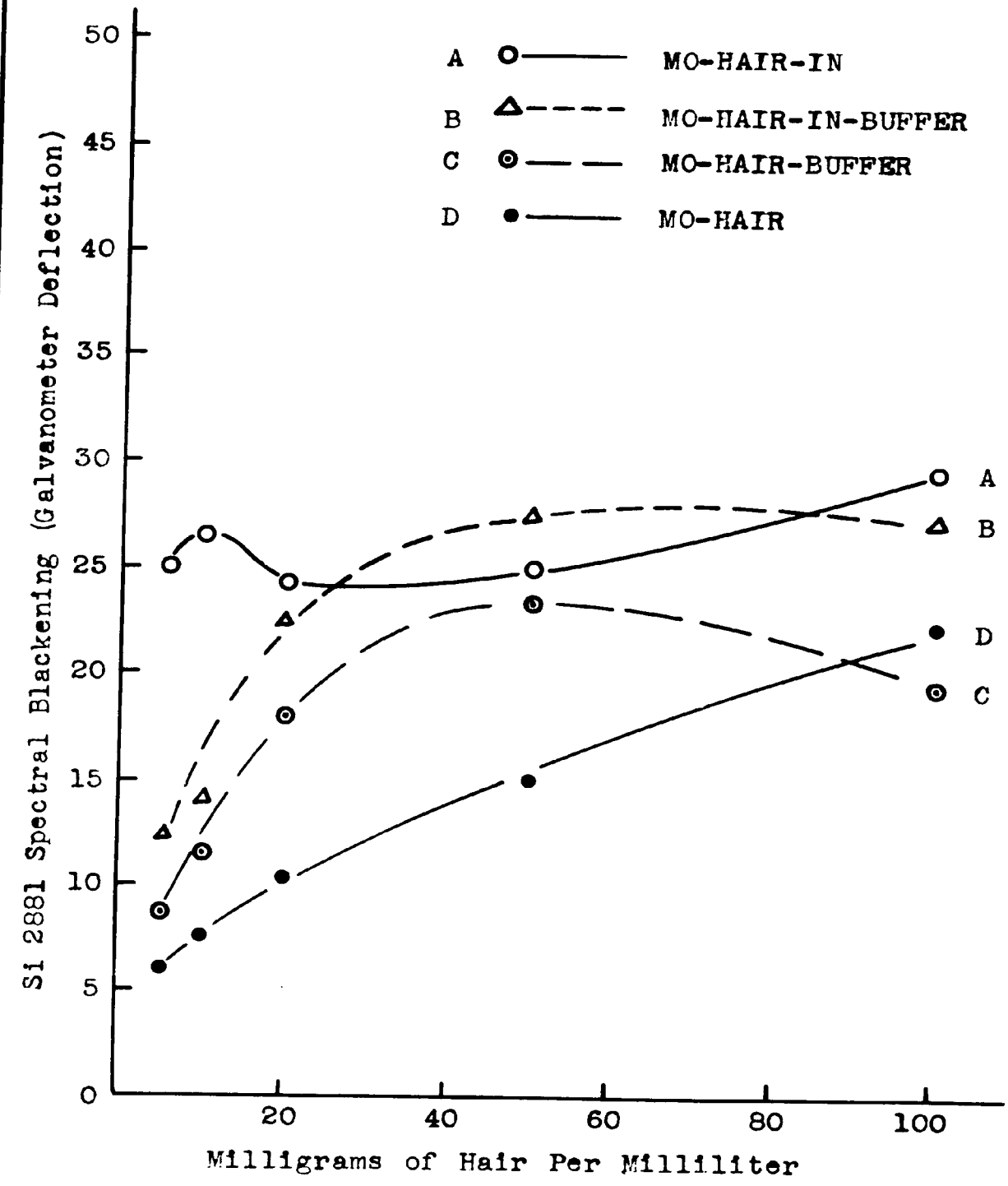


FIGURE 67

SPECTRAL RESPONSE OF SILICON TO SAMPLE DILUTION

TABLE X

Dried Precipitate Sensitivities

	Sensitivity in Micrograms*		
	Fe 2599	Mg 2852	Si 2881
Hair - Mo	0.12	0.10	0.20
Hair - Mo - Buffer	0.09	0.05	0.15
Hair - Mo - In	0.09	0.02	0.05
Hair - Mo - In - Buffer	0.06	0.02	0.05

* The number of micrograms of element necessary to produce a spectral blackening of 0.01 density.

to 0.01 or more. This is the same standard that was used for porous cup work. The enhancement is still observable at this concentration level, but it is not as marked as at the higher concentrations. Even at this level, however, the addition of indium or ammonium nitrate approximately doubles the sensitivity.

5. REPRODUCIBILITY

The spectral enhancement that indium and ammonium nitrate impart to the sample solution is very appealing. However, the chosen method of analysis must not only be sensitive but also reproducible. A comparison of reproducibility was made, therefore, among the four types of solutions under consideration. These were:

1) Hair solution plus internal control molybdenum.

2) Hair solution plus indium for spectral enhancement plus internal control molybdenum.

3) Hair solution plus ammonium nitrate buffer plus internal control molybdenum.

4) Hair solution plus ammonium nitrate buffer plus indium for spectral enhancement plus internal control molybdenum.

The standard deviations of ten repeats with each solution are listed in Table XI. Likewise in Figures 68, 69, and 70 are given representative analytical curves for these solutions.

TABLE XI

Dried Precipitate Precision

	Precision for Indicated Line Pairs (Ten Repeats per Sample Solution)					
	Fe2599/Mo2816	Fe2599/In2753	Mg2852/Mo2816	Mg2852/In3039	Si2881/Mo2816	Si2881/In2753
Mo-Hair	σ=.22		σ=.14		σ=.44	
Mo-Hair-Buffer	σ=.09		σ=.10		σ=.11	
Mo-Hair-In	σ=.27	σ=.39	σ=.26	σ=.26	σ=.18	σ=.38
Mo-Hair-In-Buffer	σ=.45	σ=.23	σ=.09	σ=.43	σ=.18	σ=.35

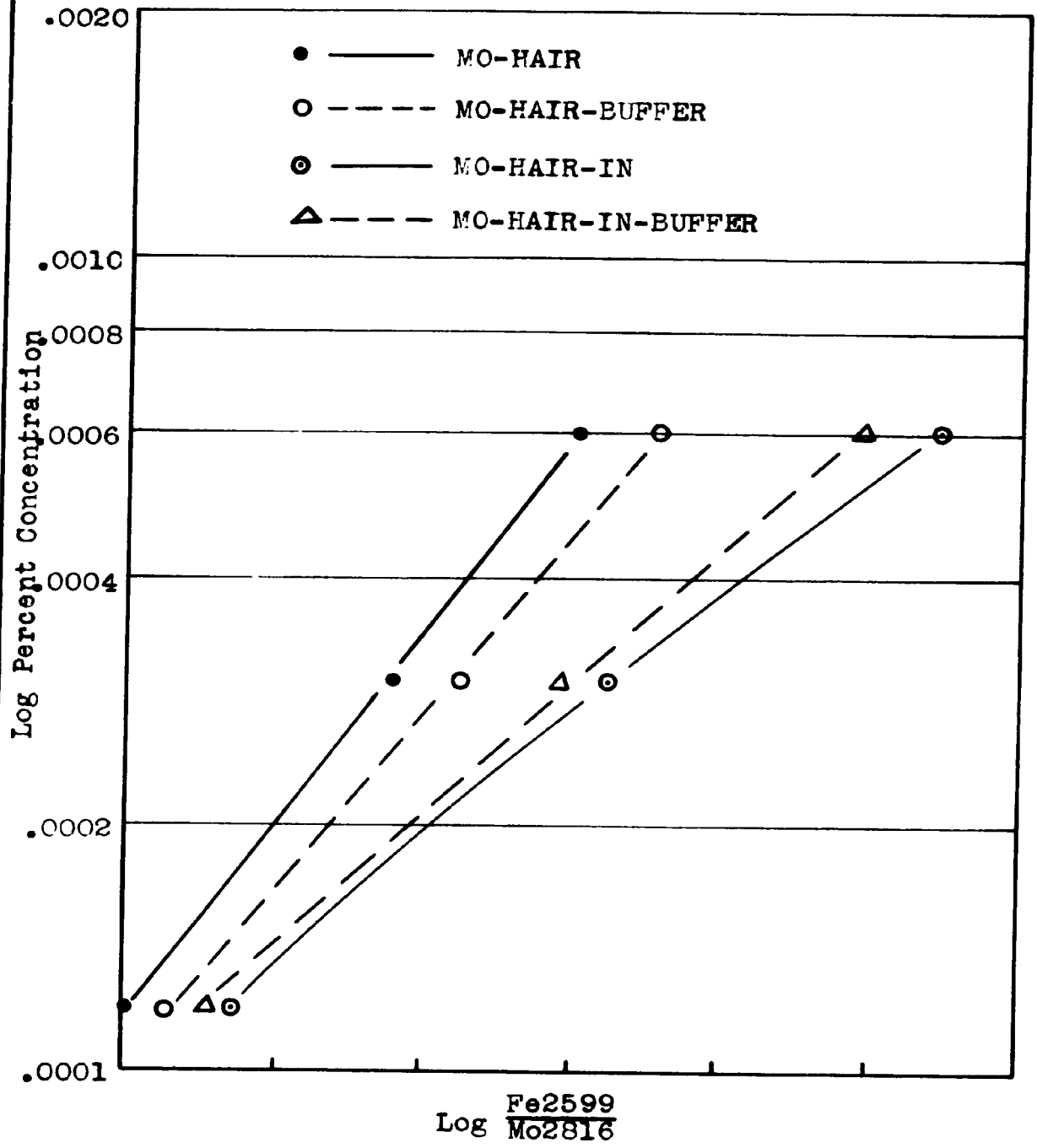


FIGURE 68

ANALYTICAL SLOPES OF IRON

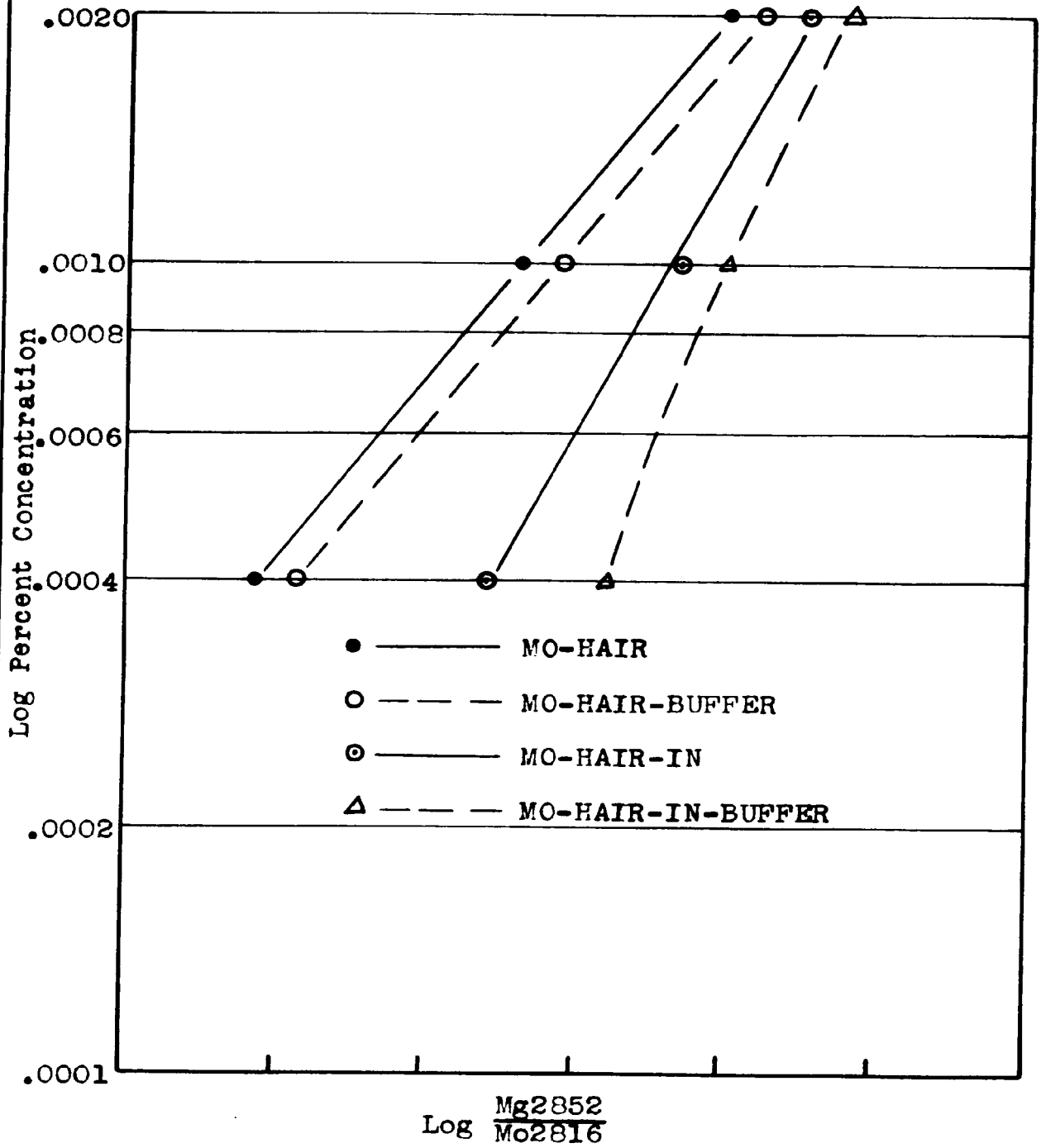


FIGURE 69

ANALYTICAL SLOPES OF MAGNESIUM

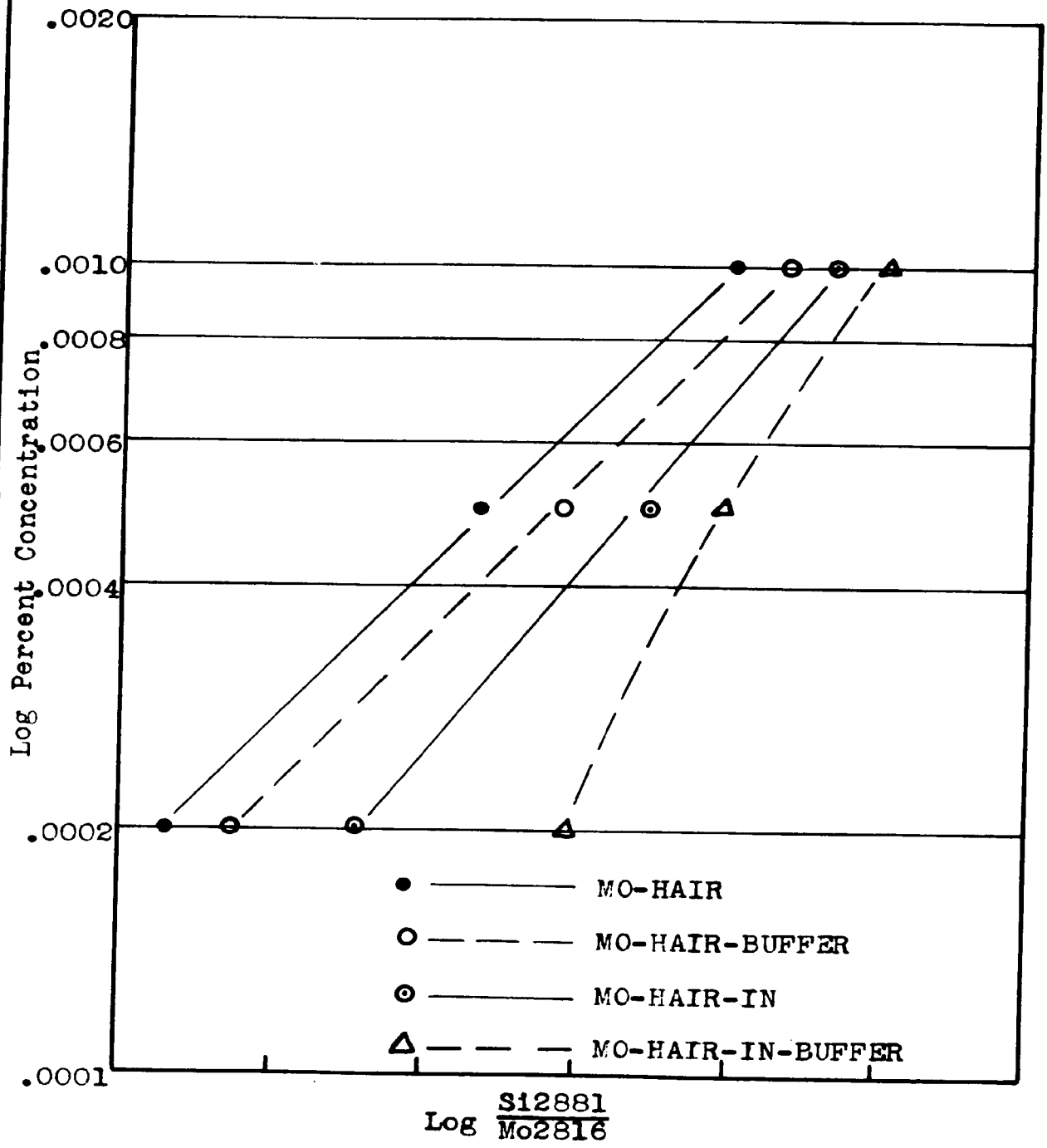


FIGURE 70

ANALYTICAL SLOPES OF SILICON

In evaluating Table XI it is evident that the best reproducibility is associated with the molybdenum-hair-buffer solution. Although the added indium is beneficial to the sensitivity, it is harmful to the reproducibility. It has already been observed that the intensity of the indium spectrum is much influenced by the drying conditions. It is consistent, therefore, that the enhancement of other spectral lines by indium be dependent upon slight variations in the drying temperature.

The analytical curves of Figures 68, 69, and 70 indicate that although indium exerts a favorable influence on the slope for iron, the slopes of the analytical curves for magnesium and silicon are adversely affected.

A careful consideration of both the standard deviations and the analytical curve slopes forces the conclusion that while indium favorably influences the sensitivity, its influence upon the reproducibility is unfavorable. Therefore, indium is not a desirable additive to biological materials except in cases where the gain in spectral intensity is considered worth the sacrifice in reproducibility.

It is noted, however, that the addition of ammonium nitrate buffer to the hair-molybdenum solution does two things. These are:

1) It improves the reproducibility

2) It improves the sensitivity

The addition of 50 mg. ammonium nitrate per ml. of hair-molybdenum solution is therefore considered desirable.

Table XII lists the standard deviations for twenty repeat runs on the molybdenum-hair-buffer sample solution. The corresponding element concentrations and percentage variations as obtained from the analytical curves of Figure 71 are also listed. These values are in agreement with those obtained by the porous cup method.

6. RECOMMENDED DRY SAMPLE PROGRAM

Minute amounts of biological material may best be analyzed in dry form if the following program is adopted:

a. Instrument for Analysis

A spectrograph of moderate dispersion and resolution.

b. Type of Source

A controlled spark discharge.

c. Parameters of Source

The most desirable source parameters are those of Source No. 3, Appendix I.

d. Preparation of Sample

Dissolve the sample in dilute nitric acid. Use approximately 0.005 ml. of 34% doubly distilled nitric acid per mg. of biological sample. (Note: Ordinary concentrated nitric acid contains 68% HNO_3.)

TABLE XII

Stock Solution Concentration

	Log Ratios of Twenty Repeats on Mo-Hair-Buffer Solution		
	Log $\frac{Fe2599}{Mo2816}$	Log $\frac{Mg2852}{Mo2816}$	Log $\frac{S12881}{Mo2816}$
	- 0.77	1.36	- 0.74
	- 0.55	1.17	- 0.63
	- 0.56	1.07	- 0.83
	- 0.65	1.28	- 0.61
	- 0.54	1.26	- 0.63
	- 0.51	1.15	- 0.71
	- 0.55	1.22	- 0.66
	- 0.56	1.29	- 0.64
	- 0.49	1.15	- 0.74
	- 0.59	1.39	- 0.63
	- 0.78	1.19	- 0.63
	- 0.56	1.28	- 0.75
	- 0.69	1.11	- 0.61
	- 0.75	1.17	- 0.76
	- 0.58	1.28	- 0.63
	- 0.68	1.21	- 0.85
	- 0.60	1.04	- 0.71
	- 0.65	1.17	- 0.83
	- 0.64	1.05	- 0.73
	- 0.53	1.16	- 0.66
Average	- 0.61	1.20	- 0.70
Standard Deviation	σ = .08	σ = .09	σ = .08
Element Percentage	0.00062%	0.0023%	0.0011%
Precision	± 3%	± 5%	± 5%

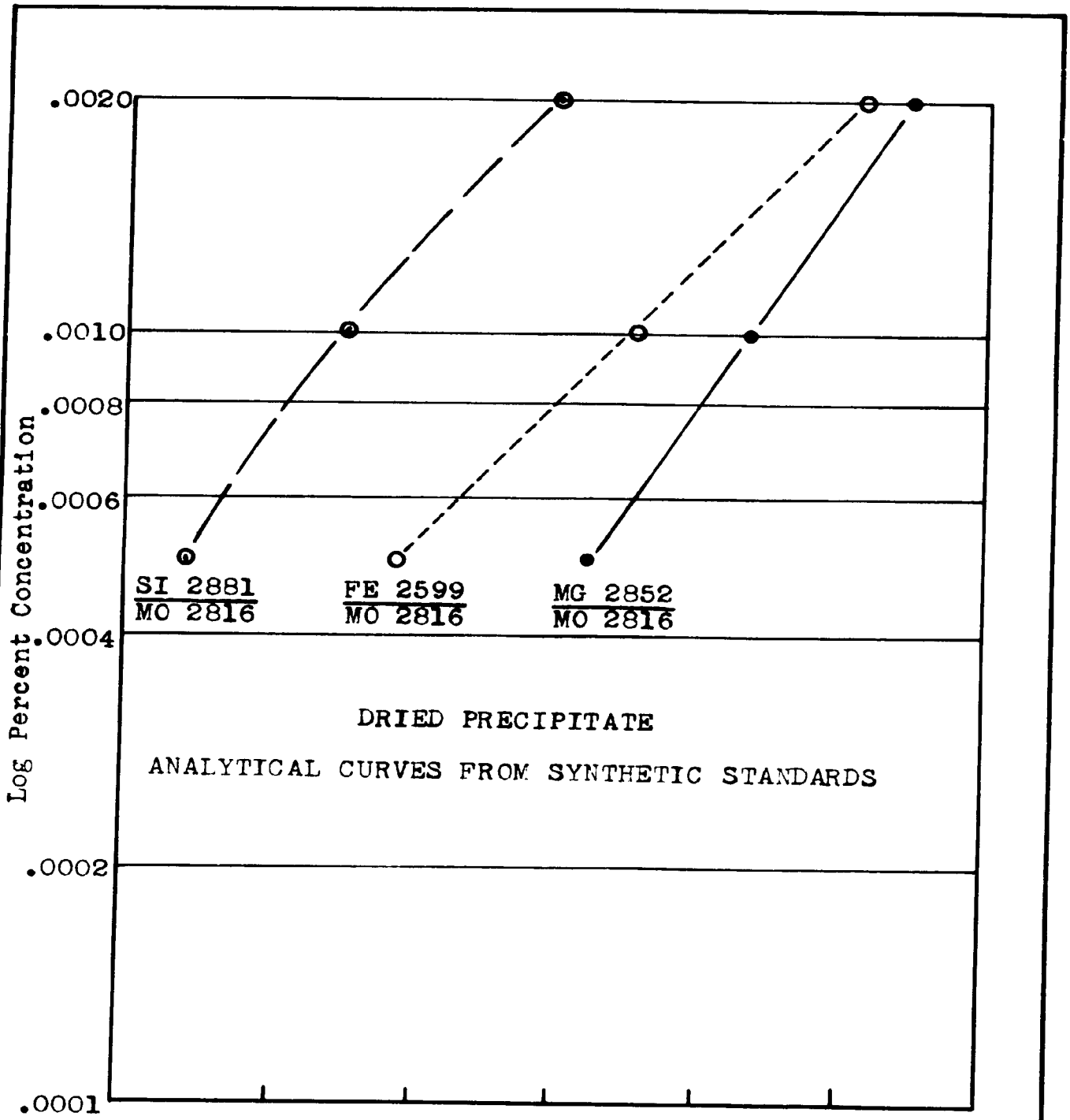


FIGURE 71

ANALYTICAL CURVES FOR IRON, MAGNESIUM, & SILICON

e. Concentration of Internal Control

Molybdenum is preferred as an internal control. Ammonium para molybdate, $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$, is a convenient source of molybdate since it is water soluble. The desired final concentration of the molybdenum is 0.0003 mg. Mo/mg. of biological sample.

f. Concentration of Ammonium Nitrate Buffer

The addition of 0.5 mg. of ammonium nitrate per mg. of biological sample is considered desirable for improving both the sensitivity and the reproducibility of the analysis.

g. Shape of Electrode Used

A flat topped electrode with a slightly beveled edge is recommended to prevent preferential sparking to the edge. This electrode shape is given in Figure 36.

h. Method of Decreasing Sample Penetration

Add two drops of melted paraffin at 160° F to the electrode top at 160° F by means of a pyrex glass stirring rod. For uniformity of drying it is recommended that these electrodes be permitted to remain on the hot plate at 160° F temperature for a period of approximately five minutes after the surface no longer appears wet.

i. Method of Drying Samples

The sample material should be placed on the ends of the sealed carbons by means of a pipette. The sample may then be dried under a vacuum of the order of 1 mm. mercury pressure, being careful to apply the vacuum slowly enough for trapped air bubbles to escape without bursting the sample drop. After concentration of the sample by this method, the electrodes should be placed in an oven at 500° F to dry for 30 minutes.

j. Exposure Time

The recommended time of exposure is two seconds with no pre-exposure. For normal biological samples weighing between 10 mg. and 100 mg., most of the sample will be excited during this time interval, while the background is still subdued.

If the preceding program of analysis is adopted, the following magnitudes of absolute sensitivity may be expected:

1) High excitation potential elements such as iron, 0.02 micrograms.

2) Low excitation potential elements such as magnesium, 0.01 micrograms.

3) Silicon, 0.01 micrograms.

The minimum amounts of material upon which one may reasonably expect to make a quantitative analysis are:

1) High excitation potential elements such as iron, 0.10 micrograms.

2) Low excitation potential elements such as magnesium, 0.05 micrograms.

3) Silicon, 0.15 micrograms.

The reproducibility of this program of analysis is suggested by the following values of precision:

1) High excitation potential elements such as iron, ± 3%.

2) Low excitation potential elements such as magnesium, ± 5%.

3) Analytically troublesome elements such as silicon, ± 5%.

The above values of precision may be expected when the weight of the analytical element existing on the electrode is as small as one microgram.

CHAPTER VI

SUMMARY

In establishing a program of analysis for biological materials, it is necessary to investigate the various parameters which influence the sensitivity and precision of analysis. Each method of analysis, of course, has its own inherent set of critical parameters.

The particular method chosen for the analysis of biological material must be capable of analyzing for twenty elements with average concentrations of 0.001% of a 10 mg. sample. After consideration of the various faults and virtues of three prominent methods of analysis (chemical, flame photometer, and spectrographic), the spectrographic method was selected as the most satisfactory method of multi-element trace analysis. Inasmuch as the controlled spark discharge consistently furnishes good reproducibility and is only slightly less sensitive than the arc, a spark source was chosen to implement the spectrographic method.

The most fundamental problem in the spectrographic analysis of biological material is, "What is the best method of introducing the sample into the discharge gap?" This problem includes not only such major topics as whether it is

best to introduce the sample in wet form or in dry form but also many sub-topics such as (a) whether it is best to introduce liquid samples at the top of the discharge gap or at the bottom and (b) when using dry sample methods, what is the best method of minimizing sample penetration into electrodes. To satisfactorily deal with this problem its scope was reduced by dividing it into two sub-problems. These were:

1) What is the best wet sample method of analyzing biological material spectrographically?

2) What is the best dry sample method of analyzing biological material spectrographically?

These smaller problems were then treated separately.

1. WET SAMPLE METHODS

Wet sample methods were investigated first by considering the virtues and defects of the rotating electrode method and the porous cup method. The porous cup method was selected as possessing inherent characteristics which were slightly less troublesome than those of the rotating electrode.

Following this selection, a survey was made of various shaped counter-electrodes and it was determined that the electrode shape of Figure 10 imparted the most satisfactory burning characteristics to the discharge.

Many methods of introducing the liquid sample into

the discharge gap were investigated. Methods of special interest were:

1) The use of a lead-in-tube and an inverted porous cup to introduce the material at the bottom of the discharge gap. The use of a reservoir at the end of the lead-in-tube enables a constant pressure head to be maintained on the porous cup.

2) The use of a vibrating pyrex rod reciprocating longitudinally in the porous cup to break up gas bubbles. By this means the sample is retained longer in the porous cup than when the porous cup is used by itself. Equally well, it permits the use of a more energetic source.

3) The use of a goose-neck reservoir to continually supply sample to the porous cup. The reservoir maintains a low level of fluid in the porous cup so that expanding bubbles that are formed do not eject the sample. This makes possible the use of a more energetic source.

Of these methods, the method employing the goose-neck reservoir proved to be the most successful in retention of sample. Each of the above methods, however, is able to retain the sample for longer exposures than when only the porous cup is used.

The ability to detect minute amounts of material depends upon the expenditure of large amounts of energy in the discharge gap. When porous cups are used, the resultant heat causes bubbles to form in the liquid sample. These bubbles expand upward so rapidly they eject the sample.

One must therefore either compromise the sensitivity requirements by using a less energetic source or develop some method of circumventing the bubble formation. There is no known method which enables the porous cup to be used with the most energetic sources. However, the method employing the goose-neck reservoir permits approximately twice as much energy to be dissipated in the gap without sample ejection as when the porous cup is used by itself. This results in more intense spectra.

It should be pointed out that although the goose-neck reservoir was designed to permit porous cups to be used with more energetic sources, it is valuable in at least one other respect. That is, use of the reservoir makes possible very long exposures without pausing to reload the porous cup. Long exposures may be taken, however, only when less energetic sources are used. The maximum exposure time possible when using Source No. 2, Appendix I, is approximately 90 seconds. This represents the elapsed time before the discharge burns a hole in the bottom of the porous cup.

2. DRY SAMPLE METHODS

An evaluation of dry sample methods was begun with a comparison of existing methods. The method involving the drying of sample drops on the ends of flat electrodes was chosen as representing the most satisfactory method of introducing the sample into the discharge gap. This method permits both the upper and the lower electrode to be sample

carriers. This method also retains the material near the surface layers of the electrode where it is readily available for consumption in a spark discharge. The sample may be retained even closer to the electrode surface by decreasing the carbon porosity with a drop or two of paraffin. This is particularly important when only small samples are available.

It was determined that for dry sample methods the best line-to-background ratios were associated with very energetic sources and short exposures. The process of timing exposures of the order of one second with a stop watch was not as convenient nor as reproducible as the process associated with the automatic electronic interval timer. The electronic timer diagramed in Figure 44 was found to function very well.

The addition of ammonium nitrate as a buffer to the dry sample improves the precision of the analysis. It is also noted that a spectral enhancement occurs because of this addition. The enhancement is stronger if indium is added as a second internal standard, but indium is so sensitive to drying conditions that the analytical precision is adversely affected. For this reason, ammonium nitrate is considered a desirable additive but indium is not.

Uniformity of sample drying is an important factor in the precision of the analysis. It was found that flat electrodes chamfered at the edge constituted the most satisfactory electrode shape. It was further determined

that the best uniformity of sample coverage on the electrode was obtained by means of vacuum drying. The sample was pipetted to the electrode surface and allowed to dry under an evacuated bell jar. The samples were then placed in an oven at 500° F for 1/2 hour for complete drying.

Table XIII lists a summary of what this study has indicated to be the best method of analysis of biological material by each of the two methods: The wet sample method and the dry sample method.

Concerning the last item of Table XIII it is noted that approximately 1/4 milliliter of sample is required to load the goose-neck reservoir for use. If the sample concentration is 100 mg/ml, this amounts to 25 mg. of sample. In the dry precipitate method the exposure is terminated before all of the material has been excited. This favors reproducibility. For a 10 milligram loading, the unexcited sample is estimated to be about 3 milligrams.

In comparing the best wet sample method and the best dry sample method it is noted that neither method is greatly superior to the other in either sensitivity or reproducibility. Since these are the most important quantities, the choice of which method is best for analysis of biological material may be made upon other factors. Primary quantities affecting the choice might be: (a) sample size, (b) source parameters, and (c) apparatus.

TABLE XIII

Comparison of Wet Sample and Dry Sample Methods

Wet Sample Method	Item for Comparison	Dry Sample Method
 porous cup counter	Shape of Electrode	 Chamfered Chamfered
None	Electrode Preparation	Add two drops of melted paraffin at 160° F.
0.0003 mg. Mo 0.01 mg. In	Recommended Internal Control per mg. of Sample	0.0003 mg. Mo
None	Recommended Buffer per mg. of Sample	0.5 mg of NH_4NO_3
5 seconds	Pre-exposure Time	None
30 seconds	Exposure Time	2 seconds
	Spark Source Parameters	
220 volts	Primary Voltage	220 volts
20 ohms	Primary Resistance	20 ohms
None added	Secondary Resistance	None added
225 microhenries	Secondary Inductance	75 microhenries
.0025 microfarads	Secondary Capacity	.045 microfarads
4 mm.	Discharge Gap	4 mm.
18,000 volts	Breakdown Voltage	18,000 volts
Twenty-two	Breaks/half-cycle	One
5 amperes	R.F. Current	20 amperes

TABLE XIII (Concluded)

Wet Sample Method	Item for Comparison	Dry Sample Method
Goose-neck reservoir	Unusual Apparatus Needed	Evacuating System with Bell Jar Interval Timer 500° F Oven
Loading the Goose-neck-reservoir	Unusual Loading Manipulations	None
	Absolute Sensitivity	
20 milligamma	Iron Class	20 milligamma
10 milligamma	Magnesium Class	10 milligamma
60 milligamma	Silicon Class	10 milligamma
	Minimum Amount of Element Necessary for Quantitative Analysis	
100 milligamma	Iron Class	100 milligamma
20 milligamma	Magnesium Class	50 milligamma
150 milligamma	Silicon Class	100 milligamma
	Precision	
± 4%	Iron Class	± 3%
± 3%	Magnesium Class	± 5%
± 7%	Silicon Class	± 5%
~ 25 milligrams	Sample Consumed per Exposure	~ 7 milligrams
~ 1/4 ml.	Unused Sample	~ 1/3 of Original Electrode Loading

Note: 1 milligamma = 10^{-9} grams.

a. Sample Size

The dry sample method permits a satisfactory analysis to be made with a smaller amount of material.

b. Source Parameters

The 0.045 mfd capacity demands of the dry sample method may exceed the capacitance of the source employed. The wet sample method provides an analysis program with 0.0025 mfd capacity which lies within the range of all normal spectrographic spark sources.

c. Apparatus

A particular experimenter may find it more difficult to obtain the 500° F oven, the vacuum drying bell jar unit, and the electronic timer for the dry sample method than the goose-neck reservoir needed for the wet sample method. Presumably, without the service of a skilled glass blower, the wet sample method might be the more difficult method to implement.

With full consideration for all the analytical and instrumental details, the author feels that the dry sample method is the most satisfactory method of analysis of biological material. It is, therefore, recommended that the dry sample method, as outlined in this dissertation, be employed in the analysis of biological material.

APPENDIX I

Parameters of Various Spark Sources

Source	No. 1	No. 2	No. 3
Primary Resistance	18 ohms	20 ohms	20 ohms
Primary Voltage	220 volts	220 volts	220 volts
Secondary Resistance	one ohm	zero ohms (circuit)	zero ohms (circuit)
Secondary Breakdown Voltage	18,000	18,000	18,000
Secondary Current	7 amperes	5 amperes	20 amperes
Secondary Inductance	75 μh	225 μh	75 μh
Secondary Capacity	.006 mfd	.0025 mfd	.045 mfd
Discharge Gap Spacing	4 mm.	4 mm.	4 mm.
Breaks Per Half-Cycle	3	22	1

APPENDIX II

Spark Circuit Calculations

In a circuit containing capacitance, inductance, and resistance in series, such as the spark discharge circuit, we may apply Kirchoff's Law. Kirchoff's Law states that around any closed path in an electric circuit, the algebraic sum of the potential differences equals zero. For the discharge circuit we may write:

$$L\frac{di}{dt} + iR + \frac{q}{c} = 0 \qquad (1)$$

Since $i = \frac{dq}{dt}$ and $\frac{di}{dt} = \frac{d^2q}{dt^2}$, equation (1) may, therefore, be written:

$$\frac{d^2q}{dt^2} + \frac{R}{L}\frac{dq}{dt} + \frac{1}{LC}q = 0 \qquad (2)$$

A general solution of this differential equation may be expressed in the form,

$$q = Ae^{D_1 t} + Be^{-D_2 t} \qquad (3)$$

where A, B, D_1, and D_2 are constants. The constants D_1 and D_2 may be identified by substituting equation (3) into equation (2). The coefficients of $e^{D_1 t}$ and $e^{-D_2 t}$ must vanish for arbitrary values of time. Hence,

$$D_1 = -\frac{R}{2L} \pm \sqrt{\frac{R^2}{4L^2} - \frac{1}{LC}} \qquad (4)$$

$$D_2 = +\frac{R}{2L} \pm \sqrt{\frac{R^2}{4L^2} - \frac{1}{LC}} \qquad (5)$$

For Source No. 2, Appendix I, R ~ 10 ohms (gap resistance), L ~ 200 x 10^{-6} henries, C ~ 2 x 10^{-9} farads. So, $R^2/4L^2 \sim 6 \times 10^8$, $1/LC \sim 2.5 \times 10^{12}$, and

$$\frac{1}{LC} > \frac{R^2}{4L^2} \qquad (6)$$

That is to say, the square roots of D_1 and D_2 will involve an imaginary and the solution will be periodic. We may therefore write the general solution of (3) in the form,

$$q = e^{-\frac{Rt}{2L}} \left[A e^{i\omega t} + B e^{-i\omega t} \right] \qquad (7)$$

where $$\omega = \left| \left(\frac{R^2}{4L^2} - \frac{1}{LC} \right) \frac{1}{2} \right| \qquad (8)$$

also $$q = e^{-\frac{Rt}{2L}} \left[K_1 \mathrm{Cos}\omega t + K_2 \mathrm{Sin}\omega t \right] \qquad (9)$$

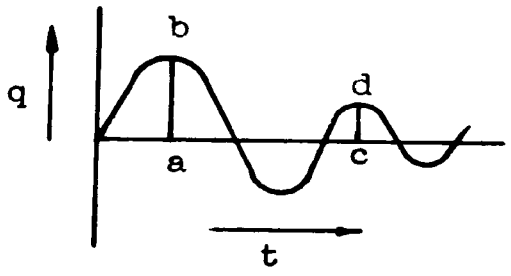
$$q = e^{-\frac{Rt}{2L}} \left[K_3 \, \mathrm{Cos}(\omega t + \phi) \right] \qquad (10)$$

where $K_1 = A + B$, $K_2 = (A - B)\, i$, $K_3 = \sqrt{K_1^2 + K_2^2}$, and $\mathrm{Tan}\, \phi = - K_2/K_1$

The natural frequency of this oscillation is $f = \frac{\omega}{2\pi}$ and the period is $T = \frac{2\pi}{\omega}$. The amplitude of the oscillation is,

$$\text{Amplitude} = K_3 e^{-\frac{Rt}{2L}} \tag{11}$$

If we compare the ratio of the amplitudes of two successive oscillations we have,



$$\frac{ab}{cd} = \frac{K_3 e^{-\frac{Rt}{2L}}}{K_3 e^{-\frac{R}{2L}(t+T)}} \tag{12}$$

or,
$$\frac{ab}{cd} = e^{\frac{RT}{2L}} = e^{\frac{R\pi}{\omega L}} = e^{\delta} \tag{13}$$

Therefore,
$$\text{Log}_e \frac{ab}{cd} = \delta = \frac{R\pi}{\omega L} \tag{14}$$

Now $\omega \sim 1.4 \times 10^6$, $L \sim 2 \times 10^{-4}$, $R \sim 10$, and $\delta \sim 10^{-1}$. Since the condenser charge q is proportional to the voltage V, the ratio of the breakdown voltage to the voltage of the succeeding oscillation should be,

$$e^{0.1} = 1.1 \tag{15}$$

The breakdown voltage is $\sim$ 18,000 volts. The minimum voltage for conduction when the gap is ionized is $\sim$ 90 volts. Therefore, $\frac{18{,}000 \text{ volts}}{90 \text{ volts}} = \frac{200}{1}$

The number of oscillations required to decrease the condenser voltage by this factor of 200:1 is given by n where,

$$\frac{200}{1} = \frac{e^{-\frac{R}{2L}t}}{e^{-\frac{R}{2L}(t + nT)}} = e^{\frac{RnT}{2L}} \qquad (16)$$

Since $R \sim 10$, $L \sim 2 \times 10^{-4}$, and $T \sim 4.5 \times 10^{-6}$, the number of oscillations n from breakdown to extinction is determined from (16) to be approximately,

$$n \cong 50 \qquad (17)$$

The energy stored in a condenser is given by $E = 1/2\ CV^2$. When the initial voltage V_i is decreased to a final voltage V_f, the energy loss of the condenser is given by:

$$E_i - E_f = \Delta E = \tfrac{1}{2}CV_i^2 - \tfrac{1}{2}CV_f^2 = \tfrac{1}{2}C(V_i^2 - V_f^2) \qquad (18)$$

Equation (18) may be rearranged to write the loss in energy in the form,

$$\Delta E = \tfrac{1}{2}CV_i^2 \left[1 - \frac{V_f^2}{V_i^2} \right] \qquad (19)$$

So,

$$\Delta E = \text{Original Energy} \left[1 - \left(\frac{V_f}{V_i} \right)^2 \right] \qquad (20)$$

Since, from equation (16),

$$\frac{V_f}{V_i} = e^{-\frac{RTn}{2L}} = e^{-\delta n} , \qquad (21)$$

we may re-write equation (20) in the form,

$$\Delta E = \text{Original Energy} \left[1 - \left(e^{-\delta n} \right)^2 \right] \qquad (22)$$

and

$$\Delta E = \text{Original Energy} \left[1 - e^{-2\delta n} \right] \qquad (23)$$

Now, the first pulse across the discharge gap is represented by one-half an oscillation or, $n = \frac{1}{2}$. Since, $n = \frac{1}{2}$ and $\delta = .1$, then,

$$e^{-2\delta n} = e^{-.1} = \frac{1}{1.1} \cong .9 \qquad (24)$$

Substituting the value from (24) into equation (23) we find,

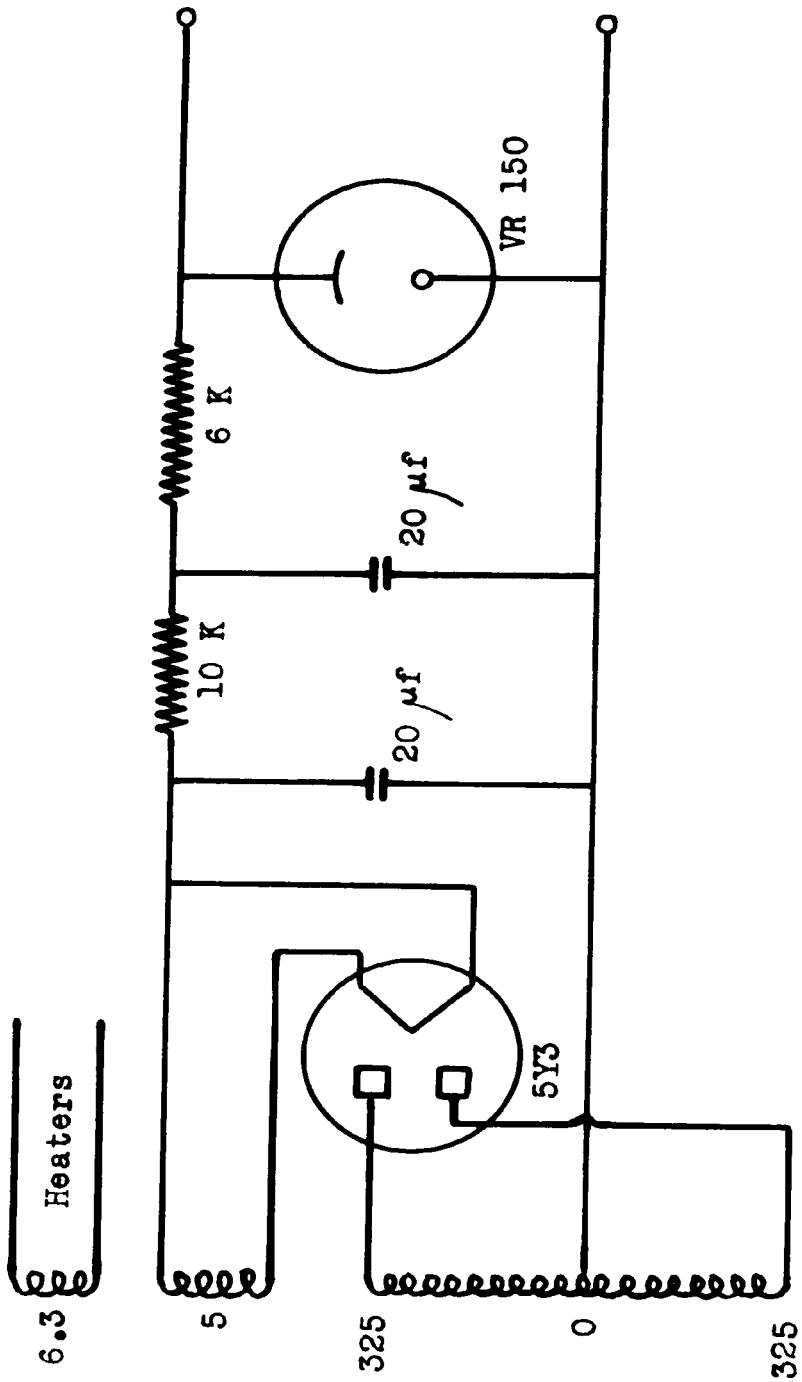
$$\Delta E = \text{Original Energy} \left[1 - .9 \right] \qquad (25)$$

So,

$$\Delta E = \text{Original Energy} \cdot \frac{1}{10} \qquad (26)$$

Thus, we conclude that the energy loss of the condenser on the first uni-directional pulse is approximately one-tenth of the original energy stored by the condenser.

APPENDIX III



POWER SUPPLY

150 VOLT OUTPUT

APPENDIX IV



ELECTRONIC INTERVAL TIMER

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