University of New Hampshire

University of New Hampshire Scholars' Repository

Doctoral Dissertations

Student Scholarship

Summer 1970

A PRACTICAL COMPARISON OF ATOMIC FLUORESCENCE FLAME SPECTROMETRY WITH ATOMIC ABSORPTION FLAME SPECTROMETRY

MICHAEL ANDREW PLEVA

Follow this and additional works at: https://scholars.unh.edu/dissertation

Recommended Citation

PLEVA, MICHAEL ANDREW, "A PRACTICAL COMPARISON OF ATOMIC FLUORESCENCE FLAME SPECTROMETRY WITH ATOMIC ABSORPTION FLAME SPECTROMETRY" (1970). *Doctoral Dissertations*. 916.

https://scholars.unh.edu/dissertation/916

This Dissertation is brought to you for free and open access by the Student Scholarship at University of New Hampshire Scholars' Repository. It has been accepted for inclusion in Doctoral Dissertations by an authorized administrator of University of New Hampshire Scholars' Repository. For more information, please contact Scholarly.Communication@unh.edu.

70-11,810

PLEVA, Michael Andrew, 1941-
A PRACTICAL COMPARISON OF ATOMIC FLUORESCENCE FLAME SPECTROMETRY WITH ATOMIC ABSORPTION FLAME SPECTROMETRY.

University of New Hampshire, Ph.D., 1970
Chemistry, analytical

University Microfilms, Inc., Ann Arbor, Michigan

A PRACTICAL COMPARISON OF ATOMIC FLUORESCENCE FLAME SPECTROMETRY WITH ATOMIC ABSORPTION FLAME SPECTROMETRY

by

MICHAEL ANDREW PLEVA

B. S., Trinity College, 1963
M. S., Trinity College, 1965

A THESIS

Submitted to the University of New Hampshire
In Partial Fulfillment of
The Requirements for the Degree of

Doctor of Philosophy

Graduate School
Department of Chemistry
August, 1969

This thesis has been examined and approved.

David W. Ellis, Thesis Director
Associate Professor of Chemistry

Alexander R. Amell
Professor of Chemistry

Albert F. Daggett
Professor of Chemistry

Charles M. Wheeler
Associate Professor of Chemistry

Clarence L. Grant
Research Professor, Engineering
Experiment Station and Adjunct
Professor of Chemistry

Aug. 15, 1969
Date

ACKNOWLEDGEMENTS

The author would like to express his deep and sincere appreciation to his research director, Dr. David W. Ellis, and to all the faculty of the Chemistry Department for their advice and encouragement. The patience, tolerance, and good humor of these gentlemen has made the author's stay at the University of New Hampshire a most pleasant one.

Sincere thanks is owed Dr. Clarence L. Grant of the Engineering Experiment Station for his helpful suggestions and guidance.

Finally, a sweeping tip of the cap is due Mrs. Pearl Libby and Miss Anne Kohl whose herculean efforts at the typewriter managed to bring order out of chaos.

Michael A. Pleva

I dedicate this work to the two women and the two men who have had the greatest influence on my life:

My wife, Karen
My mother, Mrs. Anna Pleva
My late father, Mr. Andrew Pleva
My closest friend and advisor,
Mr. Alexander Shapiro

TABLE OF CONTENTS

	Page
LIST OF TABLES	vii
LIST OF FIGURES	x
ABSTRACT	xi
INTRODUCTION	1
EXPERIMENTAL	15
PART I	15
Atomic Fluorescence	15
Optimization of Variables in Atomic Fluorescence	17
Optimization of Group I Variables	19
Optimization of Group II Variables	27
Optimization of Group III Variables	31
PART II - DETERMINATION OF CALCIUM IN BLOOD SERUM	32
Experimental Considerations	32
Preparation of Calcium Solution	34
Digestion of Blood Serum	34
Preparation of Strontium Solution	35
Atomic Fluorescence Flame Spectrometric Analyses	35
Atomic Absorption Flame Spectrometric Analyses	39
PART III - DETERMINATION OF METALLIC CONSTITUENTS IN FERTILIZERS	45
Experimental Considerations	45
Preparation of Standard Solutions	46
Preparation of Margruder Fertilizer Samples	47
Atomic Absorption Spectrometric Analyses	48
Atomic Fluorescence Spectrometric Analyses	61

	Page
DISCUSSION	78
ACCURACY	78
PRECISION	81
SENSITIVITY	83
DETECTION LIMIT	86
STABILITY	92
ANALYSIS TIME	94
MULTI-ELEMENT ANALYSIS CONSIDERATIONS	94
SAMPLE VOLUME	95
TREATMENT OF DATA	96
EXPENSE OF EQUIPMENT	96
SUMMARY	97
BIBLIOGRAPHY	98
APPENDIX I	102
APPENDIX II	113

LIST OF TABLES

Number		Page
I	Coding of the Group I Variables for the Design Matrix	21
II	Design Matrix for an Optimization Run of Group I Variables	22
III	Analysis of Variance Table for Group I Variables Optimization Run	23
IV	Composite Design Matrix for Two Runs	25
V	Composite Analysis of Variance for Group I Optimization Studies	26
VI	Settings for Optimization Studies of Group II Variables	28
VII	Analysis of Variance for a (2^5 + 1) One-half Factorial Optimization Experiment for the Group II Variables	29
VIII	Determination of Calcium	37
IX	Regression Analysis Table for Data of Table VIII	38
X	Determination of Calcium	40
XI	Determination of Calcium	42
XII	Regression Analysis Table for Data of Table XI	43
XIII	Blood Serum Analyses for Calcium	44
XIV	Determination of Iron	49
XV	Regression Analysis Table for Data of Table XIV	50
XVI	Determination of Copper	52

Number		Page
XVII	Regression Analysis Table for Data of Table XVI	53
XVIII	Determination of Zinc	54
XIX	Regression Analysis Table for Data of Table XVIII	55
XX	Determination of Manganese	56
XXI	Regression Analysis Table for Data of Table XX	57
XXII	Determination of Magnesium	59
XXIII	Regression Analysis Table for Data of Table XXII	60
XXIV	Determination of Calcium	62
XXV	Regression Analysis Table for Data of Table XXIV	63
XXVI	Determination of Copper	65
XXVII	Regression Analysis Table for Data of Table XXVI	66
XXVIII	Determination of Iron	67
XXIX	Regression Analysis Table for Data of Table XXVIII	68
XXX	Determination of Zinc	69
XXXI	Regression Analysis Table for Data of Table XXX	70
XXXII	Determination of Manganese	71
XXXIII	Regression Analysis Table for Data of Table XXXII	72
XXXIV	Determination of Magnesium	73
XXXV	Regression Analysis Table for Data of Table XXXIV	74

Number		Page
XXXVI	Determination of Calcium....................	75
XXXVII	Regression Analysis for Data of Table XXXVI..................................	76
XXXVIII	Results of Fertilizer Determinations by Atomic Absorption and Atomic Flourescence....	77
XXXIX	Analysis of Calcium in Chemtrol Standard Blood Sample................................	88
XL	Analysis of Metal Constituents in Fertilizer Samples - Part I.................	89
XLI	Analysis of Metal Constituents in Fertilizer - Part II........................	90

LIST OF FIGURES

Figure		Page
1.	Types of Atomic Fluorescence..................	3
2.	Atomic Fluorescence Experimental Block Diagram	16
3.	Reproducibility of Calcium Calibration Curve..	93
4.	Effect of Concentration and pH on Reaction Yield...	103
5.	Effect of Variables X_1 and X_2 on the Reaction Yield, Y............................	106

ABSTRACT

A PRACTICAL COMPARISON OF
ATOMIC FLUORESCENCE FLAME SPECTROMETRY WITH
ATOMIC ABSORPTION FLAME SPECTROMETRY

by

MICHAEL A. PLEVA

The work performed has indicated that in actual analytical determinations, the techniques of atomic absorption flame spectrometry and atomic fluorescence flame spectrometry using a 450 watt xenon arc source are similar in terms of accuracy, precision, and sensitivity. It is not possible from this work to recommend the newer technique of atomic fluorescence flame spectrometry as being superior to the more established atomic absorption flame spectrometry. In fact, in a few cases, the technique of atomic absorption spectrometry was significantly better than atomic fluorescence flame spectrometry.

The work has also indicated that a xenon arc lamp in atomic fluorescence flame spectrometry gave detection limits which were comparable to those obtained in atomic absorption flame spectrometry. In difficult cases, such as analyses at detection-limit concentrations, the direct-readout nature of the atomic fluorescence signal could prove advantageous over the difference signal which is obtained from atomic absorption work. In these cases, the use of more intense line sources in atomic fluorescence flame spectrometry, rather than

the continuum source used in this work would be expected to achieve a lower detection limit for atomic fluorescence flame spectrometry over atomic absorption flame spectrometry.

INTRODUCTION

Three major types of flame spectrometry are pertinent to this work: emission spectrometry, absorption spectrometry, and luminescence spectrometry. Emission spectrometry refers to those methods whereby radiation is emitted from molecules, atoms, or ions, without a prior radiational process. The specific example of emission spectrometry which will be considered is flame emission spectrometry. Absorption spectrometry includes all methods in which absorption of radiation is measured, specifically atomic absorption flame spectrometry. Finally, luminescence spectrometry refers to those methods whereby radiation is emitted from a species excited by prior radiational means. Atomic fluorescence flame spectrometry, then, may be defined as the study of the emission spectra produced by the radiational excitation of neutral atoms in a flame. The method is analagous to flame emission spectrometry in the sense that an atomic emission spectrum is recorded but differs from emission spectrometry in general in the sense that electromagnetic radiation produced the excitation, rather than thermal energy.

Atomic fluorescence is similar to atomic absorption spectrometry in that both methods employ radiational excitation. Atomic absorption requires the measurement of the difference between two signals while atomic fluorescence depends upon the magnitude of the fluorescence signal measured above background. In this regard, West[1] has stated that "for a given population of atoms in any atom reservoir of low background, the sensitivity of atomic fluorescence measurements is inherently greater than that of atomic

absorption because of the benefits which accrue from signal amplification and from the proportional relationship which exists between signal strength and the intensity of the source of excitation in fluorescence studies." Thus atomic fluorescence has the potentiality of achieving greater sensitivity than atomic absorption while employing comparable equipment and techniques.

There are four types of atomic fluorescence, and these have been described by Winefordner and Vickers:[2] resonance fluorescence, direct-line fluorescence, stepwise-line fluorescence, and sensitized fluorescence. Resonance fluorescence almost always consists of resonance radiation from the first excited state back to the ground state. Thus the exciting and emitting wavelengths are the same. This type of fluorescence has received the most attention, and at present is the basis for almost all of the analytical applications of atomic fluorescence.

Direct-line fluorescence is produced by transitions from a higher to a lower excited state other than the ground state. An example cited by Winefordner and Vickers[2] is the Tl fluorescence at 5350Å following excitation at 3776Å.

Stepwise-line fluorescence involves excitation to an excited state, radiationless decay to some lower excited state and then emission (fluorescence) to the ground state. An example[2] of this is the Na 5890Å fluorescence following excitation at 3303Å. These first three types of atomic fluorescence are diagrammed on Figure 1.

The fourth type of atomic fluorescence, sensitized fluorescence, is different from the previous three. In this case one atomic species is excited by the absorption of appropriate electromagnetic radiation. This excited species subsequently transfers its newly-acquired excess energy to

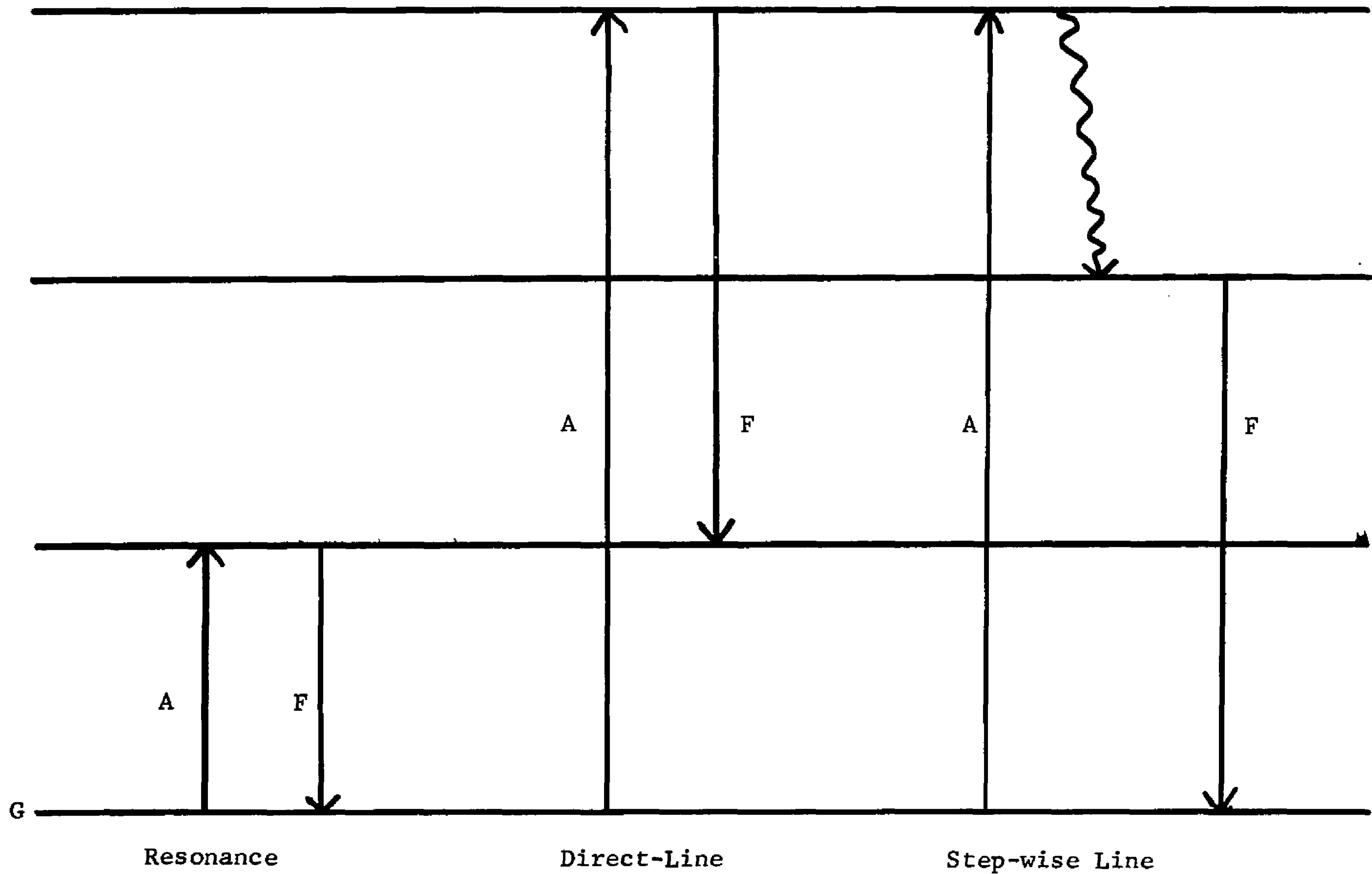


Figure 1. Types of Atomic Fluorescence

a different atomic species which then emits its characteristic radiation. An example[3] of sensitized fluorescence can be found in a system containing Tl and Hg when the Hg is at much higher concentration than the Tl. The Tl 5350Å and 3776Å line emissions may be seen when this system is aspirated into a flame, even though at the exciting wavelength of 2537Å, Tl does not absorb radiation. Hg, however, does absorb radiation at this wavelength. The Hg atoms subsequently transfer this energy to the Tl atoms, and the Tl emissions are seen.

The theoretical relationship between the concentration of the fluorescent species and the fluorescent signal obtained has been investigated thoroughly by Winefordner and Vickers.[2] Winefordner has been able to show how the emitted intensity in emission spectrometry, the absorbed intensity in atomic absorption, and the emitted intensity in atomic fluorescence flame spectrometry may be predicted in terms of spectral and flame compositional parameters.[4] For example, in emission spectrometry, the intensity of emission is evaluated from spectral parameters, such as the transition probability and the statistical weights of the states involved in the transition as well as from excitation parameters, such as the temperature of the source of excitation. In atomic absorption spectrometry the actual intensity absorbed is not generally measured. Rather, a ratio of the intensity of light absorbed to the initial light intensity is measured. The intensity of the light absorbed is obtained from the difference of the initial intensity and the transmitted intensity. The ratio is related to the path length and the absorptivity. In luminescence spectrometry the intensity of radiation emitted in all directions may be evaluated from the path length in the flame, the intensity of incident radiation, the absorptivity,

and the power efficiency, which may be defined as the ratio of the radiant power emitted by luminescence to the radiant power absorbed.

On the basis of theoretical considerations, the technique of atomic fluorescenc flame spectrometry is closely related to both flame emission and atomic absorption spectrometry. For low concentrations all three techniques respond linearly to changes in the ground-state concentrations of the atoms being excited. However, while the signals of flame emission spectrometry and atomic fluorescence flame spectrometry are directly proportional to a change in atomic ground-state concentration, the signal of atomic absorption flame spectrometry is inversely affected by an increase in atomic ground-state concentration, reflecting the difference measurement which characterizes this technique. In all three techniques at high atomic concentrations, a change in atomic ground-state concentration does not produce a linear change in signal response, mainly because of self-absorption by unexcited atoms in the flame.

In addition to similarities to flame emission and atomic absorption flame spectrometry, in the case of atomic fluorescence flame spectrometry for dilute solutions of atoms in flames, there exists a very close similarity between the relationships established for molecular fluorescence and for atomic fluorescence. Reviews of the subject[3,5], have summarized the theoretical implications of molecular fluorescence in the following equation:

$$F = 2.303\Phi I_o \epsilon_A l p C$$

where F = the resonance fluorescence signal

Φ = quantum efficiency; the ratio of the number of atoms which fluoresce to the number of

atoms which underwent excitation per unit time

I_o = intensity of the excitation source

ϵ_A = atomic absorptivity at the wavelength of excitation

l = pathlength in the flame

p = a proportionality factor relating to the fraction of the total fluorescence observed by the detector

C = concentration of the solute in the solution being analyzed

For atomic fluorescence flame spectrometry, West[5] has demonstrated that the intensity of the atomic fluorescence signal may be framed in almost the same terms as that of molecular fluorescence. For atomic fluorescence

$$F = 2.303\phi I_o \epsilon_A l p a C_f$$

In the case of atomic fluorescence it is necessary to relate the concentration of the solute atoms to the actual concentration of free atoms in the flame. This may be accomplished by the use of an atomization efficiency factor a, so that

$$C_f a = C$$

where C = solute concentration

a = atomization efficiency factor

C_f = concentration of free atoms in the flame

Thus, these equations are very nearly the same, and in fact, for a given set of operating conditions and for a given sample matrix, both of the above equations assume the

form:

$$F = K C$$

Thus there is established a linear relationship between the fluorescence signal intensity and the solute concentration of the fluorescing species. Recall again that this relationship exists only for dilute concentrations of atoms in the flame. At higher solute concentrations, deviations from this simple direct linear relationship result in a "flattening out" of the fluorescence response curve; i.e., a "plateau" is reached.

For many, but not all elements, the linear region of a calibration curve, in both atomic fluorescence and atomic absorption, includes the region of maximum sensitivity for purposes of analysis. To simplify the handling of the data, however, this work will restrict itself to the linear regions of all calibration curves, in both atomic absorption and atomic fluorescence flame spectrometry.

Almost all of the work performed to date in the field of atomic fluorescence flame spedctrometry has involved the exploration of the method itself. Much of the early work was concerned with the detection of atomic fluorescence signals for various elements and the establishment of their detection limits.[7-17] More recently, work in this area has generally attempted to achieve a better fluorescence response and/or to lower the detection limit for a given element. For example, much work has been devoted toward the improvement of the excitation source. Sources studied have included high intensity hollow cathode lamps[12], Osram and Phillips metal vapor arc lamps[7,8,9,14], continuous xenon arc sources[10,14,15], and recently, electrodeless discharge tubes.[7-9,12,13,15]

All atomic fluorescence work performed in this study employed the use of a xenon arc source. The use of this

source has offered a single, convenient, trouble-free intense source of radiation for the wavelength region studied, namely 2100Å to 4300Å. This region included the resonance lines for all of the elements studied in this investigation and most of the elements studied by atomic fluorescence flame spectrometry. The use of this source also permitted a scanning over the fluorescence wavelength region, thereby offering an evaluation of the amount of background and noise in the wavelength region of interest. This is in contrast to the technique of atomic absorption flame spectrometry where it has been necessary to "tune" the system to the wavelength of interest, thereby making it more difficult to separate signal from noise.

Another possible source of signal improvement involves the type of burner used. While most of the work in atomic fluorescence has employed total-consumption aspirator burners[7-10,12,13,15], with only a few examples of studies with chamber-type aspirator burners[11,14], there has been much searching for proper fuels to be used. Thus hydrogen/oxygen flames[7-9], hydrogen/air flames[7,8], hydrogen/entrained-air flames[15], hydrogen/entrained-air/argon flames[10], acetylene/air flames[11,14], premixed acetylene/air flames[13], and propane/air[14] flames have all been used. In the hydrogen/entrained-air/argon flame the argon was present to assist in the aspiration of the sample solution.

A third region of possible signal improvement is in the use of preconcentrators which achieve an enhancement of the fluorescence signal by the concentration of the sample solution before the solution is introduced into the flame. Preliminary unpublished work in this laboratory has indicated that the use of such a preconcentration device might prove to be useful in a practical sense in analyses involving both flame emission and atomic fluorescence flame spectrometry.[58]

Finally, the composition of the solution matrix and possible influences of matrix constituents upon the fluorescence response of a given element have been investigated by Dagnall[11,14], Goodfellow[13], and Demers and Ellis.[21] The conclusion of these workers was that the technique of atomic fluorescence can be made as free from inter-element effects as atomic absorption spectrometry. In this regard, recent work by Fassel[55], using fuel-rich oxyacetylene flames, has indicated that flame emission spectrometry likewise may be made relatively free from inter-element effects.

It is already agreed[54] that flame emission spectrometry is a complementary rather than a competing technique to atomic absorption flame spectrometry at the present time. It may be argued that atomic fluorescence flame spectrometry might prove a useful complement to the older flame emission method also. Fassel has stated[55] that detection limits have been reached for a number of elements, among them Ag, Cd, Zn, Fe, and Mg, at a level of one part per million or higher by flame emission spectrometry. Fassel attributes this relatively poor limit of detectability to several factors. One factor is the inability of the flame to dissociate molecules in the flame. A second factor is the inability of flames even of temperatures as high as 3000°K to populate adequately the lowest excited states in some atoms. In this latter case, the use of atomic fluorescence or atomic absorption flame spectrometry with their more efficient means of atomic excitation should result in an improvement in detection limits in the analysis of those metals which have been traditionally difficult for flame emission spectrometry. Thus, atomic absorption and atomic fluorescence flame spectrometry may be considered as competing techniques, each serving to complement flame emission spectrometry. It then seems logical to compare not

flame emission spectrometry with atomic fluorescence flame spectrometry, but rather atomic absorption flame spectrometry with atomic fluorescence flame spectrometry.

As a result of these above considerations, it is felt that, although the technique of atomic fluorescence flame spectrometry will justly continue to be investigated as an entity in itself and some of the details in the technique will undoubtedly be improved, it is not premature to consider a comparison of this technique with atomic absorption flame spectrometry. A comparison of this kind has recently appeared in the literature, but this comparison did not involve a flame.[59] It is felt that the technique of atomic fluorescence flame spectrometry, using those components which would be most readily and practically available in the average laboratory, has been quite well established. It remains, therefore, to actually determine how well the atomic fluorescence method will fare as a competing technique to atomic absorption flame spectrometry when an actual set of determinations is carried out.

Since the time that Walsh[22] successfully overcame the various problems inherent in the technique, atomic absorption flame spectrometry has enjoyed a tremendous growth and popularity, until at the present time the technique is widely used for elemental analyses in all kinds of matrices for about forty-five elements. The popularity of atomic absorption is undoubtedly due to the many advantages it enjoys: high sensitivity, high precision and accuracy, few spectral interferences, simplicity of operation, and relatively low cost.

The principle of the method has been suggested earlier. Neutral atoms of the element being analyzed absorb monochromatic radiation from a line source whose intensity can be

monitored. For dilute solutions, the amount of light transmitted to the detector can be related to the concentration of the analyte by the familiar Lambert-Beer relationship:

$$\text{Log } I_o/I = abC$$

where I_o = intensity of the line source

I = intensity of the transmitted light

b = pathlength of the absorbing species

C = concentration of the absorbing species

a = a proportionality constant known as the absorptivity

For a given set of operating conditions:

$$\text{Log } I_o/I = A = kC$$

where A is a logarithmic function defined as the absorbance. Thus an analytically-useful relationship may be established between the concentration of the analyte and a function of the measured detector response.

There has also been a great infusion of literature concerning atomic absorption spectrometry. However, no attempt will be made in this thesis to deal with the historical and developmental aspect of atomic absorption flame spectrometry. An excellent source dealing with the subject is the book by Robinson[23]; another text which deals with more recent material is by Slavin.[24] These two sources make an adequate introduction to the subject for the interested reader. To make a valid comparison of atomic fluorescence with atomic absorption, it is essential that well characterized systems be studied. In addition, standard methods for analyses by

atomic absorption should be available.

One of the earliest successes of atomic absorption spectrometry was for the determination of calcium in blood serum. Prior to the time of atomic absorption spectrometry, the classical method of determining calcium in blood serum was the Clark and Collip[25] modification of the Kramer and Tisdell[26] method. The Clark-Collip method involved precipitation of the calcium as the oxalate followed by repeated centrifuging, draining, and washing. A solution of the precipitate in sulfuric acid was then titrated with a standardized permanganate solution. For all of this tedious effort the results were hardly gratifying. The reproducibility of the results depended on the exact way in which the operations were performed and because of the appreciable solubility of calcium oxalate in the wash liquid, the results usually tended to be low.[27]

Flame photometric methods required prior removal of the protein in the serum by precipitation[28], or prior separation of the calcium as its oxalate.[29] When the flame photometric measurement of the calcium signal on directly diluted serum was attempted[30-32], its accuracy was questioned[33], and the general conclusion was reached[34] that flame photometric methods yielded results about four per cent higher than those obtained by the Clark-Collip oxalate-permanganate method.

The advent of atomic absorption spectrometry has largely eliminated these problems. The early work of Willis has set the pattern in this area.[35] He found that the various spectral interferences could be controlled and that an accuracy and precision equal or better than that of the Clark-Collip method could be achieved. Willis flatly stated that the atomic absorption method was at least as accurate as any other method of analysis for calcium. Further, atomic absorption had the advantage of speed and accuracy. Additional

studies by Willis[36] and by Zettner and Seligson[37] have improved some of the experimental details to the point where the analysis for blood serum calcium through the use of atomic absorption spectrometry is a quite reliable and acceptable method at the present time.

Another example of the use of atomic absorption is the determination of various metallic constituents in fertilizers. Allan[38-40] and David[41] have done much work in this area, and the technique has been perfected to the point where at the present time, an atomic absorption technique for the determination of copper, iron, magnesium, manganese, and zinc in commercial fertilizers and raw materials has been adopted by the Association of Agricultural Chemists as the official method of choice for determinations of this kind.[42]

Many other examples of the use of atomic absorption spectrometry for the determination of trace metallic components exist. Such matrices as soils, plants, urine, rock samples, lubricating oils, and milk have all been studied.[43]

For this work, it has been decided to concentrate upon the two matrices mentioned earlier, the determination of blood serum calcium and the multi-element determination of metallic components in fertilizers, as two areas where the technique of atomic fluorescence flame spectrometry will be compared with atomic absorption flame spectrometry. These two determinations are worthy choices, not only because of the weight of established evidence of their reliability by means of atomic absorption spectrometry, but also because it is felt that these are typical analytical determinations. It is the purpose of this work to make a comparison between atomic fluorescence and atomic absorption flame spectrometry on as "real-life" a set of determinations as possible. Since quantitative determinations are being made, the most important

criterion of comparison will be those of accuracy and precision, but comparisons will be made on as many different levels and aspects of the two methods as is reasonably practicable.

The approach used to make comparisons between the two spectroscopic techniques must be as rigorous as is practicable. Evaluations of the two methods on such matters as accuracy, precision and sensitivity should not be left to some sort of a semi-subjective basis but should be as quantitative as possible. In the case of atomic fluorescence, the optimum operating parameters are not as well known as for atomic absorption. An optimization of the experimental variables in atomic fluorescence would not only insure the best operating conditions for comparison with atomic absorption, but might also be useful and informative in an absolute way with regard to the relative importance of experimental variables in atomic fluorescence.

In view of all of these considerations, an experimental design technique and the related appropriate analysis of variance statistical methods were employed. The general considerations of Box[44] in the field of variable optimization were followed, while the actual experimental work resembled that of Cellier and Stace.[45] The necessary statistical evaluations and related decision-making were similar to the treatment of the subject found in the NBS Handbook 91[46] on experimental statistics.

EXPERIMENTAL

PART I

Atomic Fluorescence

The equipment used for the atomic fluorescence studies has been outlined in block diagram form (Figure 2). The continuum source was an Osram 450-watt xenon arc lamp enclosed in an air-cooled lamp housing (Schoeffel Instrument Co.) and powered by a regulated D. C. supply (Sola Electric Co.). The burner used was a V-10 total consumption burner (Ditric Corp.). The rest of the equipment included a Jarrell-Ash 0.5 meter grating monochromator equipped with a grating blazed at 3000Å and variable slits, an E. M. I. (Electro-Magnetic Industries) No. 6255B photomultiplier tube thermoelectrically cooled to -15°C, a phase-sensitive amplifier (E.M.C. Model RJB), and a variable-span recorder (Leeds and Northrup, Speedomax Azar). The high voltage necessary for the photomultiplier tube was furnished by a regulated power supply (412A, Fluke Mfg. Co.). The reference to the phase-sensitive amplifier was achieved by arranging a General-Electric miniature bulb (type 82) such that the light the bulb emitted was chopped by the mechanical chopper before the light reached a Raytheon EM 1502 Cd/Se photocell. The photocell was powered by two 45-volt Eveready batteries, while the lamp had its own 6-volt power supply (ATR Rectifier-Power Supply). The final piece of equipment involved the use of a motor-driven syringe (Sage Instruments Inc., Model 255-1) to force-feed the sample into the burner. The drive was found to be very helpful not only in assuring a constant rate

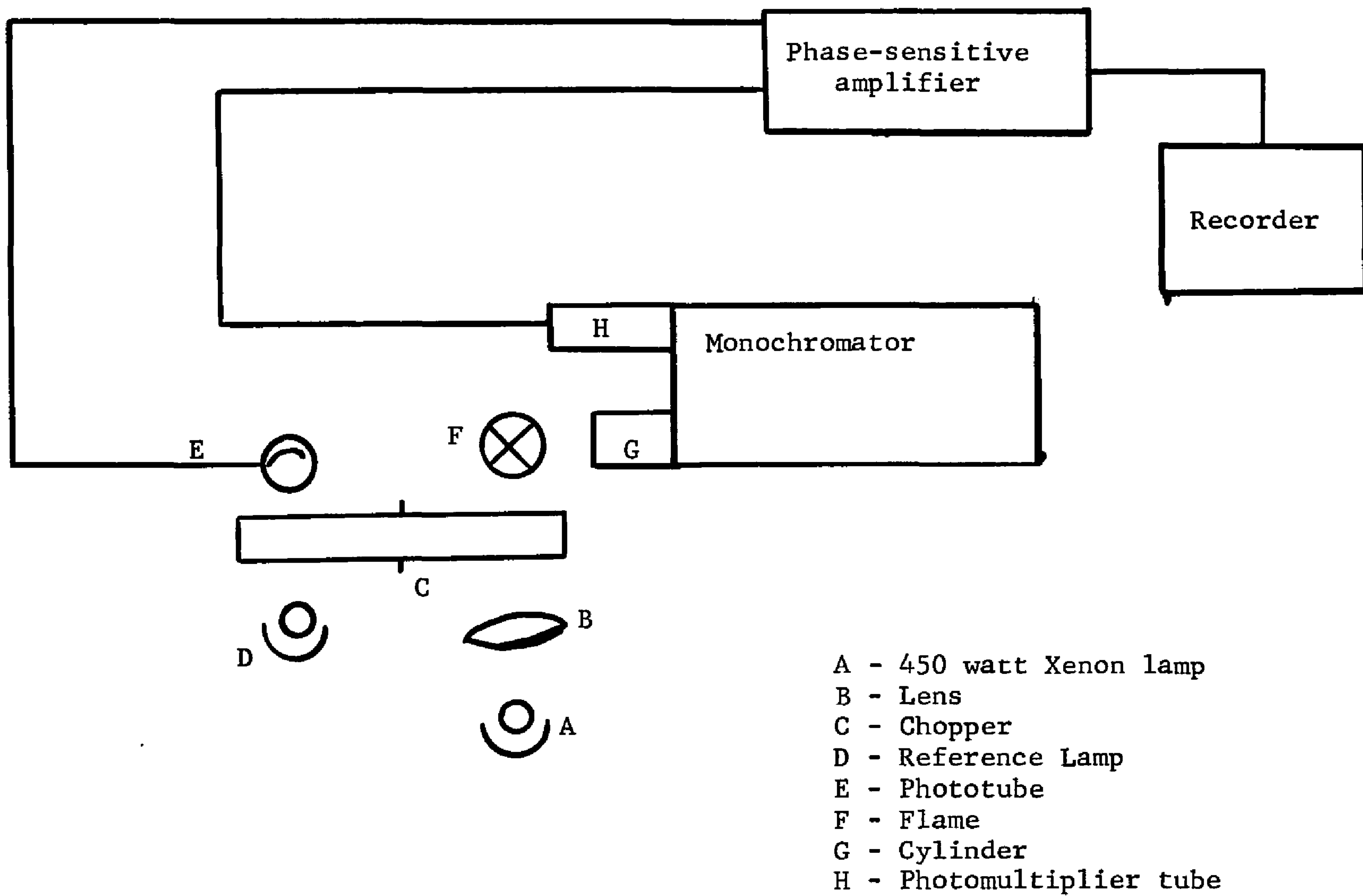


Figure 2. Atomic Fluorescence Experimental Block Diagram

of sample introduction, but also in reducing the intolerably audible noise of the flame caused by the high hydrogen gas flow rate without the syringe drive system.

The flame used for this work was the hydrogen-entrained air flame which had been found to be particularly well suited for this kind of work.[15] A preliminary survey of possible flames included the use of a hydrogen/oxygen flame and a hydrogen-argon/entrained-air flame. Neither was found to be as sensitive as the hydrogen/entrained-air flame.

Optimization of Variables in Atomic Fluorescence

In the actual operation of the instrument, it became evident that there were a number of variables associated with the instrument which had to be set at their optimum levels. These variables were:

1) the distance of the burner from the monochromator slit
2) the portion of the flame profile on which the xenon lamp was focused
3) the height of the flame on which the xenon lamp was focused
4) the flow rate of hydrogen gas into the burner
5) the aspiration rate of the analyte solution
6) the photomultiplier voltage
7) the monochromator slit width
8) the amplifier time constant
9) the photomultiplier output load resistor

In addition to these nine variables, the experimental wet chemistry associated with the determination of calcium in blood serum required the addition of two more variables whose levels had to be determined. These two

variables were the organic content of the serum and the amount of strontium suppressor to be added. Thus it was necessary to optimize eleven variables in all. In terms of a factorial experiment, even a one-half 2^{11} factorial design required too many data points. It was therefore imperative to experimentally reduce these variables to a more manageable number.

The eleven variables were first separated into two groups. One group, dealing with the electronic aspects of the system, included the monochromator slit width, the photomultiplier voltage, the photomultiplier output load resistor, and the amplifier time constant. These four variables were labelled the electronic variables. The seven remaining variables were labelled the flame variables. Of the seven flame variables it was quickly learned that in order to alleviate experimental difficulties, it was necessary to separate two variables from the seven and deal with these two variables immediately. These were the lateral position of the burner from the monochromator slit and the focusing of the xenon lamp on the flame profile. It required a great deal of effort in order to change the setting of these variables in a random fashion, in accordance with the requirements for factorial design experiments. Thus, for practical experimental considerations, it was decided to optimize these two variables first, then to deal with the remainder of the flame variables, and finally to treat the electronic variables. Then the total eleven variables were subdivided into the following three groups:

Group I: Distance from monochromator slit
Xenon lamp focusing on flame profile

Group II: Flame height (illuminated by lamp)
Hydrogen gas flow rate
Sample aspiration rate into flame

Sr content of blood serum sample

Organic content of blood serum sample

Group III: Photomultiplier output load resistor

Monochromator slit width

Amplifier time constant

The decision to optimize the groups in the order shown was based on the assumption that Groups I and II would show the greatest difference from any previous work which was performed using the same equipment.[15,21] Hence the electronic variables might possibly be close to an optimum setting using previous operating conditions.

Optimization of Group I Variables

For a more detailed explanation of the terminology and techniques used in the following sections, the appendix should be consulted.

The optimization of the Group I variables was done using a 100 p.p.m. calcium solution prepared by dilution of a standard calcium solution. (The preparation of the standard calcium solution is discussed on page 34.) The remaining variables were set as follows:

Group II: Burner height - 5.5 cm. from burner tip to mid-slit

Hydrogen gas flow rate - 19 liters/min

Syringe feed - 8 ml/min

Group III: Photomultiplier voltage - 1400 volts

Slit width - 0.170 mm

Time constant - 3 seconds

Photomultiplier output load resistor - 33,000 ohms

The settings selected to study the two variables are found in Table I. The 10.7 cm lateral distance of the burner from the slit was actually 2.0 cm from the light tube used in the assembly; when the burner was moved nearer to the light tube, no improvement in the detector response was noted.

Consider the reasons for the investigation of the Group I variables. Variable A was concerned with the comparative influences of absorption efficiency of the primary radiation and self-absorption of the emitted radiation. Thus, for example, with the lamp focused on the edge of the flame (Variable A in its +1 position), there would be very little self-absorption of the emitted radiation, but there would also be comparatively little absorption of the radiation from the xenon arc lamp. This would necessarily produce a low level of excitation and subsequent fluorescence. At the -1 setting of Variable A, the situation would be somewhat reversed. Here the potentiality for absorption of the lamp's radiation and subsequent production of fluorescence would be expected to be at a maximum, but at the same time, the potentiality for self-absorption would also have increased.

It was expected that there would be an optimum setting for the lateral distance of the burner from the monochromator slit where the maximum amount of fluorescence signal and the minimum amount of useless background flame intensity and scattered light would be admitted to the monochromator. Hence Variable B was included in the design.

A typical design matrix which was used for this optimization is found in Table II. The analysis of Variance Table corresponding to this experimental run is found in Table III. The conclusion drawn from this data was that changing the settings of both the lateral position of the burner and also the positioning of the lamp on the flame did

Table I

Coding of the Group I Variables for the Design Matrix

Variable A	-1	0	+1
Lamp positioning on flame	center of flame	midway between +1 and -1	edge of flame nearest to slit
Variable B			
Lateral burner distance from monochromator slit	11.7 cm	11.2 cm	10.7 cm

Table II

Design Matrix for an Optimization Run of Group I Variables

Variable A	Variable B	Interaction AB	Lack of Fit	Detector response Y
-1	-1	+1	+1	18
-1	-1	+1	+1	19
+1	-1	-1	+1	14
+1	-1	-1	+1	16
-1	+1	-1	+1	25
-1	+1	-1	+1	34
+1	+1	+1	+1	14
+1	+1	+1	+1	18
0	0	0	-4	22
0	0	0	-4	20

Table III

Analysis of Variance Table for Group I Variables Optimization Run

	Effects	Sum of squares	Degrees of Freedom	Mean squares	F ratio
Total		4322.000	10		
Correction factor	mean = 20.0	4000.000	1		
Variable A	-8.500	144.500	1		13.63
Variable B	+6.000	72.000	1		6.79
Interaction AB	-5.000	50.000	1		4.72
Lack of Fit		2.500	1		N.S.‡
Error		53.000	5	10.600	

F(1,5) at .95 = 6.61

at .99 = 16.26

‡Not significant

produce a meaningful difference on the signal response. Furthermore, the algebraic sign of the effect indicated in which position the variable achieved its highest response. Thus in the case of Variable A, the negative sign of the effect term implied that the highest response was achieved with Variable A in its -1 setting, with the lamp trained on the centermost part of the flame. Apparently the increased self-absorption of the fluorescence signal with the lamp at the centermost part of the flame was more than negated by the increase in absorption of the excitation radiation and subsequent fluorescence by the sample.

It was on this basis that the -1 setting was the optimum setting for Variable A. No advantage was anticipated in going to a more "negative" setting, that is, to train the lamp on the part of the flame farthest from the monochromator. This would have the result of cutting down the excitation and fluorescence potentialities while at the same time increasing the self-absorption of the emitted radiation to a higher level than for the -1 setting. In a similar fashion the geometry of the equipment limited the lateral positioning of the burner, Variable B, at its +1 position, and thus the +1 position was taken as optimum for Variable B.

This optimization run was repeated, and a composite of the data was made. This is shown in Table IV, and the corresponding Analysis of Variance table is shown in Table V. It might be noted that in this composite data, there was a strong interaction effect. This significant interaction F ratio implied that the solid angle of useable light reaching the monochromator, and hence the photomultipilier detector, reflected by Variable B, was significantly influenced by the positioning of the lamp on the flame, or Variable A. Thus

Table IV

Composite Design Matrix for Two Runs
Optimization of Group I Variables

	A	B	AB	Lack of Fit	Block	Y
Run 1	-1	-1	+1	+1	+1	18
	-1	-1	+1	+1	+1	19
Run 2	-1	-1	+1	+1	-1	16
	-1	-1	+1	+1	-1	16
Run 1	+1	-1	-1	+1	+1	14
	+1	-1	-1	+1	+1	16
Run 2	+1	-1	-1	+1	-1	12
	+1	-1	-1	+1	-1	12
Run 1	-1	+1	-1	+1	+1	25
	-1	+1	-1	+1	+1	34
Run 2	-1	+1	-1	+1	-1	24
	-1	+1	-1	+1	-1	28
Run 1	+1	+1	+1	+1	+1	14
	+1	+1	+1	+1	+1	18
Run 2	+1	+1	+1	+1	-1	11
	+1	+1	+1	+1	-1	9
Run 1	0	0	0	-4	+1	22
	0	0	0	-4	+1	20
Run 2	0	0	0	-4	-1	17
	0	0	0	-4	-1	15
Sum	-74	+40	-44	-10	+40	

Table V

Composite Analysis of Variance for Group I Optimization Studies

	Effects	Sum of squares	Degrees of Freedom	Mean squares	F ratio
Total		7198.000	20		
Correction factor	mean = 18.0	6480.000	1		
Variance A	-9.250	342.250	1		65.19
Variance B	+5.000	100.000	1		19.05
Variance AB	-5.500	121.000	1		23.05
Lack of Fit		1.250	1		N. S.‡
Block effect	+4.000	80.000	1		
Error		73.500	14	5.250	

F(1,14) at .95 = 4.60
at .99 = 8.86

‡ Not significant

Variable A's influence on the amount of intensity of signal plus scattered radiation dictated a specified placement of the burner from the slit for best results. A statistical test, known as a least significant difference calculation, was run at point (-1,+1) on the composite data. This test demonstrated that the improvement in signal response at this position was significantly higher than for any other combination of settings and, accordingly, this position was chosen as the preferred setting of the two variables. This setting did not necessarily represent a true optimum, but it was the best of the points tested and since it was physically impractical to pursue the path of steepest ascent, this point was chosen.

Optimization of Group II Variables

The coding of the variables is shown in Table VI. The levels in Table VI were chosen after preliminary work indicated that Variables A, C, and D were reasonable starting levels. It was decided to limit Variable B, the flow rate of hydrogen gas, at its +1 setting because of the audible noise involved, and because of the rapid consumption of fuel.

Table VII then shows the results of the initial optimization run for the Group II variables. Only the main effects have been presented. Interaction terms were found to be not significant at the 95% probability level. It was concluded from Table VII that at the stated variable levels, the effect of varying the hydrogen flow rate and the solution aspiration rate produced no significant change, while changing the burner height, varying the strontium content, and varying the organic content of the serum did produce meaningful effects on the response signal. Furthermore, the effects which were the most pronounced were the organic content and the burner height,

Table VI

Settings for Optimization Studies of Group II Variables

	-1	0	+1
Variable A burner height	4.75 cm burner tip to mid-slit	4.00 cm	3.25 cm
Variable B Flow rate of Hydrogen gas	15 liter/min	19 liter/min	23 liter/min
Variable C syringe feed rate	3 ml/min	5 ml/min	7 ml/min
Variable D Sr addition	1000 ppm	2000 ppm	3000 ppm
Variable E Organic content in serum	"none"	"one-half"	"full"

Table VII

Analysis of Variance for a(2^5+1) One-half Factorial Optimization Experiment for the Group II Variables

	Effects	Sum of squares	Degrees of Freedom	Mean squares	F ratio
Total		46,480.000	34		
Correction Factor	mean = 34.53	40,537.529	1		
Variance A	+19.375	3,003.125	1		55.25
Variance B	- 0.375	1.125	1		N. S.‡
Variance C	- 1.125	10.125	1		N. S.
Variance D	- 7.500	450.000	1		8.28
Variance E	+12.250	1,200.00	1		22.09
.	.	.	.		.
.	.	.	.		.
.	.	.	.		.
Lack of Fit		0.596	1		N. S.
Error		924.000	17	54.353	

$F(1,17)$ at .95 = 4.45

at .99 = 8.40

‡ Not significant

with the amount of strontium releasing agent assuming a secondary importance.

There were four replicates made of these Group II optimization studies, with each replicate incorporating a change in variable settings as indicated from the previous experiment. In each case the results were basically the same as in the first attempt, but complications were met as interactions not significant at one choice of variable settings did become significant when certain settings were changed. Following the suggestions of Davies[50], a Path of Steepest Ascent experiment was run to find the optimum conditions. To check a possible non-linearity in the response surface, an experiment was run which incorporated the concepts of a central composite design. The final result of this work was to set the Group II variables as follows:

Burner height: 3.00 cm from burner tip to mid-slit
Hydrogen flow rate: 19 liters/minute
Organic content: no digestion of serum
Strontium addition: 2500 ppm as the suppressor
Aspiration rate: 5 ml/minute

These settings represented the best estimate of the optimum settings for the Group II variables that could be deduced from the experimental runs. As both the path of steepest ascent and central composite experiments yielded results which were unsatisfactory, no definitive optimum was obtained. Thus the final results were a compromise between the results of the experimental design and operator experience.

Optimization of Group III Variables

After the complications experienced in the Group II optimization work, the Group III optimization quickly revealed from preliminary studies that an output load resistor of 33,000 ohms was the only reasonable setting for that variable, and an amplifier time constant of 3 seconds likewise gave the best results. Thus these two variables were left unchanged. A 2^2+1 factorial experiment on the two remaining variables, similar to that used in the Group I studies, revealed that the slit width was optimum at 0.170 mm and that the photomultiplier voltage gave the best signal to noise ratio at 1150 volts.

EXPERIMENTAL

PART II

DETERMINATION OF CALCIUM IN BLOOD SERUM

Experimental Considerations

As has already been mentioned, an approach similar to that of Willis[35] was to be the basic format in this work. Willis' method involved the dilution of the blood serum sample with a solution of strontium ion. Depending on the particular matrix a strontium solution of 1000 to 5000 ppm has been suggested to remove anion interferents, the most serious being phosphate, by preferentially replacing the calcium which is bound up with the interferent.[23,24] The question of the influence of serum protein on the calcium signal was not as easily resolved as that of anion interferents. The presence of protein in the sample does affect the calcium signal by complexing with the free calcium species and thereby reducing the concentration of free calcium atoms in the flame. Removal of the protein prior to aspiration, most usually by precipitation of the protein with trichloroacetic acid[56], has been the preferred technique for removing the protein influence, but this has not necessarily alleviated the situation as some of the calcium has been found to be coprecipitated along with the protein and lost in the subsequent filtration. Willis[35] has suggested that, provided the flame conditions are properly controlled, the protein may be broken down and burned as a fuel itself, thereby freeing the calcium for excitation and fluorescence. Willis[35] also found that the protein influence

on the calcium signal could be nullified through the addition of ethylenediaminetetraacetic acid to the sample. The real drawback in using ethylenediaminetetraacetic acid has been that burner clogging increases when the resulting solution has been aspirated into the flame.

It was decided to treat the organic material present in the serum as another variable to be optimized. It was hoped, for the sake of speed and convenience, that intermediate wet chemical steps, such as prior precipitation and filtration, would be found unnecessary. There have been suggestions[35,37] that direct dilution of the blood serum sample with a competitive cation such as Sr or La would adequately eliminate the ionic interferences while the dilution and proper flame conditions would allow the protein to be broken down and burned, thereby leaving an unhindered sample solution and also an unclogged burner. Indeed, Willis[35] has stated that, provided conditions were correct, the serum could be directly diluted with water and analyzed. Thus the following two solution variables were incorporated into the total optimization scheme in the atomic fluorescence flame spectrometric method:

1) the possible depressive effect of organic material in the diluted blood serum sample
2) the minimum amount of strontium necessary to achieve elimination of ionic interferences in the blood serum

A preliminary scan of blood serum solutions at the fluorescence wavelength of 4227Å indicated that a 20:1 dilution of the blood serum was the best dilution ratio for this work. A more concentrated solution was found to be viscous enough to threaten possible burner clogging and also demanded more blood serum than seemed to be practical. A more dilute

solution began to encroach on the noise limit of the signal.

The blood serum which was used for optimization studies was human serum donated by a local hospital.[48] Subsequent analyses, once the operating conditions had been set, were performed on blood serum samples which had been analyzed at the same hospital and also on a freeze-dried serum, Chemtrol[49], which had been previously analyzed.

Preparation of Calcium Solution

A standard 1000 ppm calcium solution was prepared by dissolving 2.4793 grams of A.C.S. grade $CaCO_3$ in 10 ml of concentrated HCl. The resulting solution was evaporated almost to dryness and then was diluted to 1 liter with 0.1 N HCl solution.

Digestion of Blood Serum

In order to study the effect of organic material present in the blood serum, it was necessary to digest some of the blood serum sample. This digested sample was then labelled as containing "no" organic materials. The undigested serum was used as the "full" organic content. An equal mixture of digested and undigested serum was used as containing "one-half" organic content.

The digestion was carried out as follows: 10 ml of serum was treated with 3 x 20 ml portions of concentrated HNO_3 in a silica evaporating dish over a flame from a bunsen burner. The resulting solution was evaporated to dryness in each case and was taken up with distilled water to a total volume of 10 ml. This resulting solution was diluted in a 20:1 ratio with an appropriate Sr solution.

Preparation of Strontium Solution

The strontium solution used to control ionic interferences was prepared in the following way: a stock solution of 3000 ppm strontium ion was prepared by dissolving 9.213 grams of $SrCl_2 \cdot 6H_2O$, 99.04% pure, and diluting up to one liter with distilled water. This solution was used to prepare other strontium solutions by appropriate dilution. No calcium or magnesium signals were obtained from this strontium solution when run as a blank by both atomic absorption and atomic fluorescence.

Atomic Fluorescence Flame Spectrometric Analyses

The approach used in the determination of calcium in blood serum samples was simple and direct. Standard calcium solutions, diluted appropriately, were prepared and run along with the diluted Chemtrol[49] sample in order to establish a working curve. In accordance with good experimental procedure, the atomic fluorescence spectra of these samples were obtained in a random fashion.

Once the data had been taken, a straight line was fitted to the data by the method of least squares. A Regression Analysis Table was used to check the linearity of the working curve. Once the linearity of the working curve had been established, the empirical equation describing the working curve was used to calculate the concentration of the unknown Chemtrol sample. The use of the least-squares fit of the data, together with the Regression Analysis Table, also permitted a quantitative empirical evaluation of the slopes of the working curves, an estimate of the sample standard deviation for the measurement system used, and other data necessary for a comparison of the two instrumental systems used in this determination.

All fluorescence spectra were obtained by scanning the monochromator over the fluorescence wavelength at the rate of 20Å/min. This scanning technique was found to be most satisfying in that an estimate of the background and noise associated with the fluorescence signal could be obtained from the background signals surrounding the fluorescence signal. Also, by using a scanning technique, there was never any problem in "tuning" the monochromator to its most sensitive setting. Thus the scanning technique insured the optimum monochromator wavelength position each time a fluorescence spectrum was run.

In the preparation of the working curve for calcium, it was decided to try to reproduce the organic blood serum matrix if possible, allowing only the calcium concentration to vary. Accordingly, all calcium calibration solutions were made up in an ion-free serum, Chemvarion.[49] This ion-free serum base was modified by adding known concentrations of calcium and strontium as experimental conditions dictated. The calcium solution which was used in making these dilutions was a 12,000 ppm calcium solution provided by Clinton Laboratories[49] which had been diluted to 120 ppm. A 30:1 dilution ratio of the strontium solution to the blood serum was used. Thus, for example, to prepare a 3 ppm standard calcium solution, 3.3 ml of the ion-free serum was mixed with 2.5 ml of the 120 ppm calcium solution and the resulting mixture was diluted to 100 ml with 2500 ppm strontium solution.

A typical determination of the calcium content of the Chemtrol standard serum is found in Table VIII along with the empirically-derived regression equation which best fits the data. The Regression Analysis Table is found in Table IX. The lack of significance of the Lack of Fit F ratio permitted the use of a linear equation to adequately represent the

Table VIII

Determination of Calcium

Sample: Chemtrol standard serum

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) detector response
1.0	9,12
1.5	11,14
2.0	15,19
2.5	19,21
3.0	20,21
3.5	26,24
$\frac{1}{30}$ (chemtrol)	17,18

Empirical equation: $y = 4.78 + 5.69\,x$

Table IX

Regression Analysis Table for Data of Table VIII

	Sum of squares	Degrees of Freedom	Mean squares	F ratio
Total	4023.000	12		
b_0(intercept)	3709.944	1		
b_1(slope)	282.993	1		132
Error	21.500	6	3.583	
Lack of Fit	8.563	4	2.141	0.597

F(4,6) at .95 = 4.53
at .99 = 9.15

relationship between the concentration of calcium in the sample and the detector response. Thus, using the equation along with the detector response for the 30:1 diluted serum, a value of 2.2 ppm for the calcium content was obtained. This implied that the calcium content was 66 ppm in the undiluted serum, as compared with the stated 80 ppm.[49] Successive repetitions of the determination failed to change this value appreciably and the conclusion was reached that the atomic fluorescence flame spectrometric determination of the calcium content of this sample, under the stated conditions, was 66 ppm.

The next set of determinations which were made by use of the atomic fluorescence method was the determination of calcium in three blood serum samples provided by a local hospital.[48] The same technique was used as for the Chemtrol determination. The results of the determination are shown in Table X.

Atomic Absorption Flame Spectrometric Analyses

The apparatus used for all atomic absorption determinations was a Jarrell-Ash Series 82-500 Atomic Absorption Flame Emission Spectrometer adapted with a 10-cm path length slot burner. The actual burner used in this work was a Hetco burner identical to that used in the atomic fluorescence work. Settings for maximum sensitivity were achieved by following the directions in the instrument manual. No further optimization procedures were used. The settings for the Jarrell-Ash instrument were as follows:

Fuel settings:

Hydrogen: 12 lbs/in^2 inlet pressure, rotameter setting 50

Table X

Determination of Calcium

Samples: Analyzed blood sera

Method: Atomic Fluorescence Flame Spectrometry

Sample	Stated value (ppm)	AF value (ppm)
A	95	87
Fe 125	1.0×10^2	95
Jakes	93	75

Air: 30 lbs/in^2 inlet pressure, rotameter setting 20

Burner settings:

Height: 2.0 cm from burner slot to mid-slit of monochromator

Distance of burner from focusing lens: 12 cm

Distance of focusing lens from slit: 17 cm

Slit width: 0.100 mm

Current for hollow cathode calcium lamp: 0.017 amperes

Calcium line for analysis: 4227Å

The solutions which were analyzed for calcium content were the same solutions which had been analyzed by atomic fluorescence. The same experimental design considerations were used in atomic absorption as in atomic fluorescence, and the corresponding atomic absorption data for the Chemtrol analysis is to be found in Tables XI and XII.

In a similar fashion, the three blood sera samples analyzed by atomic fluorescence were also analyzed using atomic absorption. The results, along with those from atomic fluorescence, are shown in Table XIII.

Table XI

Determination of Calcium

Sample: Chemtrol standard serum

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
2.0	6.25, 6.25	0.0280, 0.0280
2.5	6.75, 6.75	0.0303, 0.0303
3.0	7.50, 7.75	0.0339, 0.0350
3.5	7.75, 8.00	0.0350, 0.0362
$\frac{1}{30}$ (Chemtrol)	6.50, 6.50	0.0292, 0.0292

Empirical Equation: $y = 1.72 \times 10^{-2} + (5.39 \times 10^{-3})(x)$

Table XII

Regression Analysis Table for Data of Table XI

	Sum of squares x 10^4	Degrees of Freedom	Mean Square x 10^5	F ratio
Total	83.0383	8		
b_o(intercept)	82.3686	1		
b_1(slope)	0.7263	1		
Error	0.0132	4	0.0330	
Lack of Fit	0.0202	2	0.1010	3.06

F(2,4) at .95 = 6.94

at .99 = 18.00

Table XIII

Blood Serum Analyses for Calcium

Blood Sample	Stated value (in ppm)	AF value (in ppm)	AA value (in ppm)
Chemtrol	80	66	66
		18%	18%
Sample A	95	87	1.3×10^2
		8.4%	37%
Sample Fe 125	100	95	1.1×10^2
		5.0%	10%
Sample Jakes	93	75	84
		19%	9.7%

EXPERIMENTAL

PART III

DETERMINATION OF METALLIC CONSTITUENTS IN FERTILIZERS

Experimental Considerations

In this determination, it was decided to adhere strictly to the methods which have been adopted by the Association of Official Agricultural Chemists[42] for the atomic absorption determinations of fertilizers, thereby assuring a reliable standard of reference. This rigid adherence to the A.O.A.C. specifications in the atomic absorption determinations acted as a double-check; the sample not only was checked against the given value, but the wet chemical techniques used in the determinations were also checked. Thus, if agreement was reached between the accepted value and the atomic absorption value, it would be concluded that any deviations in the atomic fluorescence determinations may be attributed to the atomic fluorescence technique or to equipment, not to the solution matrix which was being examined.

The samples which were examined for trace metallic constituents were Margruder standard samples No. 6801 and No. 6901.[51] These were samples which had been examined by a number of laboratories, by various methods, and whose results had been recorded. The results stated are average values. No confidence limits about these values were available.

Preparation of Standard Solutions

The preparation of 1000 ppm standard solutions used in preparing the necessary working curves of the six metals studied were made up as follows:

Iron: 1.000 gram of pure iron wire, electrolytic quality, was dissolved in approximately 10 ml of concentrated HCl. The resulting solution was evaporated almost to dryness and was then diluted to 1 liter with 0.1 N HCl.

Magnesium: the same procedure as for iron, except that 1.000 gram of pure magnesium ribbon was used.

Copper: 1.000 gram of pure copper, electrolytic quality, was dissolved in a minimum amount of HNO_3. To this solution was added approximately 10 ml concentrated HCl and the resulting solution was evaporated almost to dryness. The resultant was then diluted to 1 liter with 0.1 N HCl.

Zinc: the same procedure as for iron was used, except that 1.000 gram of pure zinc metal was used.

Manganese: 1.590 grams of 99.50% pure MnO_2 (Fisher ACS certified) was dissolved in about 10 ml of concentrated HCl and the solution was then treated in the same way as the iron sample.

Calcium: 2.479 grams of pure (ACS grade) $CaCO_3$ was dissolved in about 10 ml of concentrated HCl; the resulting solution was treated in the same way as the iron sample.

It should be noted that no standard A.O.A.C. technique for the atomic absorption determination for calcium was given. Thus the preparation and treatment of the calcium solutions and their subsequent analysis does not necessarily have the sanction of the A.O.A.C.

From these stock solutions,dilutions to the desired concentration ranges were made. No pipet less than 25 ml was used. In any case which required the delivery of an odd number of milliliters, all solutions were delivered from a buret.

Preparation of Margruder Fertilizer Samples

The suggested A.O.A.C. procedure was as follows:

"1.000 gram of the Margruder sample was dissolved in approximately 10 ml of concentrated HCl. The solution was evaporated almost to dryness, 20 ml of 0.5 N HCl was added to redissolve the residue, and the resulting solution was filtered through fast filter paper (S&S 595). The filter paper was washed with five 10 ml portions of deionized water, the washings and filtrate being collected in a 100 ml volumetric flask. Dilution of the resulting solution to the 100 ml mark produced the solution which was then labelled the standard fertilizer sample."

It must be noted again that the above procedure was the procedure followed only for the first five of the metal ions in this determination. Calcium, not being subject to these conditions, was treated differently if such treatment was deemed necessary.

It must also be noted that for the determination of iron, zinc, copper, and manganese, the Margruder sample No. 6801 was used. The determination of magnesium and calcium

was accomplished using the Margruder sample No. 6901.

Atomic Absorption Spectrometric Analyses

Iron. The atomic absorption experimental settings were as follows:

Height of burner: 5.5 cm, burner slot to mid-slit
Iron hollow cathode current: 0.022 amperes
Fuel Settings:
Hydrogen: 35 lbs/in^2; rotameter setting 50
Air: 30 lbs/in^2; rotameter setting 20
Analysis line: 2483Å

It was found that it was necessary to dilute the standard fertilizer sample by a factor of one-half to bring the iron content into the linear portion of the working curve. The working curve data and subsequent determination of the iron content of the fertilizer sample is shown in Tables XIV and XV. The average value obtained for the iron content of the fertilizer was 40 ppm iron, as compared to the stated value of 41.04 ppm.

Copper. The experimental settings for the copper determination was as follows:

Burner height: 2.0 cm from burner slot to mid-slit
Copper hollow cathode current: 0.022 amperes
Copper analysis line: 3247Å
Fuel settings:
Air: 30 lbs/in^2; rotameter setting 20
Hydrogen: 12 lbs/in^2; rotameter setting 50

In the determination of the copper content of the Margruder sample, no dilution of the standard fertilizer sample was needed. The data for the determination are shown

Table XIV

Determination of Iron

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
5	6,6	0.0269, 0.0269
10	13,13	0.0605, 0.0605
20	23,23	0.1135, 0.1135
25	32,28	0.1674, 0.1427
$\frac{1}{2}$ (standard sample)	24,21	0.1192, 0.1024

Empirical Equation: $y = -3.80 \times 10^{-3} + (6.19 \times 10^{-3})(x)$

Table XV

Regression Analysis Table for Data of Table XIV

	Sum of Squares x 10^4	Degrees of Freedom	Mean Square x 10^4	F ratio
Total	829.1827	8		
b_o	633.5088	1		
b_1	191.3500	1		
Error	3.3024	4	0.8256	
Lack of Fit	1.0215	2	0.5108	N.S.‡

F(2,4) at .95 = 6.94

‡Not significant

in Tables XVI and XVII. The value for the copper content was found to be 5.5 ppm as compared with the stated value of 5.46 ppm.

Zinc. The experimental settings for the zinc determination were the same as those of the copper settings except that the hollow cathode current was 0.012 amperes and the analysis line was 2138Å. It was found that a 20-fold dilution of the standard fertilizer sample was necessary. The data for the determination are presented in Tables XVIII and XIX. The average value for the zinc content was found to be 23 ppm as compared with the stated value of 23.29 ppm.

Manganese. The experimental conditions for the manganese determination of the Margruder sample were the same as the copper conditions except that the hollow cathode current was 0.027 amperes and the analysis line was 2802Å. A dilution ratio of one half of the standard fertilizer sample was necessary.

The data for the determination are to be found in Tables XX and XXI. The average value for the manganese content of the fertilizer was found to be 17 ppm as compared with the stated value of 17.18 ppm.

Magnesium. The magnesium content of the Margruder No. 6901 sample was determined after previous work on the No. 6801 sample had established the linear region of the working curve. Thus, only two points, one at either end of the curve, were run to re-establish the working curve.

The experimental settings were as follows:

Burner height: 3.0 cm from burner slot to mid-slit

Fuel Settings:

Air: 30 lbs/in^2; rotameter setting 20

Hydrogen: 33 lbs/in^2; rotameter setting 50

Table XVI

Determination of Copper

Sample: Margruder fertilizer sample No. 6801

Method: Atomic Absorption Flame Spectrometry

(x) Concentration (ppm)	Detector response % absorption	(y) Absorbance
1	2,2	0.0088, 0.0088
3	7,7	0.0315, 0.0315
5	12,12	0.0555, 0.0555
8	17,19	0.0809, 0.0915
10	23,24	0.1135, 0.1192
fertilizer sample	12,13	0.0555, 0.0605

Empirical Equation: $y = -3.55 \times 10^{-3} + (1.17 \times 10^{-2})(x)$

Table XVII

Regression Analysis Table for Data of Table XVI

	Sum of Squares $\times 10^4$	Degrees of Freedom	Mean Square $\times 10^4$	F ratio
Total	503.0783	10		
b_o	356.0510	1		
b_1	145.8212	1		
Error	0.7242	5	0.1448	
Lack of Fit	0.4819	3	0.1606	1.11

$F(3,5)$ at $.95 = 5.41$

Table XVIII

Determination of Zinc

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
0.5	12,11	0.0555, 0.0506
0.8	18,17	0.0862, 0.0809
1.0	22,22	0.1079, 0.1079
1.5	29.5,31	0.1518, 0.1612
2.0	38.5, 36.5	0.2111, 0.1972
$\frac{1}{20}$ (fertilizer sample)	24,24,24,24.5	0.1192,0.1192, 0.1192, 0.1221

Empirical Equation: $y = 4.00 \times 10^{-3} + (1.01 \times 10^{-1})(x)$

Table XIX

Regression Analysis Table for Data of Table XVIII

	Sum of Squares $\times 10^4$	Degrees of Freedom	Mean Square $\times 10^4$	F ratio
Total	1753.8041	10		
b_o	1464.1000	1		
b_1	287.5061	1		
Error	1.6886	5	0.3377	
Lack of Fit	0.5094	3	0.1698	N.S.‡

$F(3,5)$ at $.95 = 5.41$

‡Not significant

Table XX

Determination of Manganese

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
0.8	7,7	0.0315, 0.0315
1.5	11,13	0.0506, 0.0605
5.0	32,31	0.1674, 0.1612
8.0	44,45	0.2518, 0.2596
10.0	52,52	0.3188, 0.3188
$\frac{1}{2}$ (fertilizer sample)	46,48,47	0.2676, 0.2840, 0.2757

Empirical Equation: $y = 7.80 \times 10^{-3} + (3.11 \times 10^{-2})(x)$

Table XXI

Regression Analysis Table for Data of Table XX

	Sum of Squares x 10^4	Degrees of Freedom	Mean Square x 10^4	F ratio
Total	3962.7559	10		
b_o	2729.1040	1		
b_1	1234.7790	1		
Error	0.9864	5	0.1973	
Lack of Fit	2.1135	3	0.7045	3.57

$F(3,5)$ at .95 = 5.41

Hollow cathode current: 0.015 amperes

Analysis line: 2852Å

It was found that a dilution factor of 1/160 was necessary to bring the fertilizer sample into the linear region of the working curve. This dilution, as all of the others was done with 0.1 N HCl.

The data for the determination are found in Tables XXII and XXIII. A value of 1.4×10^2 ppm was obtained for the magnesium content of the fertilizer sample, as compared with the stated value of 138 ppm.

Calcium. When calcium was studied in this analysis, preliminary work on the No. 6801 Margruder sample indicated that agreement between the atomic absorption and atomic fluorescence methods could only be achieved if a releasing agent such as strontium were added. As no value for the calcium content of the No. 6801 sample was available, it was impossible to know whether or not the agreement between the two techniques was coincidence or not. Thus the availability of the No. 6901 sample permitted an absolute check on the calcium determination technique which had been worked out.

The analyzed calcium solution was modified from the standard fertilizer sample only in that the diluted calcium sample contained strontium ion, introduced as $SrCl_2 \cdot 6H_2O$. A 4800 ppm strontium concentration in the undiluted sample was found to give agreement between the two methods. A dilution ratio of 1/320 was found to be necessary to enable work in the linear region of the working curve.

The experimental conditions were:

Burner height: 2.0 cm from burner slot to mid-slit

Hollow cathode current: 0.015 amperes

Table XXII

Determination of Magnesium

Sample: Margruder fertilizer sample no. 6901

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
0.5	19	0.0915
	20	0.0969
	17.5	0.0835
	18	0.0862
1.25	33.5	0.1772
	32	0.1674
	33	0.1739
	32	0.1674
$\frac{1}{160}$ (fertilizer sample)	25	0.1249
	26	0.1308
	26	0.1308
	25	0.1249

Empirical Equation: $y = 3.41 \times 10^{-2} + (1.10 \times 10^{-1})(x)$

Table XXIII

Regression Analysis Table for Data of Table XXII

	Sum of Squares x 10^4	Degrees of Freedom
Total	1498.5112	8
b_o	1362.4200	1
b_1	135.4060	1
Error	1.7752	6

Fuel settings:

Air: 30 lbs/in^2; rotameter setting 20

Hydrogen: 12 lbs/in^2; rotameter setting 50

Analysis line: 4227Å

The data for the determination are to be found in Tables XXIV and XXV. The value obtained for the calcium content in Margruder sample No. 6901 was 1.2×10^3 ppm calcium content, as compared with a stated value of 1225 ppm.

Atomic Fluorescence Flame Spectrometric Analyses

The atomic fluorescence flame spectrometric determination of metal components in fertilizers was carried out on the same solutions used in the atomic absorption experiments, the sole exception being the determination of magnesium content. In this particular determination, it was found necessary to add strontium ion in an amount equivalent to 2000 ppm in the undiluted standard fertilizer sample. Only then could agreement be reached between the atomic absorption and atomic fluorescence methods.

From the work done on the blood serum calcium determinations with regard to optimization of the various settings in the atomic fluorescence equipment, it was learned that by far the most sensitive variable was the height of the burner, that is, the portion of the flame which was exposed to the xenon lamp. Thus, in this work it was decided to set all variables at their optimum settings, as determined earlier, and to vary only the burner position as necessary. Preliminary experiments employing this approach gave results which were highly satisfactory.

The settings which were used were:

Table XXIV

Determination of Calcium

Sample: Margruder fertilizer sample no. 6901

Method: Atomic Absorption Flame Spectrometry

(x) concentration (ppm)	Detector response % absorption	(y) Absorbance
1.25	4	0.0177
	4	0.0177
	4.5	0.0200
	4.5	0.0200
5.00	13	0.0605
	15	0.0706
	15	0.0706
	15	0.0706
$\frac{1}{320}$ (fertilizer sample)	11.5	0.0531
	11.5	0.0531
	11.0	0.0506

Empirical Equation: $y = 2.60 \times 10^{-3} + (1.31 \times 10^{-2})(x)$

Table XXV

Regression Analysis Table for Data of Table XXIV

	Sum of Squares x 10^4	Degrees of Freedom
Total	200.3991	8
b_o	151.3800	1
b_1	48.2653	1
Error SS	0.8188	6

Flow rate of hydrogen: 19 liters/min

Aspiration rate: 5 ml/min

Photomultiplier voltage: 1150 volts

Slit width: 0.170 mm

Distance of burner from slit: 10.7 cm

The only change which was found necessary in the above settings was the use of a slit width of 0.400 mm in the zinc determination.

The burner heights which were used were:

Copper: 7.5 cm from burner tip to mid-slit

Magnesium: 7.5 cm

Manganese: 5.0 cm

Calcium: 4.5 cm

Iron: 5.0 cm

Zinc: 5.0 cm

The appropriate data for these six determinations are presented in Tables XXVI to XXXVII. A table of the Margruder fertilizer content as determined by atomic fluorescence flame spectrometry is shown in Table XXXVIII, along with the stated values and the values determined by atomic absorption flame spectrometry.

Table XXVI

Determination of Copper

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
1	8,9
3	15,19
5	26,34
8	41,40
10	51,46
fertilizer sample	28,30

Empirical Equation: $y = 4.72 + 4.48\,x$

Table XXVII

Regression Analysis Table for Data of Table XXVI

	Sum of Squares	Degrees of Freedom	Mean Square	F ratio
Total	10,561.0000	10		
b_o	8,352.1000	1		
b_1	2,133.0114	1		
Error	53.5000	5	10.7000	
Lack of Fit	22.3886	3	7.4629	N.S.

$F(3,5)$ at .95 = 5.41

Table XXVIII

Determination of Iron

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
5	7,7
10	15,14
15	16,17
20	23,22
25	32,25
$\frac{1}{4}$ (fertilizer sample)	12,12,11,13

Empirical Equation: $y = 2.50 + 1.02x$

Table XXIX

Regression Analysis Table for Data of Table XXVIII

	Sum of Squares	Degrees of Freedom	Mean Square	F ratio
Total	3726.00	10		
b_o	3168.20	1		
b_1	520.20	1		
Error	26.00	5	5.200	
Lack of Fit	11.40	3	3.800	N.S.‡

$F(3,5)$ at .95 = 5.41

‡Not significant

Table XXX

Determination of Zinc

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
10	11,12
20	13,12
50	17,16
100	19,17
fertilizer sample	13,13

Empirical Equation: $y = 11.34 + (7.30 \times 10^{-2})(x)$

Table XXXI

Regression Analysis Table for Data of Table XXX

	Sum of Squares	Degrees of Freedom	Mean Square	F ratio
Total	1773.000	8		
b_o	1711.120	1		
b_1	52.224	1		
Error SS	3.500	4	0.875	
Lack of Fit	6.156	2	3.078	3.52

$F(2,4)$ at .95 = 6.94

$F(2,4)$ at .90 = 4.32

Table XXXII

Determination of Manganese

Sample: Margruder fertilizer sample no. 6801

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
0.5	5
	6
	7
	7
2.0	19
	17
	16
	20
$\frac{1}{20}$ (fertilizer sample)	9
	8
	10
	9

Empirical Equation: $y = 2.33 + 7.83\ x$

Table XXXIII

Regression Analysis Table for Data of Table XXXII

	Sum of Squares	Degrees of Freedom
Total	1465.000	8
b_o	1176.120	1
b_1	276.097	1
Error	12.750	6

Table XXXIV

Determination of Magnesium

Sample: Margruder fertilizer sample no. 6901

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
0.5	10
	11
	9
	10
1.25	27
	28
	25
	25
$\frac{1}{160}$ (fertilizer sample)	18
	19
	17

Empirical Equation: $y = -1.00 + 21.86\ x$

Table XXXV

Regression Analysis Table for Data in Table XXXIV

	Sum of Squares	Degrees of Freedom
Total	3165.000	8
b_o	2628.128	1
b_1	532.862	1
Error	8.750	6

Table XXXVI

Determination of Calcium

Sample: Margruder fertilizer sample no. 6901

Method: Atomic Fluorescence Flame Spectrometry

(x) concentration (ppm)	(y) Detector response
1.25	7
	8
	6
	7
5.00	26
	28
	25
	26
$\frac{1}{320}$ (fertilizer sample)	22
	20
	18

Empirical Equation: $y = 0.58 + 5.14\,x$

Table XXXVII

Regression Analysis Table for Data of Table XXXVI

	Sum of Squares	Degrees of Freedom
Total	2959.000	8
b_o	2211.128	1
b_1	741.336	1
Error	6.750	6

Table XXXVIII

Results of Fertilizer Determinations by Atomic Absorption and Atomic Fluorescence

Element Determination	Stated value (ppm)	AA value % deviation	AF value % deviation
Cu	5.46	5.5 0.7%	5.4 1.1%
Fe	41.04	40 2.5%	37 9.9%
Zn	23.29	23 1.2%	23 1.2%
Mn	17.18	17 1.0%	17 1.0%
Mg	138	1.4×10^2 1.4%	1.4×10^2 1.4%
Ca	1225	1.2×10^3 2.0%	1.2×10^3 2.0%

DISCUSSION

ACCURACY

In any analytical determination, a question of primary importance is accuracy. How well do the results of a determination approach the true value? Often the true value is not known and, therefore, an estimate of the systematic error must be inferred by other means. For example, recovery experiments may be used. If the errors can be attributed to random variations, then the precision of the measurement process by which the value was obtained can be used as an estimate of the accuracy. Unfortunately, it is often difficult to prove that only random variations are present.

This discussion considers only errors in the measurement systems; i.e., atomic absorption and atomic fluorescence. Thus, the comparison will be restricted to "instrumental error." Errors associated with the sampling of the fertilizer and blood aliquots and the error associated with the chemical treatment of the fertilizer and blood samples were not investigated.

The data for the determination of the metal constituents in fertilizer samples are shown in Table XXXVIII. The poorest accuracy was obtained for the determination of iron. The atomic fluorescence value of 37 ppm is 9.9% low, while the atomic absorption value of 40 ppm is 2.5% low. From literature considerations[42,39] an air-acetylene mixture is the fuel mixture preferred in the determination of iron, rather than the air-hydrogen or hydrogen/entrained-air fuel mixture used in this work. The hotter air-acetylene flame

tends to break up refractory compounds in the flame to a greater extent than the air-hydrogen flame. Thus, although the results obtained are not surprising, the question of agreement or disagreement among these results still must be resolved.

The question of accuracy was judged by comparing the experimental value for the fertilizer with the stated value by a Student's t test.[46] The sample standard deviation value which was used for this and all subsequent comparisons was the square root of the Error Mean Square value, in units of concentration, in the Regression Analysis Table. The use of this value of the standard deviation then permitted a t value to be calculated and compared with tabular values. A decision was then made as to whether or not the experimental value was different from the stated value.

It should be noted that the direct use of the data in the Regression Analysis Table in the calculation of the standard deviation value was not possible. The Error Mean Square values in these tables are in detector response units, not concentration units. Thus, in the atomic fluorescence iron determination, for example, it was necessary to transform all detector measurements (see Table XXVIII) into concentration terms through the use of the empirical equation fitted to the data. It was then possible to calculate a standard deviation value for the atomic fluorescence system of 2.2 ppm.

Then:

$$41.04 = 37 \pm t(2.2)$$

$$\text{or} \quad \pm t = 1.8$$

At a 95% level of confidence, the t value for the 5 degrees of freedom associated with error is 2.57. It cannot be concluded, therefore, that the two values are different; i.e.,

at a 95% level of confidence, the atomic fluorescence value obtained for the iron content in the fertilizer by this determination was not significantly different from the true stated value.

In a similar fashion, the atomic absorption determination yielded a _t_ value of 0.7 which did not exceed its tabled _t_ value of 2.8. The conclusion drawn on the basis of these experiments was that both methods gave results which were not significantly different from the true stated value. As all of the values for the other determinations were in closer agreement with the stated value than the iron determination, the other values were accepted as being no different from the stated value.

From Table XIII, it may be seen that there was very little agreement among the values for the various blood serum calcium determinations. The percent deviations ranged from 5% to 37%. The results were clearly unacceptable by either method.

It was felt, in retrospect, that a large portion of these deviations was the fault of poor wet chemical techniques. With so little blood serum available, perhaps 5 ml, in sample A, Sample Fe125 and sample Jakes determinations, it was necessary to pipet amounts such as 3.3 ml. Even when a good quality pipet was used, this volume could not be delivered to better than $\pm$0.02 ml, thereby introducing a deviation of six parts per thousand in this step alone. It is perhaps noteworthy that when improved wet chemical techniques were used in the fertilizer determinations, a better accuracy was obtained.

Not all of the deviation in the calcium blood serum determination can be laid at the door of technique, however. In the Chemtrol determination, ample serum was available for

a large number of determinations, and while there was good agreement between the atomic absorption value and the atomic fluorescence value, both determinations were in poor agreement with the stated value. Although some of the deviation was undoubtedly caused by the transfer of small volumes and the subsequent wet chemical preparation steps, it was felt that the deviations encountered in the Chemtrol determination were caused, in part, by the complex matrix. Apparently, the simplifying assumption, dilution of the sample with a releasing agent prior to analysis in the flame, was not justified in this case. Also, the assumption that the matrix in the blood serum sample could be duplicated in the working curve by the addition of standard calcium solution to the ion-free Chemvarion base must have been faulty, as the results seem to bear out. It seems imperative, therefore, that some preliminary step such as prior precipitation of the protein or the addition of a chelating agent such as ethylenediaminetetraacetic acid, along with the addition of the strontium or lanthanum releasing agent, be inserted into the technique. It is possible that a hotter flame rather than the cool burning air-hydrogen flame might help break down refractory compounds in the flame and increase the calcium concentration in the flame and hence the atomic absorption and atomic fluorescence signals.

PRECISION

It was still possible to derive useful information from the calcium blood serum determinations, even though the consistently low results of the Chemtrol sample indicated a systematic error. A check of the reproducibility of the measurement system used in the determination of the calcium

content in the Chemtrol sample should indicate whether or not one system had a better precision than the other. This information was of interest, not only in the blood serum determination, but also in the fertilizer analyses.

In making a comparison of precision between the two systems, a direct comparison of the Error Mean Square value between the two methods could not be made, as the error mean square term in the atomic absorption analysis was a logarithmic function, while the variance terms in the atomic fluorescence analyses were linear terms, not logarithmic. The resolution of this problem, however, lay in the calculation of the relative standard deviation, expressed as a percent, for each method. This is a unitless quantity defined as:

$$RSD = (s/u)(100)$$

where s = the sample standard deviation for the method

u = the mean value of the detector response

The square of this relative standard deviation term is a term which is unitless and which is directly related to a variance term. It was then possible to compare these squared values of the relative standard deviation for a given method by an F test.

Thus in the atomic absorption determination of blood serum calcium the average value for the absorbance was 3.2×10^{-2} (see Table XI). From Table XII, since the Lack of Fit sum of squares term was not significant at the 95% level, the Error Sum of Squares term and Lack of Fit sum of squares were pooled together to give a Total Error Sum of Squares value of 3.4×10^{-6} with six degrees of freedom. The use of Total Error Sum of Squares value includes not only the error associated with differences between replicates, but also all other errors which cause a scatter of points about the working

curve. This Total Error Mean Square value would, for example, contain errors associated with the preparation of the standard solutions. The total Error Mean Square value was therefore 5.6×10^{-7}, and the standard deviation $s = 7.5 \times 10^{-4}$. The relative standard deviation, expressed as a percent, was:

$$RSD = 100 \times 7.5 \times 10^{-4}/3.2 \times 10^{-2} = 2.3\%$$

By an analagous calculation from Tables VIII and IX, the relative standard deviation value for the atomic fluorescence determination was calculated to be 9.8%. Thus the atomic absorption exhibited a variance-related value of $(2.3)^2$ and the atomic fluorescence exhibited a variance-related value of $(9.8)^2$. The atomic absorption squared term had six degrees of freedom associated with it, while the atomic fluorescence squared term had 10 degrees of freedom associated with it.

Then, $$F = (9.8)^2/(2.3)^2 = 18$$

and $$F(10,6) \text{ at } .95 = 4.06 .$$

The conclusion which was drawn was that, at the 95% probability level, the atomic absorption measurement system had a better precision than the atomic fluorescence system.

SENSITIVITY

For a determination involving a linear calibration curve as an integral part of the final determination, it is obvious that the steeper the slope, the better the concentration resolution; i.e., a small change in concentration will result in a greater difference in detector response than for a curve with a less steep slope. However, associated with any working curve is a measure of imprecision, the standard deviation associated with the fitting of the curve. When

the value of the standard deviation becomes larger, the concentration resolution decreases. Another way of stating this is to say that with a larger value of the standard deviation the value of the slope becomes less definite and thus the confidence intervals placed around the slope, or any value on the curve itself, must become larger. The definition for sensitivity[47,57] which was employed here is

$$SENS = \text{slope } b_1/\text{sample standard deviation, } \underline{s}.$$

The comparison of the sensitivity, or concentration resolution, could easily be made at this point, as the previous work had provided all of the necessary data. For the atomic absorption determination of the Chemtrol calcium content, the value for the slope b_1 was 5.4×10^{-3} ppm^{-1}. The sample standard deviation had already been calculated to have a value of 7.5×10^{-4}. The sensitivity value was therefore

$$SENS = 5.4 \times 10^{-3}/7.5 \times 10^{-4} = 7.2 \text{ ppm}^{-1}.$$

Recall that this data had associated with it four degrees of freedom. For the atomic fluorescence determination, the value for the slope was 5.7 and the sample standard deviation for the calibration curve was 1.7. Thus the sensitivity value for the atomic fluorescence determination was

$$SENS = 5.7/1.7 = 3.3 \text{ ppm}^{-1}.$$

The atomic fluorescence determination had associated with it six degrees of freedom.

The comparison to be made between the two sensitivities is made by a K term.[47] This K term involves the sensitivity values for each determination along with a $\underline{t}$ value for the number of degrees of freedom associated with each determination.

Thus: $$K = (SENS)_{AA}(\underline{t}_{AA}) \ / \ (SENS)_{AF} \ (\underline{t}_{AF})$$

For the atomic fluorescence case, the number of degrees of freedom associated with the *t* value was 6; for 95% confidence *t* = 2.45. For atomic absorption, the *t* value, for four degrees of freedom, at the 95% probability level, was 2.78. Therefore,

$$K = (7.2)(2.78)/(3.3)(2.45) = 2.5.$$

On the basis of this calculation, the atomic absorption method would be judged as being more sensitive than the atomic fluorescence technique.

DETECTION LIMIT

There is one final bit of information which can be extracted from these calculations. Since the units of the sensitivity value are those of reciprocal concentration, it is possible to take the reciprocal of the sensitivity and obtain a concentration term that corresponds to a detection limit. It is also necessary to include a probability level at which this calculation is made. Thus for the atomic fluorescence determination of calcium, the sensitivity value was 3.3. For the six degrees of freedom, the t value at 95% confidence is 2.45. The detection limit for calcium using atomic fluorescence in this experiment was

$$D.L. = 2.45/3.3 = 0.7 \text{ ppm.}$$

For atomic absorption, the appropriate t value is 2.78 and the detection limit was

$$D.L. = 2.78/7.2 = 0.4 \text{ ppm.}$$

The above calculations for the detection limit should be viewed critically for the following reason. The best way to make this detection limit calculation is to use a solution with the lowest concentration value which will give a good useable signal. Replicate determinations will then give rise to a set of values from which may be calculated a standard deviation more in line with the actual working conditions at the low end of the curve. This value of the standard deviation can be used in the calculation of the sensitivity and the detection limit. The assumption inherent in the calculations of the detection limits above is that the standard deviation will be the same at the detection limit as the standard deviation along the whole calibration curve itself.

Since a homogeneity of the standard deviation is a ncessary criterion for any statistical calculations of any sort, this detection limit calculation is useful as a first approximation, although it is likely that the use of the standard deviation for the whole line will give rise to a more optimistic detection limit value than is perhaps justified.

Consequently, in Tables XXXIX, XL, and XLI are found all of the comparisons which have been made in the blood and fertilizer determinations. As the blood analysis has already been fairly well discussed above, the fertilizer data will now be considered.

If the precisions of the two techniques are considered, one finds that in two cases, those of the Mn and Zn determinations, the atomic fluorescence precision was significantly poorer than that of atomic absorption. In all other cases, there was no difference in the precisions of either measurement system. Only in the calcium determination was the atomic absorption value for the relative standard deviation larger than that of the atomic fluorescence data.

It is reasonable to conclude that the improved sensitivity of atomic fluorescence for calcium was brought about by the extended linearity of the atomic fluorescence working curve. This fact permitted analyses at higher concentration levels than in the case of atomic absorption.

When the comparisons of sensitivities were noted, the main feature of this data was the enormous K value for the AA/AF Zinc ratio. This suggested that the atomic fluorescence technique was very poor in the determination of zinc and was clearly surpassed by the atomic absorption methods. The low sensitivity of zinc by atomic fluorescence was caused by the low xenon lamp intensity in this spectral region.

The determination of zinc in the fertilizer samples

Table XXXIX

Analysis of Calcium in Chemtrol Standard Blood Samples

	Atomic Absorption Value	Atomic Fluorescence Value
True value 80 ppm	66 ppm	67
% deviation	17.5%	16.3%
RSD	2.3%	9.8%
Comparison of Precisions	$F(AF,AA) = (9.8)^2/(2.3)^2 = 18.4$ $F(10,6)$ at .95 = 4.06	
Sensitivity	7.2 $(ppm)^{-1}$	3.3 $(ppm)^{-1}$
Comparison of Sensitivities at 95% level	$K(AF/AA) = 2.5$	
Detection Limit at .95 level	0.4 ppm	0.7 ppm

Table XL

Analysis of Metal Constituents in Fertilizer Samples - Part I

Element true value	AF value % deviation	AA value % deviation	AF Relative Standard Deviation	AA Relative Standard Deviation	Comparison of Precisions
Cu	5.4 ppm	5.5 ppm	11%	6.5%	$\frac{AF}{AA} = 2.7$
5.46 ppm	1.1%	0.7%			F(.95) = 3.44
Fe	37 ppm	40 ppm	12%	9.6%	$\frac{AF}{AA} = 1.6$
41.04 ppm	9.9%	2.5%			F(.95) = 4.15
Zn	23 ppm	23 ppm	8.7%	4.2%	$\frac{AF}{AA} = 4.2$
23.29 ppm	1.2%	1.2%			F(.95) = 3.58
Mn	17 ppm	17 ppm	12%	3.8%	$\frac{AF}{AA} = 10$
17.18 ppm	1.0%	1.0%			F(.95) = 4.15
Mg	1.4×10^2 ppm	1.4×10^2 ppm	6.7%	4.2%	$\frac{AF}{AA} = 2.5$
138 ppm	1.4%	1.4%			F(.95) = 4.28
Ca	1.2×10^3 ppm	1.2×10^3 ppm	6.4%	8.4%	$\frac{AA}{AF} = 1.8$
1225 ppm	2.0%	2.0%			F(.95) = 4.28

Table XLI

Analysis of Metal Constituents in Fertilizer - Part II

Element	AF Sensitivity	AA Sensitivity	Comparison of Sensitivities, K 95% level	AF Detection Limit 95% level	AA Detection Limit 95% level
Cu	1.4 $(ppm)^{-1}$	3.0 $(ppm)^{-1}$	$\frac{AA}{AF} = 2.1$	1.8 ppm	0.9 ppm
Fe	0.47 $(ppm)^{-1}$	0.73 $(ppm)^{-1}$	$\frac{AA}{AF} = 1.6$	5.5 ppm	3.8 ppm
Zn	0.057 $(ppm)^{-1}$	19.4 $(ppm)^{-1}$	$\frac{AA}{AF} = 310$	48 ppm	0.1 ppm
Mn	5.4 $(ppm)^{-1}$	5.0 $(ppm)^{-1}$	$\frac{AF}{AA} = 1.0$	0.4 ppm	0.5 ppm
Mg	18.1 $(ppm)^{-1}$	20.4 $(ppm)^{-1}$	$\frac{AA}{AF} = 1.1$	0.1 ppm	0.1 ppm
Ca	4.8 $(ppm)^{-1}$	3.5 $(ppm)^{-1}$	$\frac{AF}{AA} = 1.4$	0.5 ppm	0.7 ppm

was the only example of a striking difference between the two techniques. One need only examine the data (Tables XVIII and XXX) to get an idea of the very low value for the slope of the zinc working curve when compared with any other determination. For the other five determinations, it was felt that difference between the sensitivities encountered were not great enough to warrant special comment.

It should be pointed out that all of these determinations using atomic absorption and atomic fluorescence were made at the lowest attenuation which the instrument permitted, and no change of the attenuation was allowed during a given experimental run.

Along with the comparisons which have been already mentioned, less quantitative considerations were given to other areas of comparison between the two methods. The areas of comparison and the comments relating to them are stated below.

STABILITY

It would be convenient to be able to achieve long-term stability of the calibration data. Such stability might make it possible to run only one calibration point once a reliable slope had been established, or better yet, if the data reproduced itself perfectly, perhaps only one working curve would suffice for a relatively long period of time.

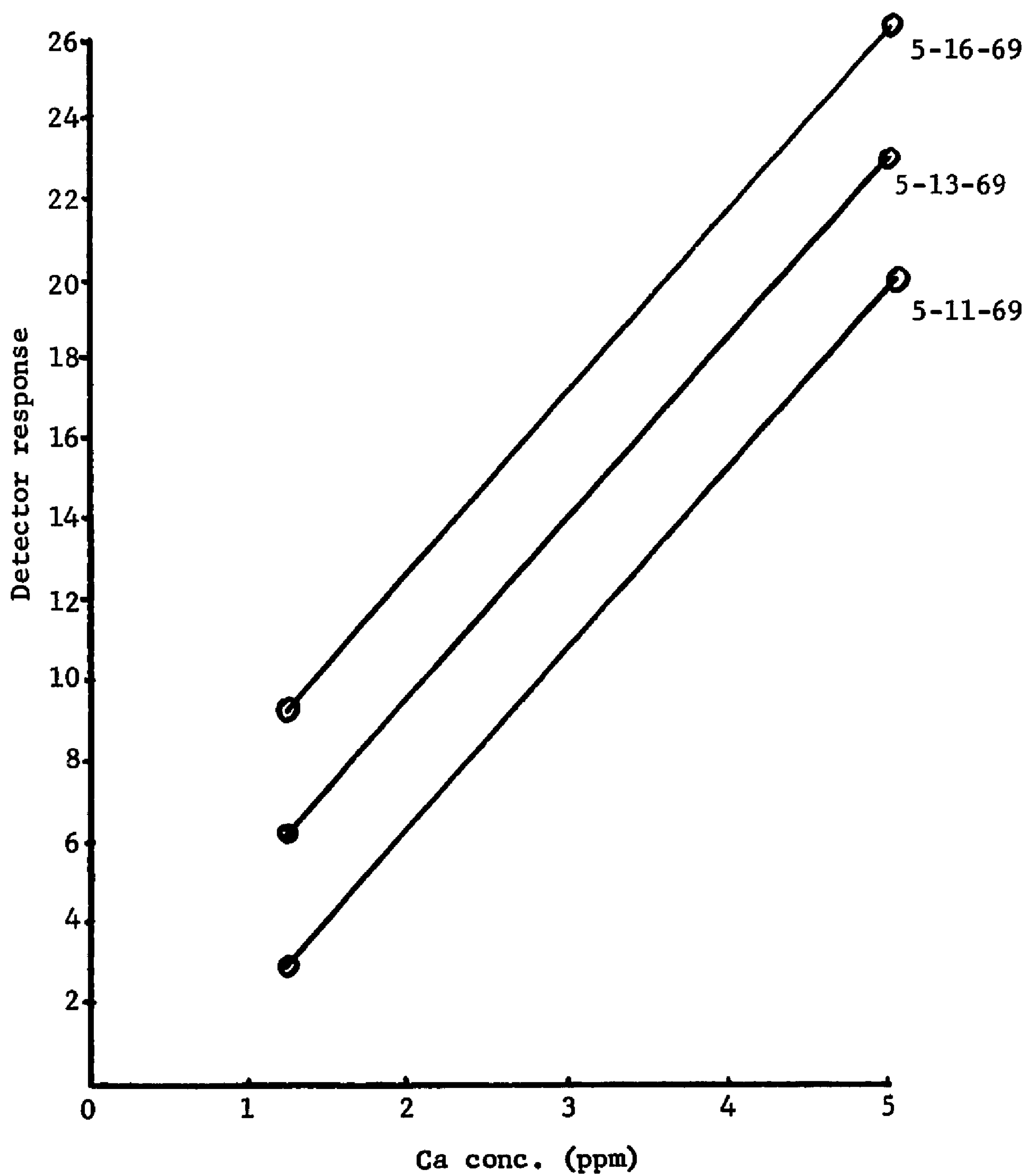
Figure 3 shows graphically the data obtained from calcium calibration curves using atomic fluorescence spectrometry over a one-week interval. It is obvious that, although the slopes were quite constant, the position of the working curve was susceptible to shifts in a vertical direction.

To check the stability of the slope for a working curve, magnesium working curves were compared from determinations four months apart and for determinations one month apart. It was noted that the values for the slope of the Mg working curve varied from a value of 28.00 to one of 25.50 over the four-month span, and the variation of the slope over a one-month span was from a value of 25.50 to one of 26.00. The conclusion reached was that the slope did not remain sufficiently constant to justify the omission of obtaining a working curve each time a determination was made. This result, therefore, cut down the feasibility of achieving a multi-element determination by atomic fluorescence spectrometry using a xenon source in a fairly short space of time.

Because of the difficulty of setting the hollow cathode current at the same value for each run, and also the difficulty in "retuning" the system to the most sensitive

Figure 3

Reproducibility of Calcium Calibration Curve
Atomic Fluorescence Spectrometry



wavelength setting, it was decided that a stability check on the atomic absorption equipment was not feasible, and no such studies were made. It was assumed that a working curve must be re-established for each determination.

ANALYSIS TIME

The length of time for a typical determination for a single element by atomic absorption and atomic fluorescence spectrometry using a xenon arc lamp was noted. It was felt that this information would be of interest from a convenience standpoint. It was found that on the average, the length of time for a 14 solution determination by atomic fluorescence flame spectrometry was approximately 50 minutes or about 3.5 minutes per sample run, while the length of time for the same determination by atomic absorption spectrometry was about 30 minutes, or about 2 minutes per sample run. The difference in time between the two methods may be attributed chiefly to the cleaning and rinsing steps made necessary in the syringe-drive mechanism used in the atomic fluorescence determinations.

MULTI-ELEMENT ANALYSIS CONSIDERATIONS

A topic of interest in this work was the feasibility of carrying out a complete analysis for six metals in one fertilizer sample all in one determination by atomic fluorescence flame spectrometry. It was felt that the ability of the atomic fluorescence equipment to scan all wavelengths might make this technique valuable.

Based on all of the considerations already presented, it is felt that a multi-element analysis is practical depending on the reliability desired in the results. For a

preliminary survey of quantitative content, such a multi-element determination would be quite practical. However, if quantitative results are desired, the above two considerations of stability and time per analysis indicate that a multi-element determination is apt to become quite lengthy in terms of total time expended.

For example, if one adopts the quite-acceptable technique of using two known concentrations to fit the working curve for the determination of a given metal, it is necessary to work with three solutions. Two of these solutions are needed to establish the working curve for the analysis of the third solution, the unknown solution. In order to achieve even a minimum of reliability in the working curve, it is felt that the two known concentrations should be run at least four times each to establish at least six degrees of freedom in the working curve. If the sample is also run four times, this makes a total of twelve runs for the determination. At the average time of 3.5 minutes per sample, it would require about 42 minutes for this one determination. If a total of six ions were studied, this would require 6 x 42 minutes, or about four and one-half hours.

SAMPLE VOLUME

Certainly an important consideration is the amount of sample volume which is necessary for an analysis to be made. The average results from all of the experiments performed indicated that the volume preferred for a four replicate sample determination was about 20 ml, or about 5 ml per sample, in atomic absorption spectrometry, and about 35 ml, or about 8 ml per sample, in atomic fluorescence spectrometry.

The scanning technique used along with the cleaning and rinsing of the syringe in the atomic fluorescence analyses were the two main reasons why a greater volume was required in the atomic fluorescence work as compared with the atomic absorption analyses.

TREATMENT OF DATA

For a statistical manipulation of the experimental data such as was performed in this work, it was found that there was no difference between the atomic fluorescence and atomic absorption techniques.

EXPENSE OF EQUIPMENT

This area of comparison was believed to be not especially significant in the comparison of atomic absorption and atomic fluorescence spectrometry. It is estimated that adequate equipment involving either technique may be obtained for about $7500.

SUMMARY

The work performed has indicated that in actual analytical determinations, the techniques of atomic absorption flame spectrometry and atomic fluorescence flame spectrometry using a 450 watt xenon arc source are similar in terms of accuracy, precision, and sensitivity. It is not possible from this work to recommend the newer technique of atomic fluorescence flame spectrometry as being superior to the more established atomic absorption flame spectrometry. In fact, in a few cases, the technique of atomic absorption spectrometry was significantly better than atomic fluorescence flame spectrometry.

The work has also indicated that a xenon arc lamp in atomic fluorescence flame spectrometry gave detection limits which were comparable to those obtained in atomic absorption flame spectrometry. In difficult cases, such as analyses at detection -limit concentrations, the direct-readout nature of the atomic fluorescence signal could prove advantageous over the difference signal which is obtained from atomic absorption work. In these cases, the use of more intense line sources in atomic fluorescence flame spectrometry, rather than the continuum source used in this work would be expected to achieve a lower detection limit for atomic fluorescence flame spectrometry over atomic absorption flame spectrometry.

BIBLIOGRAPHY

1. West, T. S., Spectroscopy Letters, 1, (12), 397 (1968).

2. Winefordner, J. D., and Vickers, T. S., Anal. Chem., 36, 161 (1964).

3. Mitchell, A. C. G., and Zemansky, M. W., "Resonance Radiation and Excited Atoms", Cambridge University Press, Cambridge, 1961.

4. Winefordner, J. D., McCarthy, W. J., and St. John, P. A., J. Chem. Educ., 44, (2), 80 (1967).

5. West, T. S., in "Trace Characterization, Chemical and Physical", Meinke, W., and Scribner, B. F., Ed., National Bureau of Standards, Monograph 100, 1967.

6. Winefordner, J. D., and Mansfield, J. M. in "Fluorescence, Theory, Instrumentation, and Practice", Guibalt, G. G., Marcel Dekker, Inc., New York, 1967, Chapter 13.

7. Winefordner, J. D., and Staab, R. A., Anal. Chem., 36, 165 (1964).

8. Winefordner, J. D., and Staab, R. A., Anal. Chem., 36, 1367 (1964).

9. Mansfield, J. M., Winefordner, J. D., and Veillon, C., Anal. Chem., 37, 1049 (1965).

10. Veillon, C., Mansfield, J. M., Parsons, M. L., and Winefordner, J. D., Anal. Chem., 38, 204 (1966).

11. Dagnall, R. M., West, T. S., and Young, P., Talanta, 13, 803 (1966).

12. Armentrout, D. N., Anal. Chem., 38, 1235 (1966).

13. Goodfellow, G. I., Anal. Chem. Acta, 35, 132 (1966).

14. Dagnall, R. M., Thompson, K. C., and West, T. S., Anal. Chem. Acta, 36, 269 (1966).

15. Ellis, D. W., and Demers, D. R., Anal. Chem., 38, 1943 (1966).

16. West, T. S., Chem. and Ind., June 18, 1966, pp. 1005.

17. West, T. S., Analyst, 91, 1079 (1966).

18. West, T. S., and Dagnall, R. M., Applied Optics, 7, 1287 (1968).

19. West, T. S., Dagnall, R. M., and Thompson, K. C., Talanta, 15, (7), 677 (1968).

20. West, T. S., Dagnall, R. M., and Browner, R. F., Talanta, 16, (1), 75 (1969).

21. Demers, D. R., and Ellis, D. W., Anal. Chem., 40, 860 (1968).

22. Walsh, A., Spectrochim Acta, 7, 108 (1955).

23. Robinson, J., "Atomic Absorption Spectroscopy", Marcel Dekker, Inc., New York, 1966.

24. Slavin, W., "Atomic Absorption Spectroscopy", John Wiley and Sons, Inc., New York, 1968.

25. Clark, E. P., and Collip, J. B., J. Biol. Chem., 63, 461 (1925).

26. Kramer, B., and Tisdall, F. F., J. Biol. Chem., 47, 475 (1921).

27. MacIntyre, I., J. Biochem., 67, 164 (1957).

28. Humoller, F. L., and Walsh, J. R., J. Lab. Clin. Med., 48, 127 (1956).

29. Powell, F. J. N., J. Clin Pathol., 6, 286 (1953).

30. Rothe, C. F., and Sapirstein, L. A., Am. J. Clin. Pathol., 25, 1076 (1955).

31. Winer, A. D., and Kufins, D. M., Am. J. Clin. Pathol., 23, 1259 (1953).

32. Chen, P. S., and Toribava, T. Y., Anal. Chem., 25, 1642 (1953).

33. Teloh, H. A., "Clinical Flame Photometry", Charles C. Thomas, Springfield, Illinois, 1959, pp 76-77.

34. Teloh, H. A., A. M. A. Arch. Pathol., 66, 474 (1958).

35. Willis, J. B., Spectrochim. Acta, 16 259 (1960).

36. Willis, J. B., Anal. Chem., 33, 556 (1961).

37. Zettner, A., and Seligson, D., Clin. Chem., 10, (10), 869 (1964).

38. Allan, J. E., Spectrochim. Acta, 17, 467 (1961).

39. Allan, J. E., Spectrochim. Acta, 10, 800 (1959).

40. Allan, J. E. Analyst, 86, 530 (1961).

41. David, D. J.Analyst, 83, 655 (1958).

42. McBride, C. H., Jour. of the A.O.A.C., 48 (2), 406 (1965).

43. Slavin, W., "Applications of Optical Spectroscopy to Analytical Biochemistry and Toxiology", Occupational Health Review, October, 1966.

44. Box, G. P. E., Biometrics, 10, 16 (1954).

45. Cellier, K. M., and Stace, H. C. T., Applied Spectroscopy, 20 (1), 26 (1966).

46. Natrella, M. G., "Experimental Statistics", National Bureau of Standards, Handbook 91, October 1966.

47. Mandell, J. and Stiehler, R. D., J. Res. Natl. Bur. Std., A53, 155 (1964).

48. Wentworth-Douglass Hospital, Dover, New Hampshire.

49. Clinton Laboratories, 1255 S. Cochran Avenue, Los Angeles, California, 90019.

50. Davies, O. L., "Design and Analysis of Industrial Experiments", Hafner Publishing Co., N. Y., 1963, Chapter 11.

51. Margruder Fertilizer Check Sample Service, P. O. Box 1558, Atlanta, Georgia, 30301.

52. Volk, W., "Applied Statistics for Engineers", McGraw-Hill, N. Y., 1958, Chapter 6.

53. Milne, W. E., "Numerical Calculus", Princeton University Press, New Jersey.

54. Winefordner, J. D., and de Galen, L., Anal. Chem., 38, 1412 (1966).

55. Fassel, V. A., and Golightly, D. W., Anal. Chem., 39, 467 (1967).

56. Oser, B. L., Ed., "Hawk's Physiological Chemistry", McGraw-Hill, N. Y., Fourteenth edition, pp 1029.

57. Skogerboe, R. K., Heybey, A. T., and Morrison, G. H., Anal. Chem., 38, 1821 (1966).

58. Ellis, D. W., presented in part at the Tenth Eastern Analytical Symposium, New York, Nov. 1968.

59. Massman, H., Spectrochim. Acta, 23B, 215 (1968).

60. Grant, C. L., taken in part from classroom lecture material, University of New Hampshire, 1966.

APPENDIX I

Optimization Studies of a Response Surface[60]

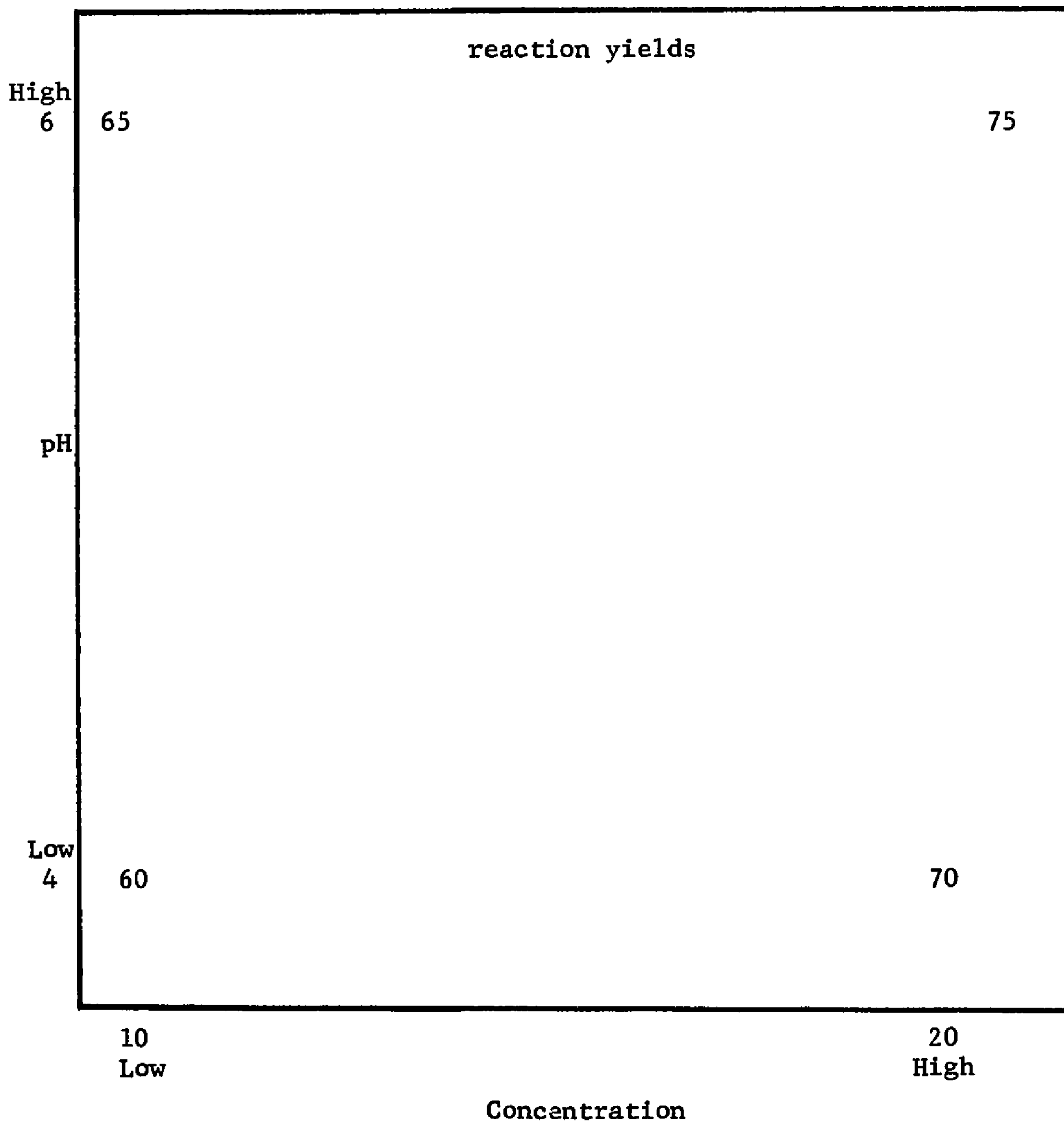
In any work of this kind, the assumption is made that a dependent variable, such as a recorder signal, is functionally related to a group of quantitatively independent variables. Examples of these may be temperature, pressure, or as in this case, concentration, burner height, organic content, slit width, etc. In this work the problem was to obtain the best possible fluorescence signal to noise ratio for a given metal. There were a number of variables that might possibly influence the recorder signal. It was necessary, then, to first decide which variables were the most important ones, and secondly, to place each variable at a setting so that the total overall effect would produce the best recorder signal possible.

A technique which encompassed both of these stated aims was that of factorially designed experiments. These factorial experiments were used for screening variables for their relative importance on the signal response, and were also helpful in achieving an optimization of the variables on the response surface. This appendix will, therefore, be concerned with so-called 2^n factorials, where n represents the number of variables to be studied, and 2 represents the number of settings to be used for each of the variables. Thus, for each variable, there will be a so-called "high" and a "low" setting.

At this point it might be helpful to illustrate what is meant by the term interaction. Figure 4 represents a situation where an experiment was performed. In this

Figure 4

Effect of Concentration and pH on Reaction Yield



experiment the yield of a reaction was influenced by a concentration change and also by a change in the pH of the solution.

A common sense analysis of the reaction yields indicated:

1) the effect of the concentration change from the low to the high setting was:

$$\text{Concentration Effect} = \frac{(70 - 60) + (75 - 65)}{2}$$

$$= \frac{(70 + 75)}{2} - \frac{(60 + 65)}{2}$$

$$= +10$$

2) the effect of the pH change from the low to the high setting was:

$$\text{pH effect} = \frac{(75 + 65)}{2} - \frac{(70 - 60)}{2} = +5$$

From these results one could predict, beginning at the Low Concentration-Low pH setting of a yield of 60, that an increase from Low to High Concentration would result in a yield of 60 + (+10) or 70, and then that starting at 70, an increase from Low pH to High pH would result in a yield of 75. In the same way, going from High Concentration to Low Concentration would result in a yield of 75 + (-10) or 65 units, and finally from High pH to Low pH the yield value would return to 60.

The reason this common sense approach worked in this example is that there were no interaction effects present; another way to state this is that the reaction surface generated by the yield values was planar; it had no hills of

unusually high yields or valleys of unusually low yields. The interaction effect was calculated in this example by looking at the diagonal effects in the response surface.

The effect of interaction was $= \frac{(75 + 60)}{2} - \frac{(70 + 65)}{2}$

$= 0$

Figure 5 represents another experimental set of results. Note that in this case, the values of the two variables X_1 and X_2 have been coded: That is, their low setting has been represented by -1 and their high setting by +1. This method of notation is quite convenient and lends itself nicely to later more complicated manipulations.

Then the effect of X_1 was $= \frac{(90 + 66)}{2} - \frac{(70 + 60)}{2} = +13$

and the effect of X_2 was $= \frac{(90 + 70)}{2} - \frac{(66 + 60)}{2} = +17$

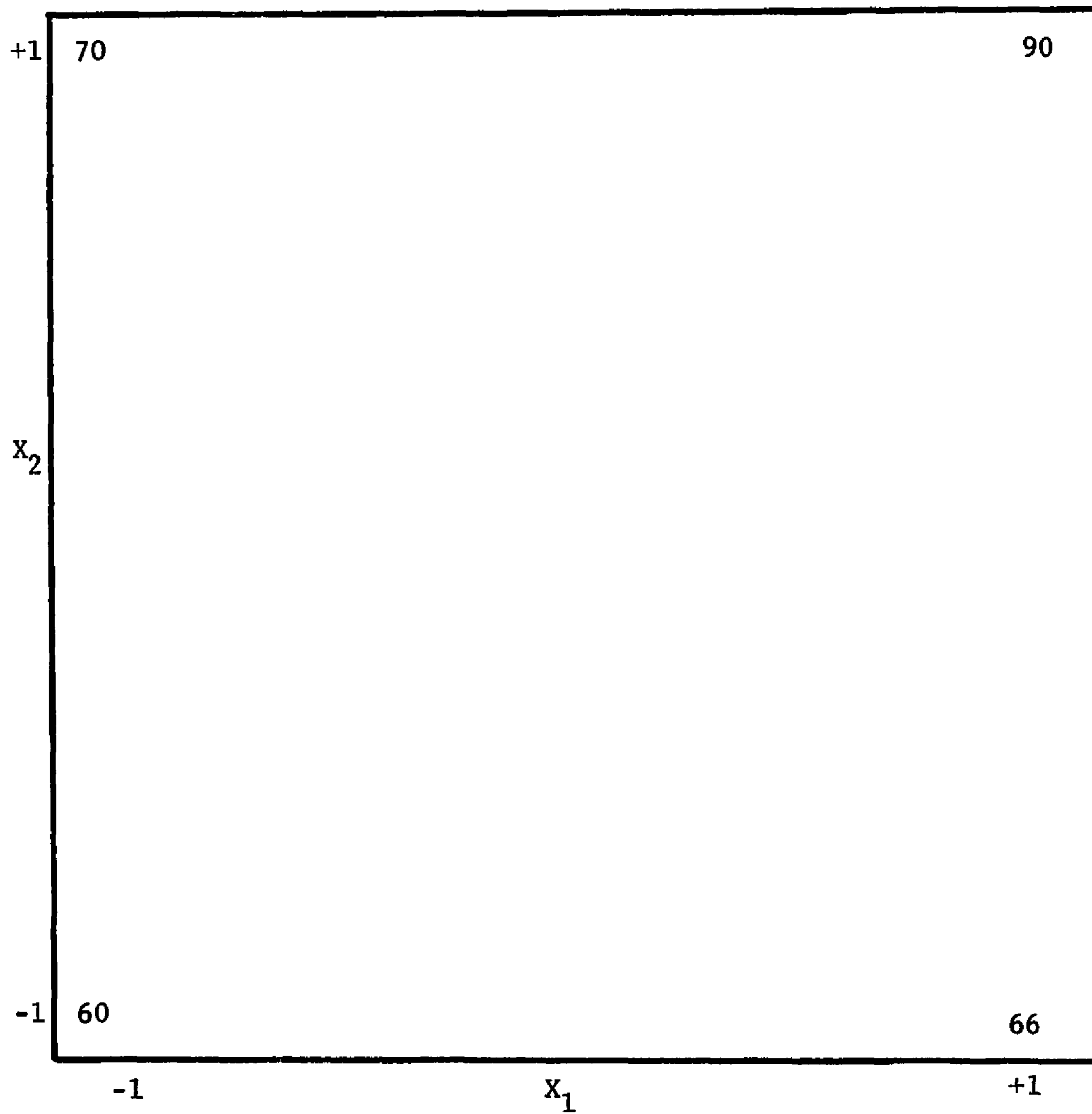
If the yield values, starting from (-1,-1), were predicted as before, a value of 73 at the position (+1,-1) would be obtained rather than the experimental value of 66; similarly, a predicted value of 77 would be obtained at the position (-1,+1) instead of the actual experimental value of 70. There would be a seven unit discrepancy between the predicted and experimental values, too high in one case and too low in the other case. The reason for this discrepancy lay in the interaction term.

The interaction effect was $= \frac{(90 + 60)}{2} - \frac{(70 + 66)}{2} = +7$

This example has indicated that it is not always possible to predict yield values correctly by employing a one-variable-at-a-time approach. The common sense separation of variable effects is applicable only to response surfaces which are planar. As there is usually no way beforehand of

Figure 5

Effect of Variables X_1 and X_2 on the Reaction Yield, Y



knowing whether or not the response surface is planar or not, the possibility of interaction of the variables must be considered and investigated. The simplifying assumption is usually made that the only interactions of physical significance are two-factor interactions, interactions involving only two experimental parameters. Thus, three and higher-order interactions are usually considered physically unrealistic and are omitted from consideration.

Using the concept of coding the high and low positions of the variable settings, it might be instructive to design a factorial experiment for the assessment of variable importance. As a simple example, the Group I variables will be considered (See Table I). There were two variables in this group, the positioning of the lamp on the flame profile, which was called variable A, and the distance of the burner from the monochromator slit, which was called variable B. There were, then, 2^2 or 4 possible permutations of the permitted variable settings, and all of these possible combinations of the variables were investigated. A scoreboard called a design matrix was set up (See Table II) to keep track of the various combinations and also to record the detector response, or Y value, which was experimentally obtained upon setting the variables at the settings prescribed in the design matrix. Thus, for example, from Table II, the low position of variable A and the low position of variable B yielded a recorder response of 18 units the first time this position was run and a response of 19 units the next time this position was run. The reason for duplicate runs at the same setting was to obtain a measure of the random error inherent in the system itself.

In a similar fashion the rest of the design matrix was filled in with experimental results. It should be stated that the numerical order in which the design matrix was filled in with experimental data was obtained in a random fashion. This randomizing of the experimental runs was a necessary criterion to insure that any difference obtained in duplicate measurements could be attributed to random non-systematic error in the system rather than some systematic variance of a variable, or some unknown condition, not being controlled during the course of the experiment.

One final point must be mentioned about the design matrix in Table II. It is noted that in the matrix, at the bottom of each vertical contrast column, are to be found two zeros. These zeros represent the experimental positioning of a so-called center point, the experimental datum obtained by positioning each variable midway between its high and low settings. This center point is helpful in giving a glimpse of the response surface at the center of the design.

Once the design matrix has been completely filled in with experimental data, the next step is to evaluate the results. Two simple formulae are needed. Applicable to all factorial experiments is the fact that the magnitude of any effect, i, $= 2 \sum X_i Y / \sum X_i^2$

Also, the sum of squares for any effect, i, is equal to $(\sum X_i Y)^2 / \sum X_i^2$. Thus, the effect of variable A calculated from Table II was:

$$\sum AY = (-1)(18) + (-1)(19) + (+1)(14) + (+1)(16) + \cdots + (0)(22) + (0)(20) = -34$$

$$\Sigma A^2 = (-1)^2 + (-1)^2 + (+1)^2 + (+1)^2 + \cdots + (0)^2 + (0)^2 = 8$$

Then the effect of A was equal to $\frac{(2)(-34)}{8} = -8.500$

and the sum of squares associated with the change of variable A from its low to its high setting was $\frac{(-34)^2}{8} = 144.500$

For variable B the effect turned out to be +6.000, and the sum of squares equalled 72.000.

To calculate the interaction effect and sum of squares, it is easiest to set up a contrast column for the Interaction AB term. It may be noted that the entries in the Interaction AB contrast column are obtained from the product of the A and B columns. The interaction effect and sum of squares term is calculated just like those of the main effects A and B.

The final column left unexplained is the Lack of Fit column. This column represents a comparison of the center point in the design matrix with the peripheral points. In a factorial experiment, if a planar response surface is assumed, then the center point should yield experimental data corresponding to values halfway between those of the low and high values. The lack of fit measurement then measures the deviation of the response surface from a planar surface.

Once all of the sums of squares data and variable effects have been calculated, the decision making step is reached. This decision making process is usually achieved by an Analysis of Variance Table (See Table III). An Analysis of Variance Table works in the following way: the first value obtained is the Total Sum of Squares value, Σy^2. The total sum of squares value is a measure of the total variability of the system. The next entry in the table is

a Correction Factor, $(\Sigma Y)^2/n$, where n equals the total number of experimental points in the experiment. The Correction Factor gets its name from the fact that it corrects the experimental values to a mean of zero. Thus the difference between the Total Sum of Squares and the sum of squares associated with the Correction Factor is known as the Total Corrected Sum of Squares. This Total Corrected Sum of Squares represents the variation in the system caused by random error and by the effect of having changed the variable conditions. Bear in mind that the purpose of this table is to be able to isolate the effect of having changed a given variable and to compare this effect with a measure of the random error in the experiment. Thus the entries in the Analysis of Variance Table are Variable A, Variable B, Interaction AB, Lack of Fit, and their corresponding sum of square values. The Error Sum of Squares may be obtained from the variation in the duplicate measurements.

The variance associated with a variable may be compared with that of the random error in the experiment. This is accomplished by comparing the Mean Square values (the sum of Squares/degree of freedom value) for the variable and error by means of the calculation of a so-called F ratio. For a variable to have a significant effect, its F ratio value must be larger than the tabled F value, which has been calculated on the basis of purely random deviation for the number of degrees of freedom involved. Thus, in Table III, variable A gave an F ratio of 13.63 compared to an F value at 95% confidence of 6.61 and a 99% confidence level of 16.26. Thus, at the 95% probability level, there is sufficient evidence to reject the hypothesis that the

variables exerted no influence. However, the hypothesis cannot be rejected at the 99% probability level. For the Lack of Fit measurement, the F value was about 0.2; thus it is concluded that there was a very low probability that the response surface was not planar; it was concluded that the response surface was planar. In a similar fashion the lateral distance from the monochromator slit, Variable B, had a significant effect on the response signal as it was varied from its low to high setting. The magnitude of the sum of squares value associated with Variable B implies, however, that Variable B did not exert as large an influence on the response as did Variable A.

It is possible to write an equation for the response surface from the data. Since the coded difference between the high and low settings in the design matrix is always equal to a value of 2, one-half of the magnitude of the effect term is used as the coefficient of the polynomial equation which fits the data. The intercept of the equation is the mean of the measurements.

Thus, for the Group I variables,

$$Y = 20.00 - 4.75A + 3.00B$$

The largest value of Y from this equation was obtained when $A = -1$, $B = +1$, in agreement with the experimental data. The value predicted from the equation was 27.75 against an experimental value of 29.50. Since the Lack of Fit term was not significant, the difference between the experimental and predicted values was attributed to random experimental error.

The above example illustrated the procedure which was followed in the optimization of the variables associated with the atomic fluorescence equipment. No attempt will be made to discuss the Group II optimization. It is the same in

principle as the Group I and Group III optimizations; however, dealing with five variables, the work involved becomes more complicated, in theory and in the laboratory. The book by Davies[50] is recommended if an understanding of more complex optimization procedures is desired. This appendix has attempted to present only a basic understanding of the principles involved in optimization studies.

APPENDIX II

Fitting and Testing of Working Curves[60]

The whole intention of this work has been concerned with the determination of the content of a given metallic element in some matrix. Thus the fitting of a working curve and the subsequent use of this curve for the analysis of an unknown sample is a very important process. It was decided to use a least squares model as the vehicle whereby a polynomial equation might be generated which would satisfy the experimental data. The fitting of the least squares model is a purely mathematical problem, and the method employed, the so-called Forward Dolittle Loop, is not discussed here. The interested reader may learn about the mechanism of this technique from Milne's book.[53] However, the determination of the adequacy of the least squares model chosen to fit the data is a statistical problem, and this aspect of the determination of the proper working curve is the topic of interest.

Consider a typical fitting of a working curve and its subsequent analysis. Table VIII gives the raw data which were taken in the establishment of a calcium working curve and the use of this same curve for the analysis of the calcium content of the Chemtrol sample. All fourteen pieces of data, the Y values, were obtained in a random fashion. This was done, as in the optimization studies, to insure statistical independence of errors.

When the actual choice of a polynomial equation for use to fit the collected data was considered, it was natural to select a linear relationship between the recorder response

and the calcium concentration. This relationship, of course, was predicted by earlier theoretical considerations of atomic fluorescence spectrometry. Thus was postulated an equation of the type,

$$Y = b_o + b_1 x$$

where x = concentration of calcium solution

y = detector response

b_o = intercept

b_1 = slope

Such an equation is called a regression equation, and b_o and b_1 are called regression coefficients. From theoretical considerations, it has been stated that the so-called "normal" equations can be employed to evaluate the regression coefficients. The normal equations for a linear model are

$$nb_o + b_1 \sum x = \sum Y$$

$$b_o \sum x + b_1 \sum x^2 = \sum xY$$

where n = the total number of points in the experiment which were used to fit the working curve; in the case of Table VIII, n = 12.

Then, from Table VIII, the value $\sum x = 27.0$

$\sum x^2 = 69.5$

$\sum Y = 211.0$

$\sum xY = 524.5$

thus,

$$12b_o + 27b_1 = 211.0$$

$$27b_o + 69.5b_1 = 524.5$$

Solving the equation, $b_o = 4.79$ and $b_1 = 5.69$. The equation, $Y = 4.79 + 5.69x$, then gave the best straight line that described the data. The next step was to decide whether or not another model was needed. For this the Regression

Analysis Table (RAT) shown in Table IX was used.

The Regression Analysis Table is similar in its function to an Analysis of Variance Table. The so-called RAT table is used in the following way: The first entry in the table is the Total Sum of Squares. As in the Analysis of Variance Table, the total sum of squares value represents a measure of the total variability in the system. The next entry, b_o, is analagous to the correction factor in an Analysis of Variance Table. The sum of squares associated with b_o corrects for the fact that the mean value of the Y values is not zero. The difference between the Total Sum of Squares and the b_o Sum of Squares is analagous to the Total Corrected Sum of Squares. The only things that are left in the system after the b_o term has been removed are the random error and the causal relationships that require any additional regression coefficients. The Error Sum of Squares is obtained from replicate measurements. A regression coefficient is then fitted and checked to see if this coefficient is adequate to explain the sum of squares relating to causal relationships; if one coefficient is not satisfactory to explain the causal relationship sum of squares, more coefficients are calculated as necessary.

Thus, in Table IX, after the sum of squares associated with the intercept b_o, the error, and the slope b_1 had been tabulated, the difference between the sum of these values and the Total Sum of Squares was a value of 8.563. This value represented the lack of fit of the experimental data with the polynomial which had been chosen to represent that data. What was done next were two things: 1) the F ratio was calculated for the slope and a decision was made regarding its significance; 2) the F ratio for the Lack of Fit was calculated and

a decision was made regarding the significance of the Lack of Fit in terms of the random error in the system. From Table IX, the slope b_1 was clearly significant while the Lack of Fit value for the F ratio did not approach the F value at the 95% decision-limit level. Thus the linear equation

$$Y = 4.79 + 5.69x$$

represented the experimental data adequately, and this equation was used to calculate the concentration of the 30:1 diluted unknown sample. For Y = 17.5, the average of the experimental runs, x had a value equal to 2.2, implying that the original sample contained (2.2)(30) or 66 ppm calcium.