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Title: The Determination of Zinc in Plant Material by Atomic Absorption Spectroscopy: A Comparison with the Accepted AOAC Colorimetric Method

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A method for determining zinc in plant material by atomic absorption spectroscopy has been developed. The proposed method utilizes .1 N HCl-99% methanol as the solvent system and is consistent with the requirements for easy sample preparation and for the determination of other trace metals in the same solution. The accuracy of the results obtained by the proposed method was compared to the results obtained for identical samples as determined by the official colorimetric method of the Association of Official Analytical Chemists. The results obtained by each method compared favorably. The proposed AAS method yielded a sensitivity of 12 ppb as compared to 38 ppb for the AOAC method. The time of analysis by the proposed AAS method is significantly shorter than that required for the AOAC colorimetric method.

The Determination of Zinc in Plant Material by Atomic Absorption Spectroscopy: A Comparison with the Accepted AOAC Colorimetric Method

A Thesis

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To Bobbie

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INTRODUCTION

The determination of trace elements in plant material requires a technique that is rapid and accurate. The small amounts of metals present in this type of matrix necessitate a method that is sensitive to concentrations in the microgram or nanogram per gram range. A method for the determination of several metals in a solution of one ashed sample is highly desirable. Atomic absorption spectroscopy (AAS) is an analysis technique that meets these requirements.

The purpose of this study is to develop a method for the determination of zinc in plant material, using a procedure that is consistent with the requirements of sample preparation for the determination of other trace metals in the same sample. The accuracy of the method is evaluated by a comparison of the results obtained by the experimental atomic absorption method with those obtained for identical samples determined by the official colorimetric method of the Association of Official Analytical Chemists.

Since its advent as an analytical method in 1955,[28,32] AAS has become a powerful tool for the determination of trace metals in many types of material. It has been used in a variety of areas including pharmaceutics, forensic analysis,[6] clinical pathology,[22] medicine and the chemical industry. The main reasons for this broad usage are the short time required for analysis, the simplicity of operation, and the sensitivity of the method. Another big advantage of AAS is the relative freedom from spectral interferences.

Approximately seventy elements can be determined using AAS, including both metals and nonmetals. Primary application of AAS is the determination of metals, however, this method lacks the sensitivity to

detect some metals at ultra trace levels. Researchers have tried numerous ways to achieve greater signal enhancement.

The first techniques which were used successfully in enhancing sensitivity were instrumental modifications of the basic flame spectrometer. These included the slotted quartz tube[26] and the long-path flame absorption cell.[18] As inefficient as the flame atomizer may be, it is still the most practical and commonly used method of introducing the sample into the radiation path. Other atomizers, including the L'vov furnace, Graphite Tube Furnace, Tantalum strip, and the Mini-Massman atomizer,[27,33] have been used to enhance the sensitivity of AAS. Electrically heated systems are not as practical as flame atomization because they are more expensive and require background correction. They are, however, methods of obtaining very good sensitivity and worthy of further study.

Experimentalists have tried many chemical means of enhancing the sensitivity obtained by AAS determinations. A common method is the chelation of the metal ion followed by extraction into an organic solvent, and aspiration into a flame. A commonly used chelating agent is ammonium pyrillodine dithiocarbamate (APDC). The metal complex formed is extracted into a suitable solvent. One of the most popular solvents is methyl iso-butyl ketone (MIBK). The accuracy of the APDC-MIBK method[24] is limited by the efficiency of the extraction and loss of analyte during the separation.

A mixture of an organic solvent and water has been shown to provide an increase in sensitivity.[1,19] Studies have been performed using a large variety of organic compounds, and the classes of compounds found most suitable were ketones, esters, and alcohols. The sensitivity enhancement caused by these compounds is a thoroughly studied subject and

the exact explanation for this phenomena is still subject to controversy.

LITERATURE REVIEW

Zinc is an essential trace element needed for growth and health. Over a century ago Raulin first demonstrated that Aspergillus niger required zinc for growth.[8] Since then the role of zinc has been extensively studied and found to be important for many reasons. While essential for life, at higher concentrations it is as toxic as other heavy metals.

Zinc has been found to be tightly bound in 17 enzymes and has been indicated in 58 other enzymes requiring zinc for their metabolic functions.[30] The first metalloenzyme discovered was carbonic anhydrase, a zinc metalloenzyme. Since then the importance of zinc enzymes in the biochemistry of the leukaemic process has been reinforced by the discovery of zinc in the reverse transcriptases from murina leukaemia. Zinc has also been implicated as a cofactor for the synthesis of nucleic acids and for DNA polymerizations. Zinc may also be required for the synthesis of proteins and RNA.[17]

Plasma-zinc levels have been thoroughly studied in healthy and diseased patients with some interesting results. Plasma-zinc is one of the most uniform biochemical characteristics of normal adult blood, with little variation found due to sex or age. The mean level is 92 micrograms zinc per 100 ml serum in humans.[4] Abnormally low concentrations of zinc were observed in alcoholic cirrhosis, other types of liver diseases, active tuberculosis, indolent ulcers, myocardial infarcts, Down's syndrome, cystic fibrosis with growth retardation, and in carcinoma of the broncus.[8] There is evidence that certain cases of nutritional dwarfism in man are associated with zinc deficiency.[15] The administration of zinc reverses this process. Rats with prenatal

deficiency of zinc were found to be more aggressive and to affiliate less than their normal counterparts.[25] No conditions have been observed involving a higher than normal plasma zinc concentration.[29]

There have been many methods devised for the determination of zinc. The most commonly used involve the use of colorimetric, spectrophotometric or electrochemical techniques.

The most reliable colorimetric method for zinc determination among the many available ones is the dithizone method. The procedure consists of complexing an aqueous solution of zinc with dithizone in CCl_4. Other interfering elements are masked with various masking agents and zinc is then re-extracted into a solution of CCl_4 and diethyldithiocarbamate (NaDDC). The absorbance of the solution is measured at 525 nm.

2-Carboxy-2'-hydroxy-5'-sulphoformazylbenzene, also called zincon, is another common reagent used for the spectrophotometric determination of zinc in plant materials. At pH 9, zincon forms a blue complex with zinc at λ_{max} = 625 nm with a molar absorptivity coefficient of 2.0 X 10^4. A large number of metals interfere making pre-separation necessary. A popular method for this pre-separation is anionic ion-exchange chromatography.

1-(2-Pyridylazo)-2-napthol (PAN) produces a red chelate with zinc, extractable into chloroform. The molar absorptivity coefficient is 5.2 X 10^4 at 550 nm. PAN has been adopted for determining zinc in the presence of copper. Among other azo reagents the following are suggested for zinc determination: 4-(2-pyridylazo)-resorcinol (PAR) [a_m = 9.2 X 10^4, 505 nm]; 5-nitrophenol-(2-azo-1')-2'-(β-acetyl-hydrazino)-naphthalene (NAAN) [a_m = 3.8 X 10^4, 646 nm]; and 1-(5-chloro-2-pyridylazo)-2-naphthol (5-Cl-PAN) [a_m = 8.4 X 10^4, 564 nm].[9,20]

There are several other instrumental methods available for the determination of zinc but only four popular methods will be discussed: polarography, X-ray fluorescence (XRF), neutron activation analysis (NAA), and atomic absorption spectroscopy (AAS). Each method has distinct advantages and disadvantages.

When the results obtained by polarographic methods are compared to those obtained by AAS, the results are in agreement. A selection of the method to be used must be based upon the time required for the analysis and freedom from possible sources of contamination or interference. Analysis by AAS is superior in both of these respects.[7]

X-ray fluorescence and neutron activation analysis do not require sample dissolution or chemical pre-treatment, thus minimizing the chance of loss of any component and probability of contamination. Interferences due to some matrix interferences occur in both methods, adversely affecting the accuracy of the results. The major limitation of X-ray fluorescence is the sensitivity for trace content at the ppm level. The standard addition method must be used which is time consuming and inconvenient for routine analysis. In neutron activation analysis, the level of ^{46}Sc in the sample must be low due to interference from the photopeak used in the determination of zinc.[12]

Atomic absorption spectroscopy yields precise results but experimental values seem to be lower than those obtained with other methods, possibly due to volatilization of zinc during the ashing process.[2] This is one of the major drawbacks in the AAS method. The analysis of zinc by AAS has been found to be dependent upon the pH of the solution being analyzed. The signal obtained is constant from pH 1-5 but starts decreasing until a pH of 10 where it stabilizes again.[11] During the dissolution process, contamination can occur if trace impurities are present

in the reagents used, or loss of zinc can occur. The major advantage of AAS over all the previously mentioned methods is the ability to rapidly determine zinc at ppm concentrations. The determination of zinc in varying types of plant material by AAS has been performed by many researchers and this paper is not the first but rather an extension of that work.[2,5,7,10,12]

Other methods are presently being studied to detect heavy metal chelates. One such method is the separation of volatile heavy metal chelates by gas chromatography and determination of the metal with flame AAS. This method has some promising possibilities.[35] Another method is the chelation of heavy metals with diethyldithiocarbamate and separation with high-performance liquid chromatography. This method shows potential use in the field of analytical chemistry because simultaneous micro-determinations of various metals can be performed in a short time.[23] The detection of zinc, however, was not reported using either method.

THEORY

Atomic absorption spectroscopy (AAS) is an analytical method based upon the measurement of a decrease in the intensity of monochromatic radiation from an external source as it passes through a medium, usually a flame, containing free ground state metal atoms. The free metal atoms may be excited to a higher energy electronic state by the absorption of radiation corresponding to the exact energy difference between the electronic ground state and a higher energy excited state. The most probable transition is from the ground state to the lowest energy excited state. The wavelength of radiation corresponding to this transition is known as the resonance line.

The amount of radiation absorbed by the neutral atoms is governed by several physical factors. The absorption is given by the following equation:

$$\int_{\infty}^{0} K\nu d\nu = \left(\frac{\pi e^2}{mc}\right) Nf$$

where:

$\int_{\infty}^{0} K\nu d\nu$ = total amount of radiation absorbed at frequency

e = charge on the electron

m = mass of the electron

c = speed of light

N = total number of atoms in the path

f = oscillator strength

The amount of radiation absorbed depends upon a number of constants, the band width of the incident radiation, the number of atoms in the radiation path, and the oscillator strength. The oscillator strength is the probability of an electronic transition between the ground and excited states--the greater the probability, the greater the oscillator strength.

In order to obtain reproducible data, it is necessary to control both the efficiency of producing atoms and the rate at which they are lost. Once this is accomplished the number of free atoms in the flame remains constant for a given concentration of sample. The actual number of atoms formed is equal to the number of atoms in the sample times the efficiency of atomization.[27] Modifications in the method or instrumentation used in AAS that affect the atomization efficiency are worthy of study.

Before one can appreciate the factors affecting atomization efficiency, a basic understanding of the many processes occurring simultaneously in the flame is necessary.

The first step in the atomic absorption process is the aspiration of a sample through the nebulizer where it breaks into droplets of various sizes. In a premix burner the sample is mixed with the fuel and oxidant gases in the premix chamber. The larger drops go down the drain and the smaller droplets pass into the flame. Once in the flame, the solvent is evaporated leaving a small solid particle called a clotlet which is then vaporized by the thermal energy provided by the flame. The vaporized salt dissociates and the metal ion formed is reduced by electrons in the flame. The radiation passing through the flame is absorbed by these neutral metal atoms.[21]

The rate of aspiration has been found to be important in AAS. A hyperbolic relationship has been found to exist between aspiration rate and nebulization efficiency. Nebulization efficiency, defined as the amount of solution actually passing into the flame divided by the total flow rate, is decreased by increasing aspiration rate. Therefore, in any system the optimum aspiration rate must be established experimentally.

Nebulization is an important parameter in the atomic absorption process. The turbulently flowing solvent is broken into drops of varying sizes with smaller droplets passing into the flame where they are vaporized. Here the primary effect of organic solvents is most pronounced. Szivos and co-workers have measured the nebulization efficiency and report 3.6 and 12.4 percent nebulization for water and methanol respectively. The explanation for this noticeable increase in nebulization is thought to be due to smaller average diameter of the methanol drop.

Nebulization, and therefore atomization efficiency, is increased when organic solvents are used. Most researchers attribute the sensitivity enhancement to decrease in drop size with the volume of aerosol reaching the flame remaining constant. For methanol a 3.9 times increase in sensitivity relative to water is found with the average measured drop diameter of methanol being 11 μm. The value calculated using the Nukiyama-Tanasawa equation is 12.2 μm with the average diameter of water droplets being 20 μm. This amounts to a 50% decrease in average drop size which is indeed significant.[31]

The reduction in average drop size observed in organic solvents may be explained by a reduction in surface tension, viscosity or density.[34] The most noticeable effect would certainly be the decrease of the surface tension of the solvent. If a sample has a high surface tension, larger drops are formed, whereas, if the surface tension is low then smaller drops are more probable. Less energy is required to evaporate the solvent from smaller drops, and smaller salt clotlets are formed. Along with the decrease in energetics, decreasing the amount of time for evaporation is also crucial for the production of free atoms. The production of smaller clotlets decreases the time needed for vaporization of the sample. The

less time used in evaporating the solvent and vaporizing the solid particles, the greater the probability of formation of neutral atoms which can absorb radiation passing through the flame from the hollow cathode lamp. The reduction in average drop size is the most important factor in the enhancement observed when organic solvents are used.

The aspiration of an aqueous solution into a flame will cause a decrease in the flame temperature of about 600°C. This drop in temperature decreases the probability of complete dissociation of the analyte salts. The aspiration of a solution of water and an organic compound will decrease the flame temperature less than is observed when water is the solvent. The difference in flame temperature was once thought to be the source of enhancement obtained by using mixtures of water and miscible organic compounds for the analysis matrix. It has been shown that the flame profiles of neutral atoms is not related to flame temperature profiles.[14] This indicates that the difference in chemical environments within the flame is more important to the formation of free metal atoms than is the flame temperature.

The aspiration of an organic solvent into a flame causes a fuel rich environment. The reducing power of this type of flame is greater than that of a stoichiometric flame. This helps reduce the formation of metal oxides or other intermediates and favors the formation of neutral atoms. The introduction of reducing components into the flame is an advantage of the use of organic solvents. Metal atoms are produced by reaction of the salt with common flame constituents. C, C_2, CH, CO, and H have been suggested as the species responsible for atom production in the acetylene-air flame.

The emission profile of C_2 radicals indicates that this species is confined to the lower region of the flame, approximately to the limits of the inner cone. The emission profile of CH radicals shows this radical

is present in the primary reaction zone. The atomic profile does not correspond to either of these profiles. CH and C radicals have theoretical reducing powers greater than that shown by the air-acetylene flame. CO and H_2 are weak reducing agents at the temperature of the flame and cannot be considered as the species responsible for reduction. However, C_2 and H radicals have approximately the same reducing power as is observed for the acetylene-air flame. Experimental evidence indicates the H radical is the agent responsible for atom formation in most flames.

The degree of atomization based on calculations assuming reduction by H radicals involving fast binary reactions more closely match the observed values than those obtained from calculations assuming dissociation equilibrium. The H radical profile resembles the atom profile rather than C_2 and CH atom profiles. Certainly this is a complex system with many reactions occurring simultaneously, all of which contribute to the formation of free metal atoms.[13]

The increase in sensitivity obtained in atomic absorption atomization efficiency by use of organic solvents is experimentally evident. Many complex factors enter into the overall process. The signal enhancement obtained using organic solvents is most dramatic in the nebulization step. The increased sensitivity cannot be entirely explained by the reduction in drop size although this is the major contributing factor. A more reducing atmosphere is produced, but this does not appear to be as significant as the reduction in drop size. A lesser temperature decrease could have some effect, but it is not the most important factor. A combination of factors contribute to the sensitivity enhancement attributed to the use of organic solvents in atomic absorption spectroscopy.

EXPERIMENTAL PARAMETERS

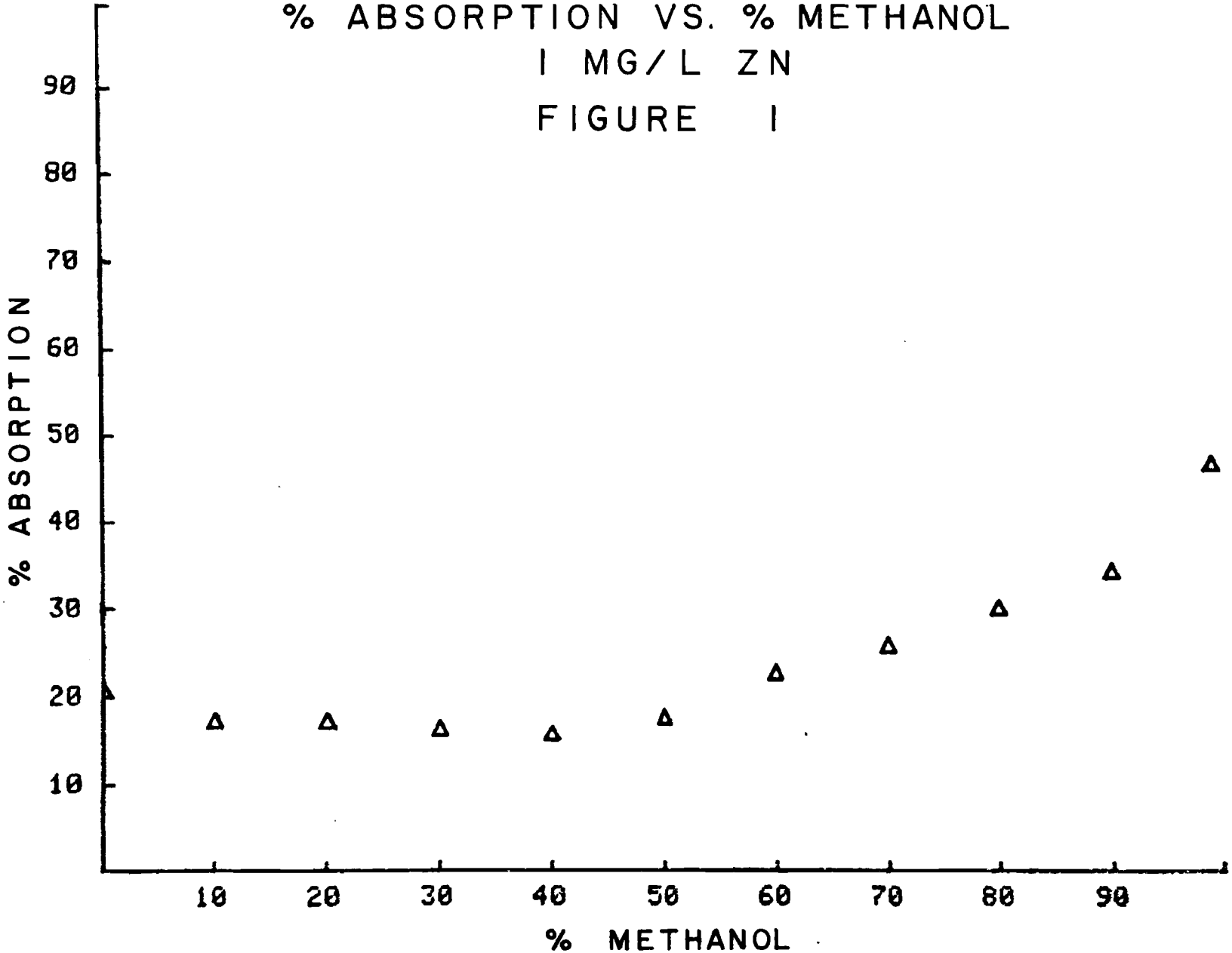
1. Percentage Methanol

The selection of a solvent is important in the preparation of a sample for AAS determination. An organic solvent can be used to increase the sensitivity of the determination. The optimum mixture of the organic solvent, methanol, used in this study was determined by preparing solutions containing equal concentrations of zinc but varying ratios of methanol to water. These solutions were aspirated into a flame and the percentage absorption was recorded. The results obtained are shown in Figure 1. The percentage absorption observed remains essentially constant for mixtures containing from 0-50% V/V methanol. This is probably due to the participation of the water molecules in the surface tension phenomena. If the percentage of methanol is increased above 50%, the percentage absorption begins increasing exponentially, with the greatest percentage absorption observed using a solution containing 99% methanol. Thus, 99% methanol and 1% water was selected as the optimum mixture of organic solvent for this study.

Methanol is a convenient solvent to use for AAS. It is infinitely miscible with water, thereby avoiding interfaces with water at which zinc might be lost, and preparation of solutions, both standards and unknown samples is relatively easy. The analysis standards prepared and used in the study were found to be stable up to six months.

2. Fuel Flow Rate

The adjustment of the proper fuel to oxidant ratio is important to obtain the maximum sensitivity and minimize chemical interferences in the flame. The optimum ratio of fuel to oxidant is determined experimentally

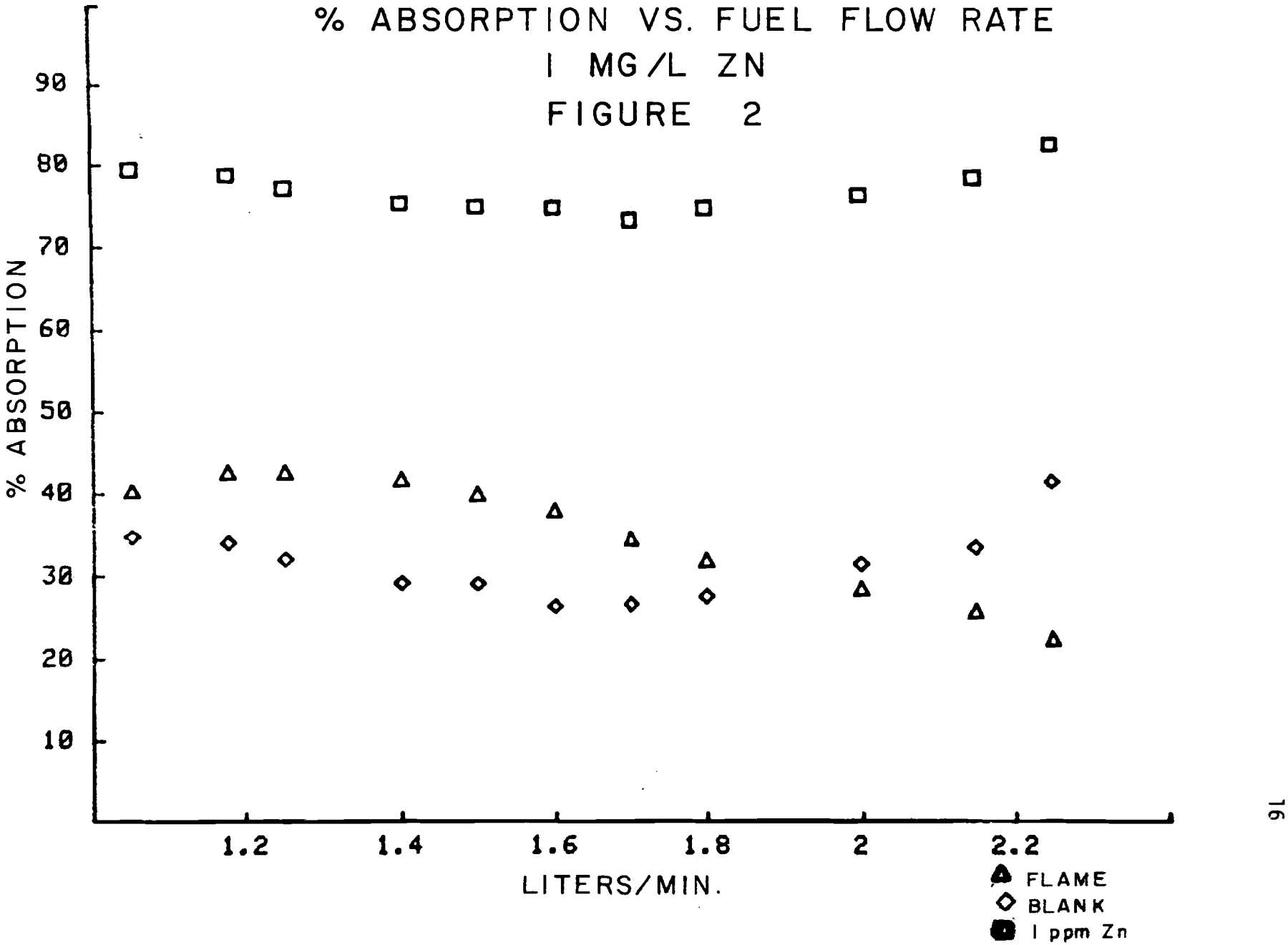

% ABSORPTION VS. % METHANOL
1 MG/L ZN
FIGURE 1
% ABSORPTION
90
80
70
60
50
40
30
20
10
10
20
30
40
50
60
70
80
90
% METHANOL

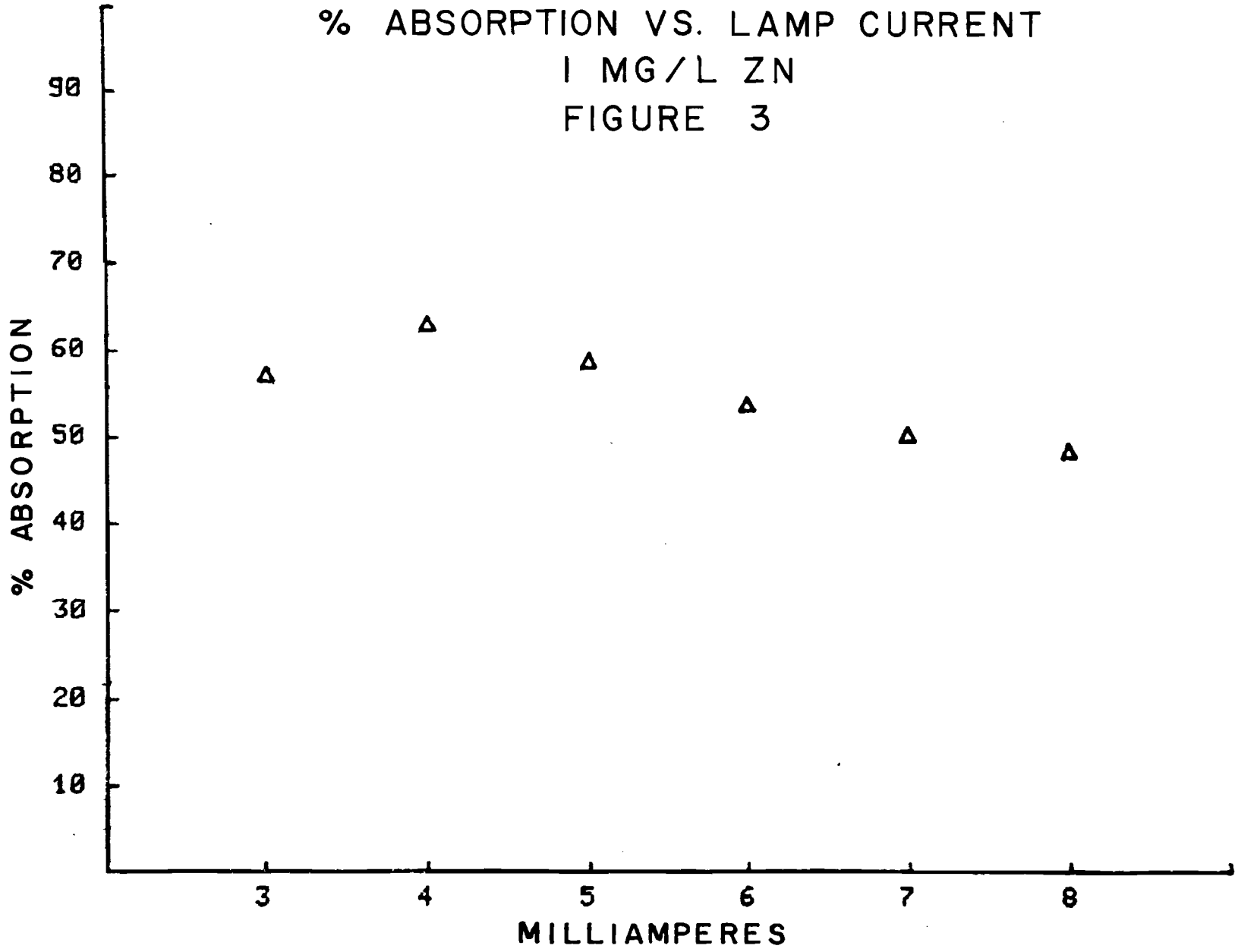
by recording the percentage absorption observed using the flame only and that obtained while aspirating a blank as the fuel flow rate is varied while maintaining the oxidant flow rate constant at 5.2 liters/minute. A plot of percentage absorption vs. fuel flow rate is shown in Figure 2. The point at which flame only and 99% methanol blank curves intersect indicates a fuel to oxidant ratio where the flame and blank have the same percentage absorption. At this setting the background due to the flame scatter or absorption is minimal. The sensitivity is also found to be maximum at this fuel to oxidant ratio. The point of intersection occurs when a fuel flow rate of 1.8 liters/minute is used. This produces a slightly fuel rich flame. Some authors used fuel rich conditions[4,5] and others used fuel lean conditions[10,24] depending on the matrix of the sample.

3. Hollow Cathode Lamp Current

The establishment of the optimum hollow cathode lamp current can be of utmost importance if reliable results and maximum sensitivity are to be obtained in atomic absorption analysis. The procedure is simple, although somewhat time consuming. The lamp current is varied by 1 milliampere and the percentage absorption recorded while aspirating a 1 ppm solution of zinc. The lamp must have time to re-equilibrate with a minimum time period of twenty minutes between trials. All other parameters must be maintained constant.

The most desirable lamp current is obtained when the signal output is constant and maximum sensitivity is obtained. Figure 3 shows a maximum response is obtained at 4 milliamperes and then decreases at higher current settings. If one chooses a lamp current too high, the possibility of self-absorption by radiation from the lamp becomes a


% ABSORPTION VS. FUEL FLOW RATE
1 MG/L ZN
FIGURE 2
% ABSORPTION
90
80
70
60
50
40
30
20
10
1.2
1.4
1.6
1.8
2
2.2
LITERS/MIN.
FLAME
BLANK
1 ppm Zn


% ABSORPTION VS. LAMP CURRENT
1 MG/L ZN
FIGURE 3
% ABSORPTION
90
80
70
60
50
40
30
20
10
3
4
5
6
7
8
MILLIAMPERES

factor. A hollow cathode lamp current of 5.5 milliamperes was selected as the optimum lamp current for this study.

4. Burner Height

The distance from the top of the burner to the center of the incident beam of radiation is an important variable to be considered in the selection of optimum conditions to be used for an element. There are several factors which enter into the selection of the optimum burner height.

One important consideration is the residence time of atoms in the flame. The main objective is to find the position in the flame where the most neutral atoms are present for the longest time. This will be the region of greatest sensitivity.

Another major factor is the effect of flame scatter and absorption upon the establishment of a reproducible baseline. For zinc, absorption obtained while aspirating a solution of 1 ppm zinc is relatively independent of burner height, but the stability of the signal does change. As can be seen in Figure 4, the absorption did not change considerably above 3 mm. The interference from the flame decreased above 3 mm. A burner height of 5 mm was selected to be used for the determination of zinc.

The optimum instrument parameters for the determination of zinc in a 99% methanol solvent are summarized in Table 1.

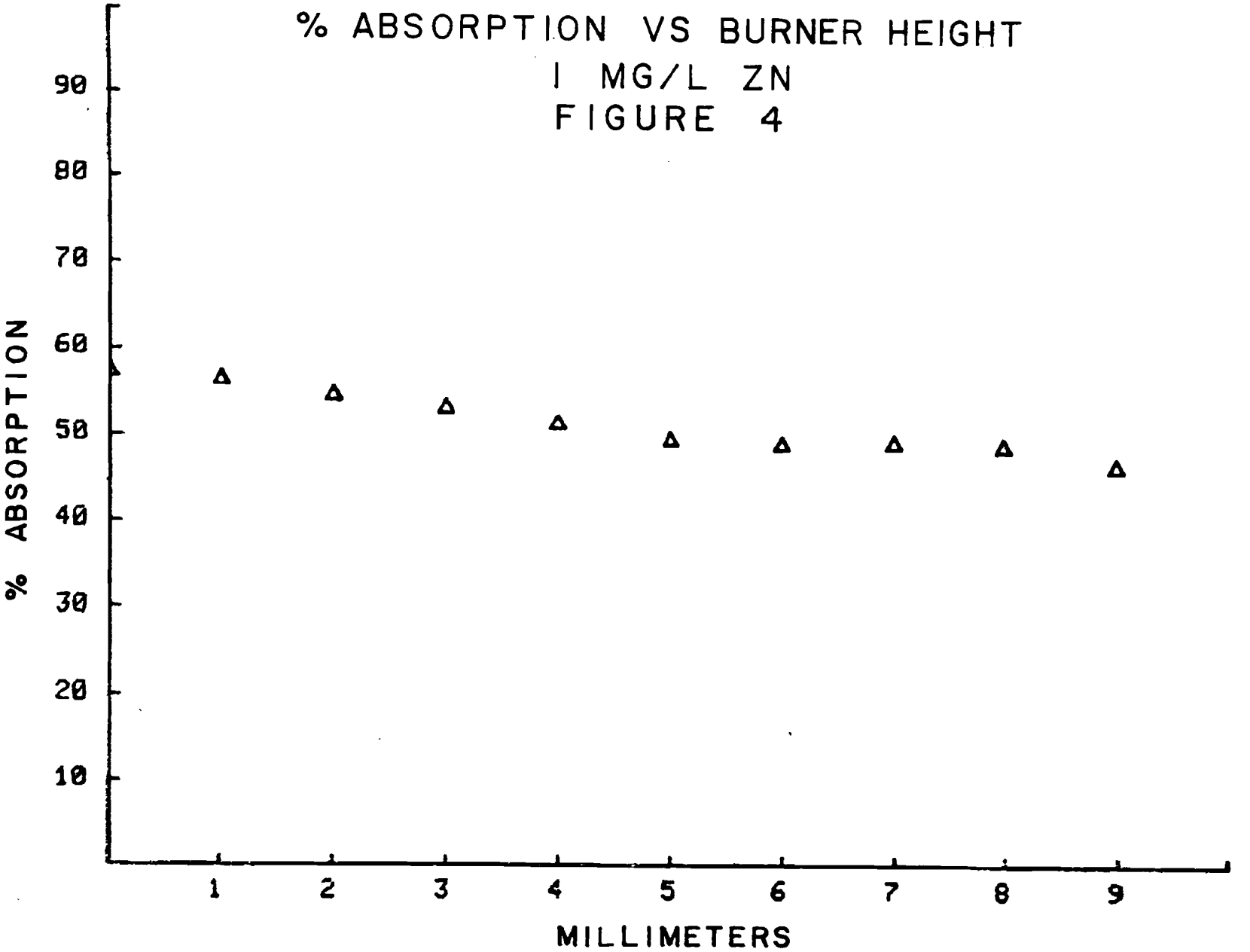

% ABSORPTION VS BURNER HEIGHT
1 MG/L ZN
FIGURE 4
% ABSORPTION
90
80
70
60
50
40
30
20
10
1
2
3
4
5
6
7
8
9
MILLIMETERS

TABLE 1

Optimum Conditions for the Determination of Zinc in a 0.1N HCl-99% Methanol Solvent

Wavelength	213.7 nm
Burner Height	5.0 mm
Lamp Current	5.5 ma
Fuel Flow Rate	1.8 l/min
Oxidant Flow Rate	5.2 l/min

EXPERIMENTAL

Throughout the study a Jarrell-Ash Model 82-500 Atomic Absorption Spectrometer equipped with a premix laminar flow 10 cm slot burner was used. The instrument had a 0.5 meter Ebert mount monochromator with a grating blazed at 250 nm and a R-213 photomultiplier tube. The instrument was connected to a Fisher Series 8000 Recorder. Gilmont #3 Flow Meters were used to monitor the fuel and oxidant flow rates. A single element zinc hollow cathode lamp supplied by Fisher Scientific Company was used as the external source of radiation.

A 1.000 gm sample of reagent grade zinc supplied by Fisher Scientific Company was dissolved in a minimal amount of concentrated HCl and diluted to one liter with distilled-deionized water to obtain a 1000 ppm solution of zinc. A 100 ppm zinc solution was prepared by diluting 10 ml of 1000 ppm zinc standard to 100 ml. Before preparation of standards and samples, all glassware was cleaned in hot aqua regia and then rinsed with copious amounts of distilled-deionized water.

Analysis standards with concentrations of 0.1, 0.2, 0.5, 1.0, 2.0, and 5.0 ppm zinc in .1N HCl-99% methanol were prepared as follows. The appropriate amount of stock solution (e.g., 0.2 ml of 1000 ppm for 2 ppm) was added to a 100 ml volumetric flask and a corresponding amount of deionized water (e.g., 0.8 ml for 2 ppm) was added to bring the total volume of aqueous solution to 1 ml. Methanol was then added to dilute to volume and the flask shaken until no reduction of volume occurred. This produced an analysis standard that was in 99% methanol by volume. This process was repeated for each of the analysis standards.

A .1N HCl-99% methanol blank solution was prepared by adding 8.33 ml of concentrated HCl and 1.67 ml deionized water and diluting to a

total volume of 1000 ml with methanol. This solution was used in working up the zinc into solution from the plant samples and also as a blank when performing the actual analysis by AAS.

There were three different types of plant material used in this study. Wheat, a cereal grain, in which only the kernel was used; soybeans, a hairy annual Asiatic legume, in which only the bean was used; and alfalfa, a deep-rooted European leguminous plant, in which the whole plant was used. All plant matter was ground to a powder to facilitate ashing.

Approximately 2 grams of plant material was weighed into 30 ml Vycor crucibles and dry ashed overnight at 500°C. The crucibles were allowed to cool after removal from the oven and 10 ml of 1.0 N HCl was added. Each crucible was evaporated to near dryness on a hot plate. The crucibles were then cooled and 10 ml of .1N HCl-99% methanol was added. The samples were filtered through Whatman 41 filter paper previously washed with hot .1N HCl-99% methanol solution into 25 ml volumetric flasks. These were diluted to volume with the .1N HCl-99% methanol blank solution. All analyses were performed at 213.7 nm on the Jarrell-Ash atomic absorption spectrometer.

A Beckman Model DB-G Spectrophotometer was employed for the Association of Official Analytical Chemists colorimetric determination. The maximum wavelength used in the analysis was 525 nm and matching quartz cuvettes were used for consistency. All glassware was treated with hot aqua regia to insure dissolution of all trace metals before any solutions were prepared.

Several reagents needed to be prepared for the AOAC analysis and are listed below.

1. Carbon tetrachloride
2. Zinc standard solutions
3. 1 N ammonium hydroxide
4. 1 N hydrochloric acid
5. Diphenylthiocarbazone (dithizone) solution
6. 0.5 M ammonium citrate solution
7. Carbamate solution
8. 0.02 N hydrochloric acid

Following are the procedures used in the preparation of the above solutions.

1. ACS grade carbon tetrachloride may be used without further purification.
2. A zinc standard solution with a concentration of 1000 ppm was prepared as mentioned earlier. A working solution of 10 µg/ml was made by diluting 10 ml of stock solution to 1 liter.
3. A 1 N NH_4OH solution was prepared from a concentrated solution of NH_4OH by diluting 67 ml of concentrated NH_4OH to 1 liter with deionized water.
4. A 1 N HCl solution was prepared by diluting 83.3 ml of concentrated HCl to 1 liter.
5. A solution of 200 mg of dithizone dissolved in 500 ml of CCl_4 was filtered to remove all insoluble matter. This solution was transferred to a 2 liter separatory funnel and 2 liters of 0.02 N NH_4OH (40 ml of 1 N NH_4OH diluted to 2 liters) was added. The solution was shaken to extract the dithizone into the aqueous phase. The phases

were separated and the aqueous phase was discarded. The CCl_4 was diluted to 2 liters with clear CCl_4 and transferred to a clean dry brown bottle.

6. A solution of 226 grams of $(NH_4)_2HC_6H_5O_7$ dissolved in 2 liters of deionized water was prepared. Between 80 and 85 ml of concentrated NH_4OH was added until the solution achieved a pH of 8.5-8.7. A CCl_4 solution containing excess dithizone was added and extracted with 100 ml portions of clear CCl_4 until the extract was green. The layers were allowed to separate and the aqueous phase was stored in a Pyrex vessel.
7. The carbamate solution was prepared by dissolving 250 mg of sodium diethyldithiocarbamate (NaDDC) in a 100 ml volumetric flask and diluting to volume. This solution was refrigerated in a Pyrex vessel and freshly prepared every two weeks.
8. To prepare the 0.02 N HCl solution 100 ml of 1 N HCl was diluted to 5 liters.

In order to avoid errors due to the variation of compositions of the solutions and to reduce measuring out reagents, two solutions were prepared from the above solutions.

Solution A -- 1 liter of 0.5 M NH_4 citrate and 140 ml of 1 N NH_4OH were diluted to 4 liters.

Solution B -- 1 liter of 0.5 M NH_4 citrate and 300 ml of 1 N NH_4OH were diluted to 4.5 liters. Just prior to using, 1 volume of carbamate solution was added to 9 volumes of the NH_3-NH_4 citrate solution to obtain the volume of solution B required.

Approximately 15 grams of plant material was dried in a 110°C oven for two hours, allowed to cool and stored in a desiccator. Two to 2.5 grams of the finely ground plant material was weighed into Vycor crucibles and ashed at 500-550°C overnight in an electric muffle furnace. The sample was moistened with a little water and 10 ml of 1 N HCl added with subsequent heating on a hot plate until all substances soluble in the HCl were dissolved. Five milliliters of hot water was added and all insoluble material was filtered out with 7 cm Whatman No. 41 filter paper previously washing with 5 ml of hot 1 N HCl and then rinsed with hot water until the washings were no longer acidic to methyl red. All filtrates were collected in 50 ml volumetric flasks and one drop of methyl red was added to the filtrate. This pink solution was neutralized with 1 N NH_4OH to produce a yellow solution. The solution was then brought to pink again by the addition of 4 ml of 1 N HCl and diluted to volume.

The analysis standards were prepared by adding 0, 5, 10, 20, and 30 ml of the 10 μg/ml working solution to 100 ml volumetric flasks. One drop of methyl red was added to each flask and neutralized with 1 N NH_4OH. Four milliliters of 1 N HCl was added and the solutions were diluted to volume. From this point on all samples and standards were treated the same throughout the analysis.

1. First Extraction

A 10 ml aliquot of the sample solution was pipetted into a 125 ml Squibb separatory funnel. Forty milliliters of solution A and 10 ml of dithizone solution were added and the mixture was shaken for 30 seconds. The shaking must be done by hand to insure adequate mixing. At this point the zinc and other dithizone-complex forming metals were extracted into the CCl_4 layer. An excess of dithizone was present as signified by

the yellow-orange color of the aqueous phase and must be present to make sure that all metals that form complexes with dithizone have done so. The drop of CCl_4 extract on the surface of the aqueous layer was shaken down into the CCl_4 layer and the total CCl_4 layer was drained into a second separatory funnel. Care was taken so as not to let any of the aqueous phase enter the stopcock bore. Five milliliters of clear CCl_4 was added to the first separatory funnel and shaken for 30 seconds. The layers were allowed to separate and the CCl_4 layer was drained into the second separatory funnel. This was done until the CCl_4 layer contained no green color. This usually required three rinses. The aqueous phase was then discarded.

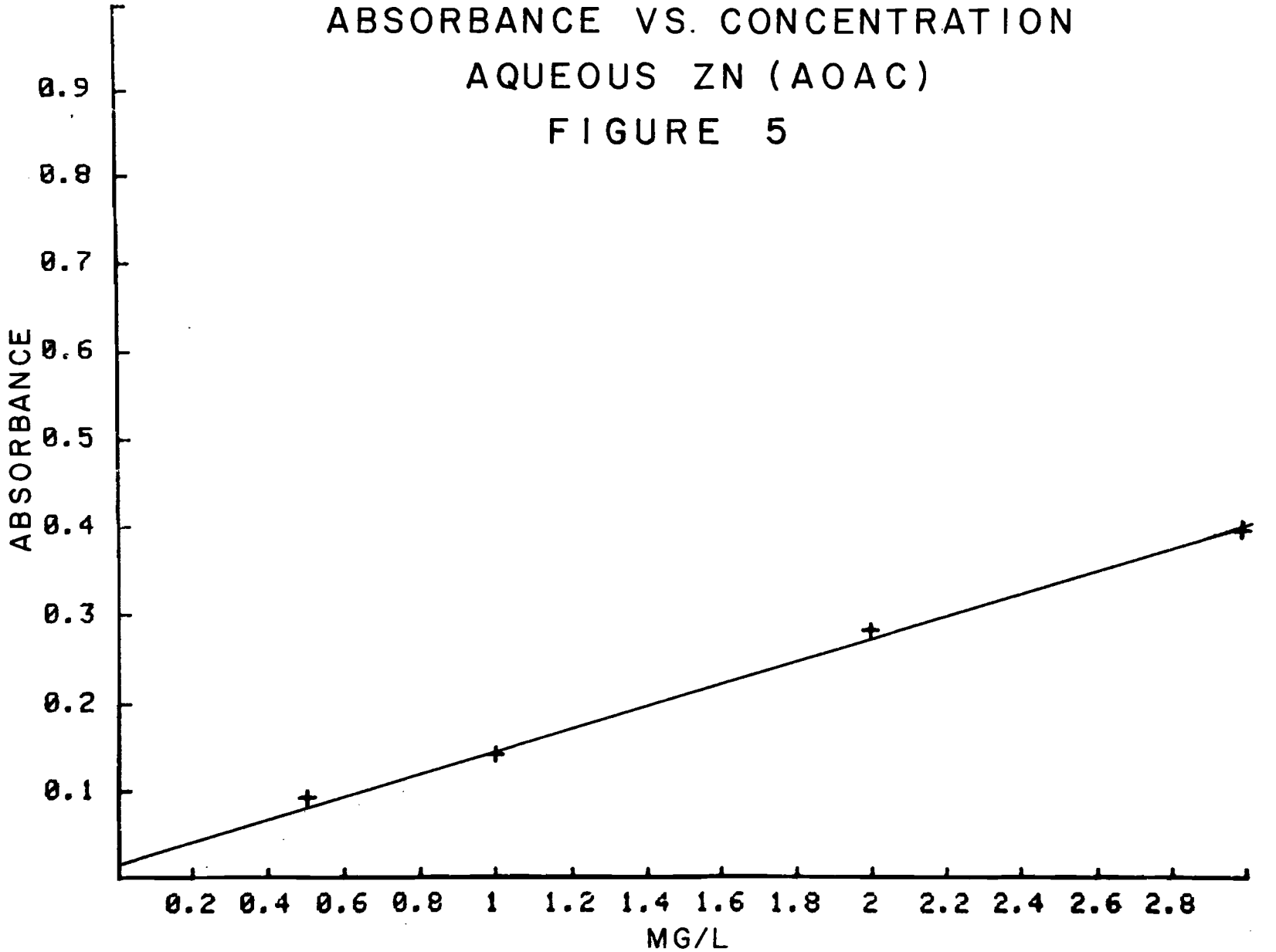
2. Second Extraction

Into the separatory funnel which contained the CCl_4 solution of metal dithizonates, 50 ml of 0.02 N HCl was added and shaken vigorously for 1.5 minutes. All timing was done with stopwatches to insure reproducible conditions. All the CCl_4 layer was shaken down into the CCl_4 layer and the layer was drained into the waste container. The CCl_4 layer contained copper and other metal-complex dithizonates with the zinc being extracted into the aqueous phase. The CCl_4 layer may be saved if the determination of copper is desired. The aqueous phase was rinsed several times with 1-2 ml of clear CCl_4 until all traces of green were gone. Again, care was taken so as not to let any of the aqueous phase enter the stopcock bore. The small film of CCl_4 on the surface of the aqueous phase was allowed to evaporate before the final extraction was performed.

3. Final Extraction

Into the separatory funnel containing the zinc in 0.02 N HCl, 50 ml

of solution B and 10 ml of dithizone reagent were added, then shaken for 1 minute and the phases were allowed to separate. At this point the copper and other metals were sequestered by the diethyldithiocarbamate and kept in the aqueous phase while the zinc-dithizonate was extracted into the CCl_4. The stopcock bore and stem of the separatory funnel were very carefully rinsed with 1-2 ml or the CCl_4 extract. The rest of the CCl_4 extract was drained into test tubes with 5 ml being pipetted into 25 ml volumetric flasks. These were diluted to the mark with CCl_4 and shaken thoroughly. The percent transmittance was read at 525 nm on a Beckman DB-G spectrophotometer. A calibration curve was constructed from the standards and the sample concentrations were determined from this graph (Figure 5). The concentration of zinc expressed as micrograms/gram of plant material was calculated from these values.[16] The sensitivity determined from the calibration curve (Figure 5) was 38 ppb for the Association of Official Analytical Chemists colorimetric method.


ABSORBANCE VS. CONCENTRATION
AQUEOUS ZN (AOAC)
FIGURE 5
ABSORBANCE
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.2 0.4 0.6 0.8 1 1.2 1.4 1.6 1.8 2 2.2 2.4 2.6 2.8
MG/L

RESULTS AND DISCUSSION

The amount of zinc in each type of plant material was calculated from the calibration curves for the proposed AAS method and the AOAC colorimetric method. The results obtained are summarized in Table 2. The precision of the AOAC spectrophotometric dithizone method and the proposed AAS method were about the same. The accuracy of both methods was approximately the same with the proposed AAS method generally giving lower values than those obtained with the AOAC method.

The sensitivity of the AAS method was determined from the calibration curve pictured in Figure 6 and found to be 12 ppb. Aqueous AAS gave a sensitivity of 33 ppb as determined from the calibration curve in Figure 7. This led to a three-fold increase in sensitivity when methanol was employed as a solvent. The dithizone method yielded a sensitivity of 38 ppb. Sensitivity is defined as the minimum concentration need to give a 1% absorption. The sensitivity of the 0.1 N HCL-99% methanol method far surpassed that of the other two methods.

Figures 5, 6, and 7 indicate that the results obtained by the proposed AAS method adhere more closely to Beer's Law than those obtained by using the other two methods. This is a distinct advantage of using the 0.1 N HCl-99% methanol solvent when working with low levels of zinc.

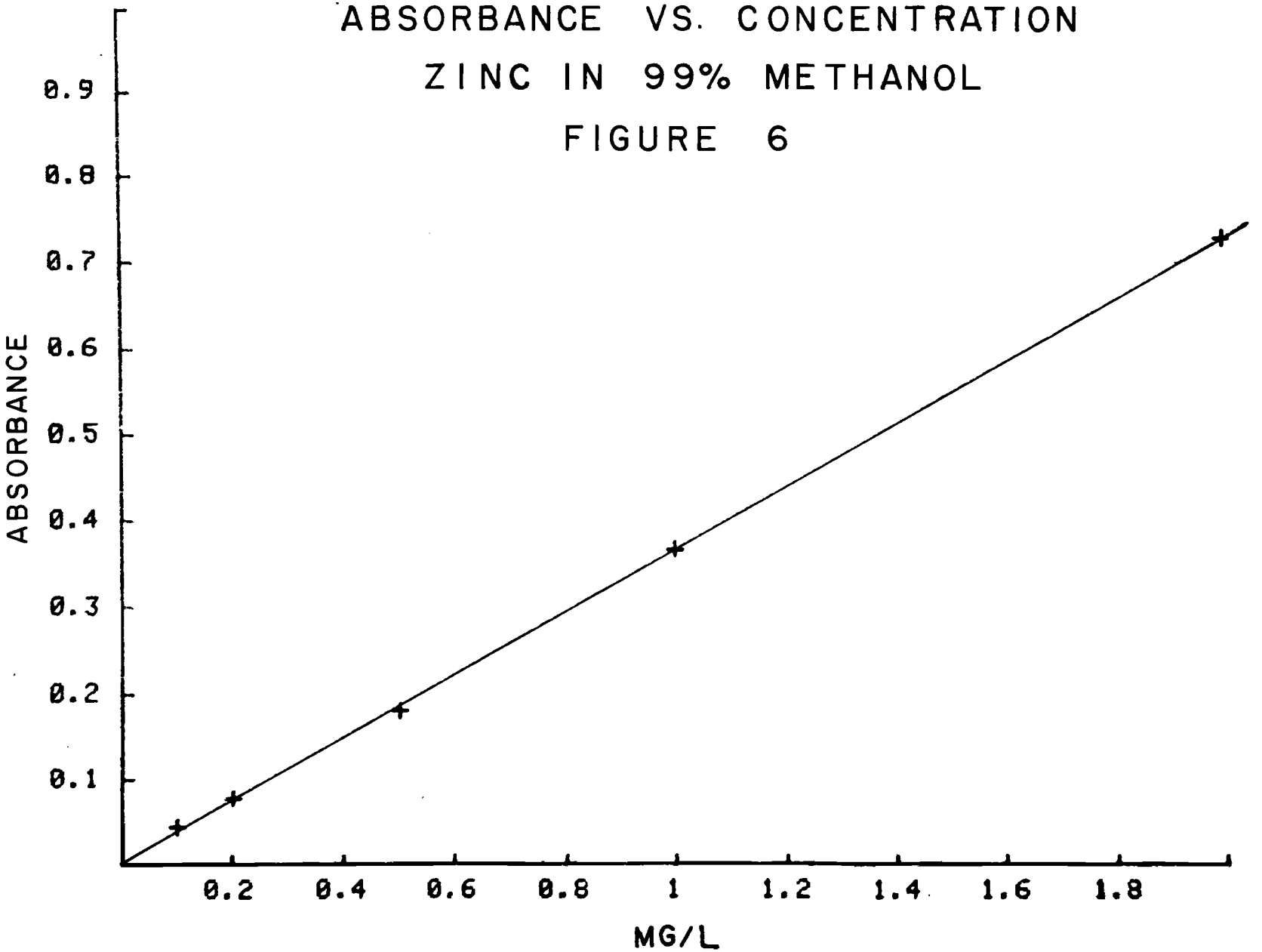
Another advantage of the AAS method is the faster analysis time over the colorimetric method. The time required for analysis of an equal number of samples is 10 minutes for AAS and 4 hours for the AOAC method. This difference in speed without loss of precision and accuracy is the major advantage of the proposed AAS method.

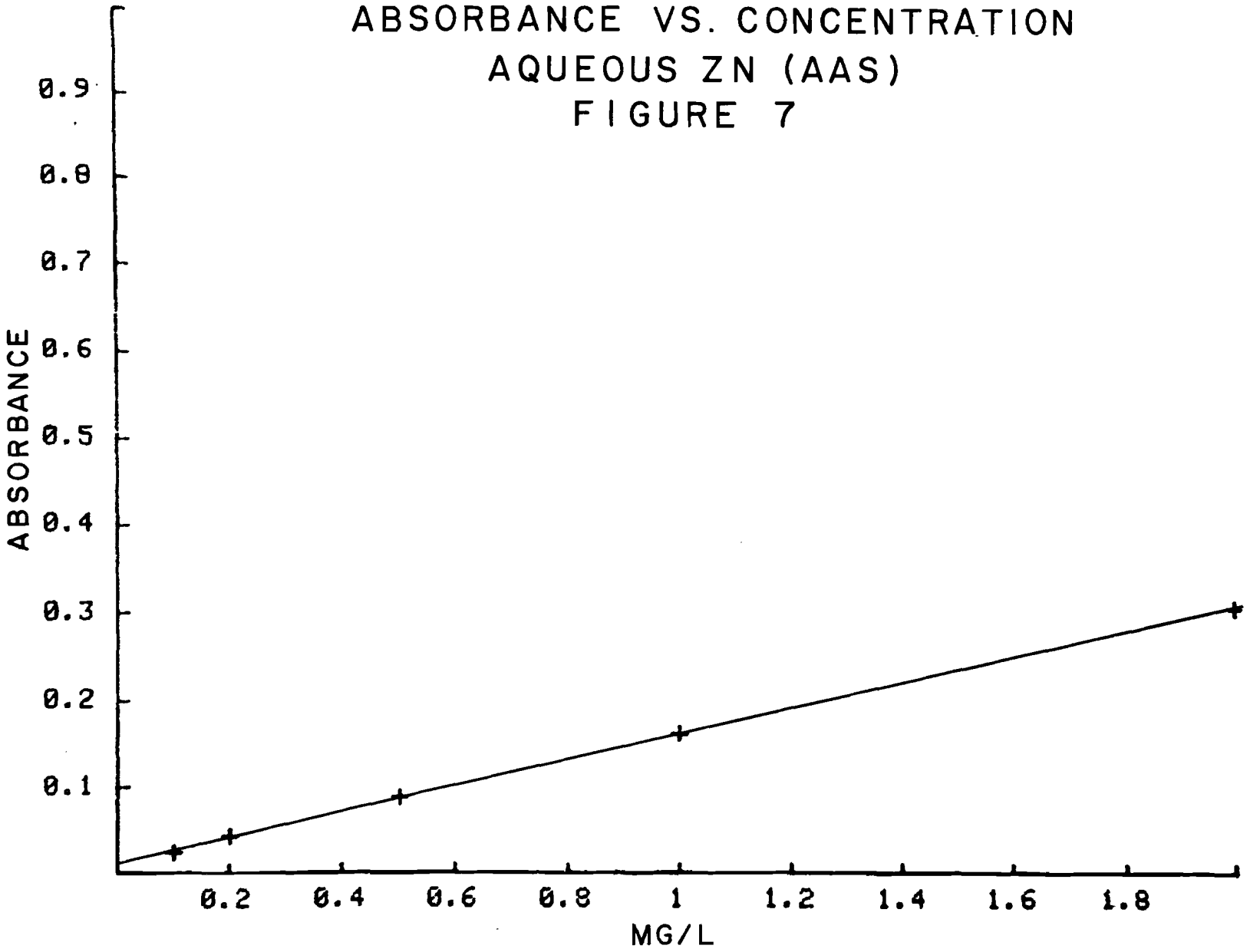
A recovery study was important in AAS to determine the efficiency of the ashing and sample preparation procedure. Two grams of plant

TABLE 2

A Comparison of the Amount of Zinc in Plant Material as Determined by Atomic Absorption and AOAC Methods

	WHEAT		ALFALFA		SOYBEAN	
	AA	AOAC	AA	AOAC	AA	AOAC
Micrograms/Gram	34	36	35	27	51	69
Standard Deviation	2	2	2	3	3	3
Coefficient of Variance	6%	5%	6%	12%	6%	4%
Number of Trials	9	4	10	5	10	4


ABSORBANCE VS. CONCENTRATION
ZINC IN 99% METHANOL
FIGURE 6
ABSORBANCE
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
MG/L


ABSORBANCE VS. CONCENTRATION
AQUEOUS ZN (AAS)
FIGURE 7
ABSORBANCE
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
MG/L

material was weighed into 30 ml Vycor crucibles and 0.5 ml of 20 ppm zinc was added. These were placed in a muffle furnace at 500°C overnight. The samples were prepared for analysis by AAS following the procedure mentioned earlier. By finding the amount of zinc in the recovery samples, knowing the size of the samples, and the mean level of zinc in the plant material from previous studies, the percent recovery may be calculated. The mean percent recovery was found to be 97% $\pm$ 3% (r.s.d.) with values ranging from 80-135%.

CONCLUSIONS

The proposed AAS method has both merits and disadvantages which will be briefly discussed. AAS using methanol as a solvent is a very useful technique because it gives a quick, simple method to determine low content metals, such as cobalt, in plant materials.

Satisfactory results may be obtained when determining zinc by AAS employing water as the solvent, however determination of zinc by the proposed AAS method does have its advantages. One of the main goals of this study was to show that zinc may be determined along with other metals in a solution of one ashed sample. When determining high content metals, such as zinc, in plants the advantage is an extension of the working range from 2 ppm to 3 ppm zinc. Above 3 ppm zinc the curve tends to deviate from linearity. The amount of zinc present in plant material is sufficiently greater than that of other trace metals, so that further dilution of the solution may be necessary to remain within the useful analysis region of the calibration curve.

The method could be improved in the following ways. (1) By using an atomic absorption spectrophotometer for the analysis, background correction could be implemented, increasing the accuracy of the method. (2) Using a smaller sample of material would be advantageous in the analysis of zinc because it would shift the unknown concentrations into the optimum portion of the working curve. Background absorption was compensated for by careful control of the fuel-oxidant ratio.

Use of a 0.1 N HCl-99% methanol mixture as the solvent in AAS does provide a solution to the problem of analyzing previously unmeasurable amounts of metals in plant material. The increase in sensitivity is well worth the slightly greater effort required in preparing solutions and

unknowns. The increase in the working curve range is indeed significant. A 10° rotation of the burner out of the optical path produces a good linear curve from 0-10 ppm zinc, thus zinc can be determined without further dilution of the solution used for the determination of metals present in lesser amounts. Overall, the method shows promise in achieving a significant step in the direction of increasing the sensitivity in routine analysis without expensive equipment modification or by using non-flame atomizers.

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APPENDIX I

Data for Determination of Zinc by AAS

Data for Determination of Zinc by AAS

Sample	Absorbance	Concentration (µg/ml)	Wt. Sample (gm)	Concentration (µg/gm)
WHEAT				
1	0.888	2.80	2.013	34.7
2	0.817	2.57	2.016	31.9
3	0.808	2.55	2.010	31.7
4	0.771	2.43	1.962	31.0
5	0.937	2.95	2.048	36.0
6	0.944	2.97	2.102	35.3
7	0.881	2.77	1.906	36.4
8	0.845	2.66	1.938	34.3
9	0.877	2.76	2.178	31.7
ALFALFA				
1	0.878	3.11	2.105	36.9
2	0.858	3.03	1.995	38.0
3	0.915	3.24	2.391	33.9
4	0.784	2.76	1.909	36.1
5	0.832	2.94	2.135	34.4
6	0.736	2.58	2.036	31.7
7	0.703	2.55	1.864	34.2
8	0.733	2.57	1.964	32.7
9	0.801	2.82	2.073	34.0
10	0.824	2.91	1.986	36.6
SOYBEAN				
1	1.286	4.11	2.191	46.8
2	1.223	3.87	1.845	52.4
3	1.068	3.28	2.263	52.0
4	1.255	3.99	1.579	44.1
5	1.319	4.23	2.120	49.9
6	1.334	4.29	2.100	51.1
7	1.380	4.46	2.112	52.8
8	1.202	3.79	1.901	49.8
9	1.369	4.42	2.015	54.8
10	1.291	4.12	1.933	53.3

APPENDIX II

Data for Determination of Zinc by AOAC

Data for Determination of Zinc by AOAC

Sample	Absorbance	Concentration (µg/ml)	Wt. Sample (gm)	Concentration (µg/gm)
WHEAT				
1	0.286	2.084	6.165	33.8
2	0.310	2.281	6.082	37.5
3	0.304	2.222	5.896	37.7
4	0.258	1.866	5.043	37.0
ALFALFA				
1	0.167	1.158	2.042	28.3
2	0.166	1.147	2.148	26.7
3	0.137	0.913	2.163	21.1
4	0.184	1.295	2.298	28.2
5	0.160	1.096	1.977	27.7
SOYBEAN				
1	0.456	3.058	2.139	71.5
2	0.446	2.988	2.307	64.8
3	0.489	3.288	2.351	69.9
4	0.536	3.617	2.600	69.6

APPENDIX III

Data for AAS and AOAC Calibration Curves

Data for AAS and AOAC Calibration Curves

AAS		AOAC	
Concentration (ppm)	Absorbance	Concentration (ppm)	Absorbance
0.1	0.031	0.5	0.092
0.2	0.063	1.0	0.143
0.5	0.150	2.0	0.284
1.0	0.301	3.0	0.398
2.0	0.634		
5.0	1.365		

APPENDIX IV

Recovery Study

Recovery Study

Sample	Wt. Sample (gm)	Amount Zn Calculated (µg)	Amount Zn Found (µg)	% Recovery
1	2.121	43.32	51.98	86.6
2	2.119	43.28	56.85	135.7
3	2.021	41.28	48.68	74.0
4	2.161	44.14	53.22	90.8

10 micrograms of zinc was added to each sample.

Average concentration of zinc in wheat = 20.4 micrograms/gram

Note: Ran out of original wheat sample so had to rerun analysis on new samples.