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Title: A Determination of Manganese in Plant Material by Atomic Absorption Spectroscopy using a Methanol-Aqueous Solvent Combination

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The effect of various organic solvents upon the absorbance of manganese was investigated. Methanol was found to give the greatest enhancement in sensitivity of the solvents analyzed. The plant material was dry ashed, dissolved in 1.0 N HCl and evaporated to near dryness. The residue was then dissolved in a solution of 1% HCl in methanol. The results obtained were comparable to those obtained using the official AOAC colorimetric methods. The procedure has been used for the analysis of samples containing from 20 to 120 μgm of manganese. A plot of absorbance vs concentration was found to be linear throughout this range and a sensitivity of 30 ngm/ml was obtained. The standards used in atomic absorption spectroscopy work were found to give consistent absorbance reading over a period of 60 days. In contrast standards used for official AOAC colorimetric analysis were found to decrease in intensity within 30 minutes or less.

A DETERMINATION OF MANGANESE IN PLANT MATERIAL BY ATOMIC ABSORPTION SPECTROSCOPY USING A METHANOL-AQUEOUS SOLVENT COMBINATION

A Thesis

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INTRODUCTION

Today many trace metals are known to be essential in the growth and diet of both humans and plants. The requirements of trace metals in enzymatic reactions have been known for years. Unfortunately, the mechanisms by which trace metals function in the biological environment are still a mystery. Therefore, a method of analysis is needed which is extremely sensitive and has a low detection limit for the determination of trace quantities of metals in biological materials.

Commonly used methods include colorimetry, atomic absorption spectroscopy, X-ray fluorescence, polarography, and neutron activation. Each of these methods has distinct advantages and some disadvantages. The selection of an analytical technique is normally based on the type of information desired, the availability and cost of equipment, time required per analysis, and degree of operator experience required.

The preparation of a sample is normally the most time consuming part of the analysis. Thus, a method of sample preparation that is rapid, accurate and would require small quantities of sample would be advantageous. In addition, most analysis for trace metals would involve the determination of more than one metal, therefore if the method can be used to analyze for more than one metal in a single solution, the method would have a universal appeal.

The official colorimetric methods of determination of trace metals in plants recommended by the Association of Official Analytical Chemists (AOAC) normally require a considerable amount of sample pre-

paration time due to the need for complexation and separation procedures required to remove possible interferences. Thus it can be seen that a more rapid type method would have distinct disadvantages if a large number of samples are analyzed on a routine basis.

The atomic absorption method of analysis has an advantage over the official colorimetric method, because the preparation time for analysis of plant materials is relatively short and is only dependent upon the form the sample is in.

The purpose of this study is to make a comparative study using two methods of analysis, namely the atomic absorption method and the official AOAC colorimetric method for determining the amount of manganese in plant materials. The results obtained by the atomic absorption method of analysis will be compared against the official AOAC colorimetric method and the relative advantages and disadvantages of both methods will be compared.

THEORY

The basic components of an Atomic Absorption Spectrometer are a source of radiation, a method of producing neutral atoms, a monochromator, a detector, an amplifier and a signal readout device.

The radiation source most commonly used for Atomic Absorption Spectroscopy is the hollow cathode lamp. The hollow cathode lamp consists of a cathode in the form of a hollow cup that is constructed from the element to be determined, or an alloy of the metal. The tungsten anode is a straight wire or shaped as a spherical disk located in close proximity to the cathode. The two electrodes are enclosed in an evacuated glass envelope containing a small amount of either argon or neon as an inert filler gas. The face of the lamp where the directed beam of radiation is emitted is usually constructed from quartz or borosilicate glass.

The hollow cathode lamp is operated by applying a potential of 350-500 volts between the anode and the cathode. A current ranging from 1 to 50 milliamperes is established due to the ionization of the filler gas. The positively charged ions are accelerated to a high velocity towards the cathode. At the point of collision between the cathode and the gaseous ion, metal atoms "sputter" away from the cathode. Further collisions of the inert gas ions and the metal atoms produce excited atoms, which in turn release energy in the form of radiation characteristic of the atom emitting the energy. The intensity of the radiation depends upon the cathode current, the metal from which the cathode is constructed, and the design of the lamp.

The process by which neutral atoms are introduced into a beam of radiation is called atomization. There are several ways in which this atomization process can be accomplished. A solution of the analyte can be aspirated into a flame, a sample can be rapidly heated in a graphite furnace or on a tantalum boat; or if the sample has an appreciable vapor pressure, a cold vapor of the metal or its hydride can be swept through a cell which has quartz end windows. Of these three methods, the most commonly used is the nebulizer chamber and burner combination.

Nebulization is the process by which a solution is broken into fine particles or droplets. The construction of a pneumatic nebulizer is such that the solution is aspirated into a premix chamber by the oxidant gas, due to the "Venturi action" of the gas passing across a small orifice. The solution is broken up into fine droplets. The droplets of solution are formed by one of three methods: impact beads, heated burner chambers, or ultrasonic nebulizers.

Nukiyama and Tanasawa[48] have developed an empirical equation relating the average droplet size emitted from a pneumatic nebulizer to several physical parameters.

$$d_o = \frac{585}{(v)}\left[\frac{\gamma}{p_f}\right]^{0.5} + 597\left[\frac{\eta}{(\gamma p_f)^{0.5}}\right]^{0.45}\left[1000\,\frac{Q_f}{Q_g}\right]^{1.5}$$

where:

d_o = average drop size in microns

p_f = fluid density in grams per cubic centimeter

η = viscosity in grams per second-centimeter

Q_g = flow rate of gas in cubic centimeters per minute

Q_f = flow rate of fluid in cubic centimeters per minute

v = velocity of the aspirating gas in meters per second

γ = surface tension in dynes per centimeter

This equation shows the average droplet size is directly proportional to the surface tension and the viscosity of the solution. Therefore the use of a solution which has a low surface tension and a low viscosity should lead to the production of smaller droplets of vapor. The use of an organic solvent with lower surface tensions and viscosities than water should lead to the formation of finer droplets than those produced from an aqueous solution. The formation of smaller droplets in the atomization process greatly enhances the sensitivity of the method.

The monochromator used for Atomic Absorption Spectroscopy includes gratings, prisms and interference filters. The basic requirement of the monochromator is to separate the analytical line from other nearby spectral lines. A high resolution monochromator is not required unless two elements present in the sample have absorption lines close together.

The type of detector used in atomic absorption varies with the type of monochromator used. Three types of detectors that have been used for AAS are phototubes, photodiodes, and photomultiplier tubes. The readout devices used for atomic absorption measurements are deflection meters, digital displays, or strip chart recorders. The advantage of using a digital display over a meter is the ease by which the desired signal can be observed. The use of a strip chart recorder is highly desirable, and can be used in combination with either a meter or display.

The atomic absorption process can be represented as the absorption of radiation of specific frequency by a gaseous neutral atom in the ground electronic state. The electrons in the atom are thus promoted from some orbital energy, E, to an higher orbital energy, E + hν. The sudden release of energy, hν, causes the electrons to fall back to the initial orbital energy, E. This process is called atomic emission.

In Flame Atomic Absorption Spectroscopy the number of atoms that are in the excited electronic state is negligible. This can be seen by the ratio of the number of atoms excited by the flame to the number in the ground state as calculated by the Boltzman Distribution equation:

$$\frac{N^*}{N_0} = \frac{g^*}{g_0} e^{-E^*/kT}$$

where N* and N_0 are the number of atoms in the excited state and ground state, respectively, g* and g_0 are the statistical weights for the energy levels involved, k is the Boltzman constant, T is the absolute temperature, and E* is the energy of activation. Walsh[76] has applied this equation to calculate the N^*/N_0 ratio for four atoms: Na, Cs, Ca, and Zn at various flame temperatures.

Table 1

N^*/N_0 Ratio

Resonance line	g^*/g_0	2000°K	3000°K	4000°K	5000°K
Cs 8521 Å	2	4.4×10^{-4}	7.2×10^{-3}	3.0×10^{-2}	6.8×10^{-2}
Na 5890 Å	2	9.9×10^{-6}	5.9×10^{-4}	4.4×10^{-3}	1.51×10^{-2}
Ca 4227 Å	3	1.2×10^{-7}	3.7×10^{-5}	6.0×10^{-4}	3.3×10^{-3}
Zn 2138 Å	3	7.3×10^{-15}	5.4×10^{-10}	1.5×10^{-7}	4.3×10^{-6}

This table shows the relationship between the temperature and the number of ground state atoms. As the temperature of the flame is increased the probability of finding excited state atoms and metal ions is more probable. The number of excited state atoms is negligible at lower flame temperatures where most of the analysis by AAS is done. At lower temperatures the probability of chemical interferences is more of a problem than at higher temperatures due to the lower quantity of energy that is available to vaporize and dissociate the salts.

When an atom in the vapor state absorbs radiation and an electron is excited to a higher energy level the intensity of the beam of radiation is decreased. The decrease in intensity of the radiation is the result of a neutral atom absorbing radiation at specific wavelengths. This can be measured by the ratio of the incident radiation prior to contact with the vapor of atoms to that of the radiation passing through the vapor. The decrease in intensity of the radiated beam can thus be equated to the number of atoms absorbing the radiation. This decrease in intensity of radiation can therefore be related to Beer's Law, where:

$$P_\lambda = P_{o\lambda} e^{-k_\lambda \ell c}$$

$P_{o\lambda}$ is the power of the source at wavelength λ, P_λ is the power of radiation at wavelength λ after passage through the vapor of gaseous atoms, k_λ is the absorptivity coefficient, ℓ is the path length through the vapor of gaseous atoms, and c is the concentration of atomic vapor.

This equation can be rewritten in terms of absorbance by dividing $P_{o\lambda}$ through both sides of the equation and when transformation from natural to common logarithms is performed the equation becomes:

$$-\log \frac{P_\lambda}{P_{o\lambda}} = k_\lambda \ell c$$

Absorbance is defined as $\log (P_\lambda/P_{o\lambda})$ and when k_λ is replaced by the absorptivity coefficient (a) the equation becomes:

$$A = a\ell c$$

This equation is the familiar Beer's law equation and states that if absorbance is plotted against concentration a linear relationship is observed if the absorptivity coefficient and path length are held constant.

The hollow cathode lamps are used for a number of important reasons in AAS; the lamp emits an intense radiation of stable intensity with a spectral line of the same frequency as the analyte element. The interferences caused by extraneous lines emitted within the band pass of the monochromator are negligible. In contrast, the continuum radiation source (e.g., tungsten filaments, quartz halide lamps, and hydrogen and xenon lamps) is a poor choice. In figure 1 the relationship between a hollow cathode lamp and a continuum is shown. In figure 1-A the spectral line width emitted by the hollow cathode source is shown centered in the band pass of the spectrometer slit. In figure 1-B the same emission signal from the hollow cathode is shown with an absorption line of the same element at the same wavelength. The intensity of the emission line signal is reduced markedly, approximately 50% in this case. In figure 1-C a continuous

FIGURE 1-A

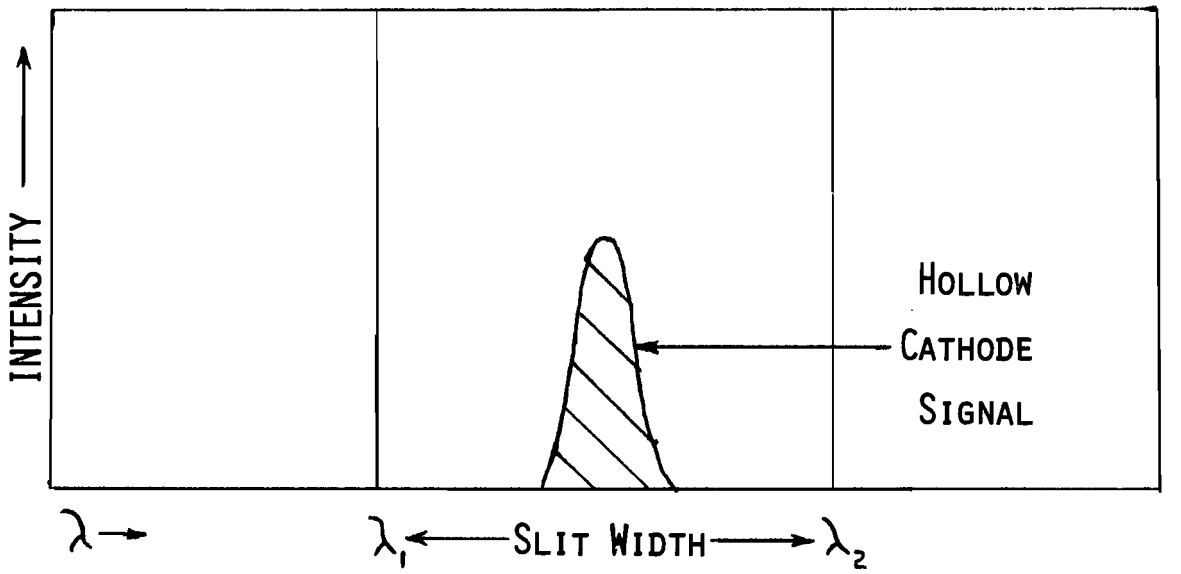


FIGURE 1-B

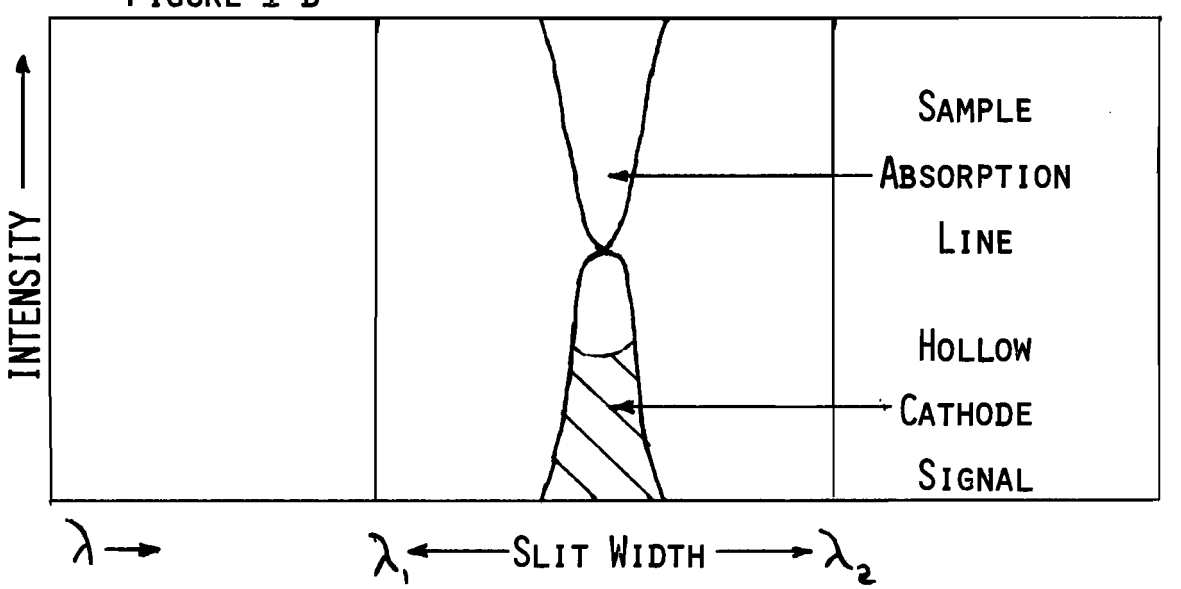
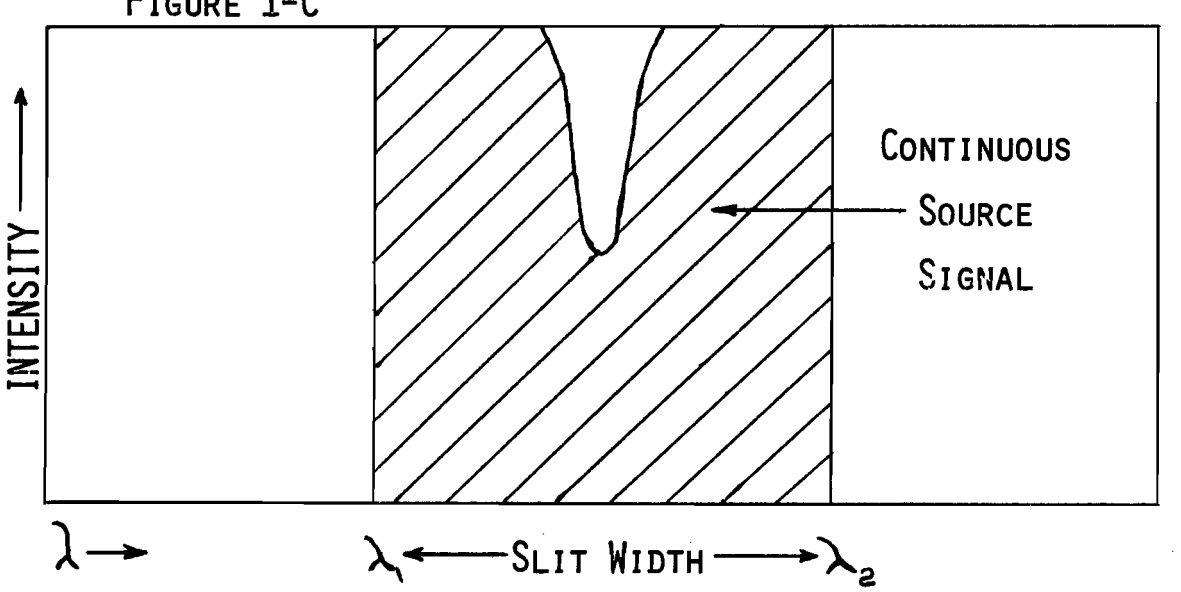


FIGURE 1-C



source signal is shown, filling the band pass of the spectral slit width minus the energy absorbed due to the absorption line. In contrast to the hollow cathode signal, figure 1-B, the absorption in this case represents a very small fraction of the total energy passing through the monochromator.

LITERATURE REVIEW

Occurrence, Metabolism and Toxicity of Manganese

Manganese is found in nature in various forms. Its major ores are the oxides, carbonates, and silicates.[10] Manganese deposits are found in Russia, India, South Africa, the United States and several other countries. The deposits found in the United States are of lower quality than those in other countries, but the purity of the refined metal has improved due to the development of the electrolytic refining process.

Manganese is mined in two ways depending on the nature of the ore. Pneumatic drills or rock-drilling machines are used, followed by blasting. The ore is refined by one of three processes: reduction of MnO_2 by carbon in an electric furnace; reduction of Mn_2O_4 with aluminum by the thermite process; and if the pure metal is desired, by electrolysis of concentrated solutions of manganous chloride. A mercury cathode is used followed by vacuum distillation of the mercury.

The largest industrial use of manganese is in the manufacture of steel. Manganese, when added to molten steel (ferromanganese and silicospiegel) deoxidizes the metal to give it the added properties of strength and hardness. Other uses of manganese include the manufacture of dry cell batteries; production of $KMnO_4$; as a pigment in glass, ceramics, paints, varnish, inks or dyes; and in agriculture to supplement manganese deficient soils.

Manganese was found to be an essential trace element in plants and animals in 1931.[11] Manganese is found in high concentrations in the parts of a plant where reproductive processes occur. Thus it is

found in higher concentrations in seeds rather than in stem, leaves or roots. Manganese is known to have a function in the activation of a number of enzymes. Enzymes known to contain manganese include: glycosyltransferase, many peptidases, phosphatase, polymerase, and specific enzymes such as galactransferase. Von Oettingen[50] reported that manganese increases the oxidase reactions in plants. The suggestion has also been made that manganese cycles between the (III) state and the (II) state in the final stages of photosynthetic processes resulting in the oxidation of water and formation of oxygen.[53]

In animals, manganese tends to concentrate in the liver, kidney and bone with the lowest portion found in blood, bone marrow, and the lungs. In humans, manganese has been suggested to be in the range of 12 mg to 20 mg with a daily requirement of 3 to 9 mg.[54,39]

Manganese is absorbed through the intestinal tract at a very low rate due to its low solubility in gastric fluid. The absorption of manganese through the skin does not occur to any significant extent but absorption into the lungs results in acute poisoning if taken in large doses over an extended period of time.

Excretion of manganese is exclusively by the feces and to some extent through the bile, with partial re-absorption into the intestines.[43] As little as less than 0.01 mg/l has been reported to be excreted in the urine. The distribution of manganese shows accumulation in those organs rich in mitochondria, mainly the liver and kidneys. The transport of manganese has been proposed to be through some type of intercellular pathway.[15]

The effect of a deficiency of manganese in man have not been shown conclusively. Although reported effects including a low level of serum

cholesterol, weight loss, slowed growth of hair and nails, and reddening of the hair are believed to be related to manganese deficiency.[15] The deficiency of manganese in animals leads to abnormalities of the bone, lack of growth, congenital ataxia (a nervous disorder) and defective eggshell formation in chickens.[11] The affects of a deficiency of manganese in plants are well known with symptoms of chlorosis occurring when sufficient quantities of manganese are absent. The problems associated with chlorosis have been reported by Phipps[54] and Somers and Shive.[68]

The toxic effects of manganese occur after prolonged exposure to the metal dust or its compounds. The disease associated with manganese poisoning is characteristic of similar heavy metals when inhaled in sufficient quantities, and is called "metal fume fever." This disease initially affects the lungs and in its chronic forms acts as a nerve toxin creating a number of physiological disorders.

Colorimetric Methods of Determination

The Association of Official Analytical Chemists (AOAC) colorimetric method for the determination of manganese in plant material requires ashing the sample at 500°C until the residue is nearly white (ca 12 hrs). The residue is then moistened with 5-10 ml of 12 M HCl and evaporated to near dryness. The ash is dissolved in 5 ml of 12 M HCl, boiled for a few minutes and diluted with water (ca 20 ml). The solution is filtered and rinsed with water followed by the addition of 15 ml of concentrated H_2SO_4 and evaporated to ca 30 ml. Five to 10 ml of concentrated HNO_3 is added, and the solution is evaporated until dense white fumes appear. Water (ca 70 ml) is added to redissolve

The determination of manganese by other colorimetric methods has been reported by many authors.[42,65,67] Methods which are often used for these determinations include the formaldoxime method, the pyridylazonaphthol (PAN) method, and the use of persulfate instead of periodate as an oxidizing agent. The oxidation of manganese (II) to permanganate by persulfate is similar to that of periodate, except that a small amount of silver or cobalt ion is required to catalyze the oxidation.

In the formaldoxime method the solution containing the manganese is added to a 1 M formaldoxime solution and made alkaline. The solution is required to be basic in order for the formaldoxime to chelate the metal ion. The solution is colorless until after exposure to oxygen when it forms a brown-red complex. The absorbance of the solution is measured at 445 nm and compared to a blank or water. This method can be made specific for manganese by adding cyanide. Cyanide acts as a masking agent to prevent the formation of other metal complexes with formaldoxime. This method was used by Tusl[71] for the determination of manganese in feeds. A sensitivity of 2 μg manganese per 25 ml of solution was reported. Bradfield[9] also used this method for the analysis of manganese in leaf and fruit crops.

The (PAN) method utilizes 1-(2-Pyridylazo)-2-naphthol as a chelating agent to form a complex with manganous ion. The chelate, which is sparingly soluble in water, can be extracted with chloroform. The chloroform extract forms a red-violet complex whose absorbance can be measured with a spectrophotometer. Vlacil[73] claims this method can be used to determine 3×10^{-6}% manganese in a 10 gm sample. The PAN method can also be made specific for manganese by adding cyanide.

Various other chelating agents which have been used[8] include carbamate and its derivatives;[38] acyl hydroxylamines, called hydroxamic acids; ethylenediaminetetraacetic acid.

Atomic Absorption Methods

Christian and Feldman[13] suggest that manganese has one of the highest sensitivities in atomic absorption spectroscopy. The spectral line, at 2794.8 Å has been determined to be the most sensitive for this element.[1] Allan[1] states that this spectral line results from the multiplet $a^6S\text{-}7^6P^\circ$.

Burners and Flames

The use of different burners and flame gases effect the sensitivity, detection limit, and can possibly lead to interferences. Golassi and Hell[26] investigated signal to background ratios, source stability, and warm up time, firing characteristics, and operating currents. They found the signal to background ratio effects the sensitivity and linearity.

The use of different burners and flame gases was studied by Perrin and Ferguson.[52] Their results suggested that the $O_2\text{-}C_2H_2$ flame using a total consumption burner was superior to two other burners and other fuel-oxidant combinations. Smith and Shrenk[66] evaluated the difference between the Beckman total consumption burner and the Jarrell Ash tri-flame burner. Their results indicated that the tri-flame burner was more useful for the reduction of interferences.

Other investigators have studied the effect of location of the light beam in the flame,[61] concentration of the elements on the height of the maximum absorbance zone,[19,56] sources of noise,[63] affect of

atomizer parameters,[74] use of a continuous source in determination of detection limits,[44] and decay of the atom population using graphite rod atomization.[60]

Ashing and Preparation of Sample

Problems associated in trace metal analysis of plant materials include contamination of the sample prior to selection of an analysis sample; methods used to grind the sample, and contamination introduced in the solutions used to prepare the sample. Samsoni[64] investigated the problems of fine dust contamination and found that dust particles could dramatically influence the results obtained for the determination of manganese in plants. He proposed a method of washing the plant material prior to analysis. His investigations also showed plant samples containing manganese could be uncontrollably contaminated by using iron or steel grinders.

Varju[72] suggested the precision of the results obtained in plant analysis could be influenced by the method of preparation. Various methods for preparation of plant samples for analysis have been studied.[80,30,75,62,34,20,54] Baker and Smith[5] found that dry ashing of the sample causes a decrease in the apparent amount of manganese in many plant materials. They proposed a wet ashing method with HNO_3, H_2SO_4 and NH_4VO_3 as a catalyst and extraction of the metal with pyrrolidine dithiocarbamate into $CHCl_3$. Various carbamates have been used for the extraction of metals used to prepare a sample for AAS determinations.[70,37]

Mineral acids seem to be the most common reagents employed to extract trace metals from materials. Dohuja, Toda, Fuway and

Yamamoto[21] analyzed National Bureau of Standards (NBS) orchard leaves using H_2SO_4, HNO_3, $HClO_4$, and HCl alone and incombination. They compared their results to those obtained after wet digestion. The combination of H_2SO_4 and HNO_3 was found to be best. Minczewski and Chwastowska[45] found that HNO_3-$HClO_4$ was satisfactory for analysis of plant materials. The digestion using HCl,[55,4,3] HNO_3,[25,31] and various other combinations of mineral acids [55,49,27] have been studied thoroghly.

Organic Solvents

Organic solvents have been used to increase the sensitivity of analysis[2,41,51]. Feldman, Bosshart,and Christian[23] studied the effects of four solvents on the absorption of manganese and found that the sensitivity was increased using the organic solvent. Crnogaroc and Seruga[16] found that in the presence of less than 20% of either ethanol, 1-propanol, isopropyl alcohol and iso-amyl alcohol the sensitivity of manganese was increased with increasing hydrocarbon chain length and increasing concentration of alcohol. Kabonove and Turkin[36] studied the effect of a wide range of organic solvents and concluded that aliphatics (heptane and octane) and aromatics (toluene) gave the maximum sensitivity.

Krolova[40] used a hexamethylenedithiocarbamate complex of manganese and other metals and extracted them into n-amyl,methyl, butyl, isobutylamyl, or iso-amyl alcohols. He found the sensitivity, when compared to aqueous solutions increased by a factor of 2 in n-amyl methyl ketone or butyl, iso-butylamyl alcohol, and by a factor of 3-4 with 3-heptanone and 2,4-dimethylpentanone. Yangagisowa and Suzuki[79] and others[38] have used complexation and extraction for the determination of manganese.

Valastnik[74] and others[38] found that changes in the concentration and temperature of the organic solvents influenced the apparent amount of metal present in a sample. Feldman and Christian[24] found that in general the absorption increased as the temperature of the solution increased. The greatest change occurred at temperatures between 20-55°C in aqueous solutions.

Moselky[47] reported that, in spark spectral analysis, line intensities increased to a maximum when solutions contained 20% by volume of an organic solvent.

Interferences

The effect of interfering ions upon the results obtained by AAS have been investigated.[57,58] Some of the ions found to cause interferences in the atomic absorption determinations of manganese have been investigated.[5,37,49,61]

Barnett[6] analyzed four elements, including manganese, in mineral acids and found the influence of interfering ions was less than that reported by Hwang and Feldman.[32] Barnett attributed the lesser interference to the difference in the burner systems used. The effect of mineral acids interferences, has also been reported by others.[7,47,81]

The interference of calcium, phosphorous, sodium, potassium, magnesium, iron, ethylenediaminetetraacetic acid (di-sodium salt), H_2O_2,[79] silicon,[25] HCl, and NH_4OAc[3] results in an apparent decrease in the absorption of radiation by manganese. Some ions increase the apparent absorption by manganese to a slight extent,[81,7] while others have a negligible effect.[6,17,22,66]

INSTRUMENTATION

I. Instrumentation

A Jarrell-Ash Model 82-500 Atomic Absorption Spectrometer equipped with; a one half meter Ebert mount monochromator, a grating blazed at 250 nm, a 90 cps electronic chopper, an AC amplifier, and a R-213 photomultiplier was used. The readout device was a Leeds and Northrup Model X/L 680 multi-range stripchart recorder. The entrance and exit slits on the monochromator were fixed at 100 and 150 microns respectively.

The sampling system consisted of a premix laminar flow 10 cm single slot burner, an air-acetylene flame was used. The fuel and oxidant flow rates were monitored with two Gilmont 10 inch flow meters. The radiation source was a Jarrell-Ash hollow cathode lamp.

A GCA-McPherson Instruments series EU-700 spectrophotometer was used for the AOAC colorimetric measurements. A tungsten filament radiation source with a 150 micron adjustable slit width and a 1 centimeter cuvette was used. The absorbance values of the solutions were recorded on a digital display to three significant figures.

METHODS

I. Hollow Cathode Lamp Current

The intensity of the emitted radiation is dependent upon the hollow cathode current and the effect of self absorption within the lamp. In order to determine the optimum hollow cathode current, a solution containing 2 μg/ml of manganese was prepared. The cathode was allowed to equilibrate for a period of 30 minutes after

each current adjustment, followed by aspiration of the 2 μg/ml manganese solution and recording the percentage absorption on a stripchart recorder. The current to the hollow cathode was increased in 0.5 milliampere steps and aspiration of the solution was repeated until an optimum current setting had been found.

II. Fuel-to-Oxidant Ration

The fuel and oxidant used in this study were acetylene and air respectively. The ratio of fuel-to-oxidant was measured by aspirating a 2 μg/ml manganese solution and recording the percentage absorption on a stripchart recorder. One parameter was varied and the other kept constant. The absorption of radiation by the aspirated sample increased to a maximum and then decreased as the fuel flow rate was varied at a constant oxidant flow. The optimum oxidant flow rate was found by varying the oxidant flow rate using the optimum fuel flow rate determined in the previous step. This procedure was then repeated until the optimum fuel-to-oxidant ratio was established.

III. Burner Height

The maximum population of neutral, ground state atoms in a flame normally exists in a small well defined region. The position of this region in the flame is determined by such variables as fuel and oxidant flow rates, viscosity, and surface tension of the aspirated solvent. The height of this region above the burner can be determined by varying the burner height and measuring the change

in the absorption of incident radiation. The burner height was determined by adjusting the burner to a setting of zero millimeters and aspirating a 2 μg/ml manganese solution into the flame. This was then repeated until a maximum percentage absorption was found. The optimum burner height was thus determined to be the height at which the maximum percentage absorption was found for the manganese.

IV. Sample Preparation for Atomic Absorption Spectroscopy

All glassware and plastic bottles were cleaned in a hot solution of Aqua-Regia, rinsed three times in distilled water, and finally rinsed twice in distilled-deionized water and allowed to dry before use.

A manganese stock solution was prepared from the pure metal (99.9%) obtained from the Baker and Adamson General Chemical Division Allied Chemical and Dye Corporation. The stock solution 1000 μg/ml was prepared by dissolving one gram of the metal in a minimum volume of concentrated (12 M) Hydrochloric acid in a 1000 ml volumetric flask and diluting to a volume with distilled-deionized water. A 100 μg/ml stock solution was prepared by taking two 50 ml aliquots of the 1000 μg/ml manganese stock solution and diluting to volume in a 1000 ml volumetric flask with distilled-deionized water. Analysis standards were prepared by diluting either the 100 μg/ml or 1000 μg/ml stock solutions. The stock and analysis solutions were stored in tightly sealed plastic bottles.

The ground grain sample is prepared for analysis by drying the grain in a porcelain dish for a period of 2 hours at 110°C

and allowing the sample to cool in a desiccator. A 2 gm sample of the dried grain is then weighed, using a Mettler single pan analytical balance, into a previously weighed 30 ml Vicor crucible. The Vicor crucibles and samples are placed into an electric muffle furnace and ashed at 500 to 510° for a period of 12 hours.

At the end of the ashing period the crucibles are taken from the furnace and allowed to cool to room temperature. Ten ml of 1.0 N Hydrochloric acid is slowly added into the ashed residue and the crucibles are heated slowly on a hot plate to near dryness. The residue must not be allowed to bake. Ten ml of a warm acidic Methanol solution (1 ml of 12 M HCl diluted with anhydrous Methanol to a total volume of 100 ml) is then added to redissolve the residue. The solution is filtered through Whatman #41 filter paper into a 25 ml volumetric flask. Two 5 ml portions of the solvent(1% HCl-99% Methanol) are used to thoroughly wash the residue and remove any metal ions retained on the filter. The solution is brought to volume by the addition of solvent. The volumetric flask is stoppered and inverted a minimum of forty times, adding more solvent if necessary due to mixing, and stored in tightly sealed plastic bottles.

V. Determination of Manganese by Atomic Absorption Spectroscopy.

The manganese content in the plant samples was determined using the optimal instrument parameters experimentally established for manganese. These optimum parameters are listed in Table 2.

The solutions of plant samples prepared as previously described were aspirated into the flame and the percentage absorption recorded

Table 2

OPTIMUM INSTRUMENT PARAMETERS

	Methanol-Aqueous	Water
Wavelength	2794 Å	2794 Å
Hollow Cathode Current	3.5 mA	3.5 mA
Fuel Flow Rate	1.25 l/min	2.01 l/min
Oxidant Flow Rate	3.41 l/min	4.60 l/min
Burner Height	5 mm	2 mm

on a strip chart recorder. Each solution was aspirated a minimum of three times. The absorbance values for the plant samples were found by taking the average of the height of the recorded peaks and converting the percentage absorption into absorbance units.

The analysis standards 0.1 to 10 μg/ml manganese are prepared by taking the necessary volume from either the 100 or 1000 μg/ml manganese stock solutions and the required volume of distilled-deionized water to prepare a solution that is 1% aqueous and diluting the remaining volume with anhydrous Methanol. An example of the method used for preparation of the analysis standards is shown in Table 3.

A plot of Absorbance vs Concentration of Manganese in μg/ml was made with the aid of a Hewlett-Packard Model 9820 A calculator-plotter. The concentration of manganese in the solutions was found using the least-squares slope from a minimum of three separate absorbance readings.

VI. Effect of Other Ions Upon the Determination of Manganese by AAS

The stock solutions of the interfering ions Cl^-, PO_4^{-3}, SO_4^{-2} and Na^+ used in this study were prepared from their corresponding acids or salts. The anion stock solutions were prepared by taking the needed volume of the concentrated acid solution and diluting to volume in a 1000 ml volumetric flask with distilled-deionized water. The anion stock solutions were standardized against a standard NaOH solution. The cation stock solution was prepared by drying NaCl in a weighing bottle for two hours, weighing out the required amounts of salt, and diluting to one liter with distilled-deionized water.

Table 3

EXAMPLE OF HOW THE ANALYSIS STANDARDS WERE PREPARED FOR ATOMIC ABSORPTION SPECTROSCOPY

Standard	Volume 1000 ppm Mn Stock Solution	Volume 100 ppm Mn Stock Solution	Volume of Distilled-Deionized Water	Volume of Methanol
0.1 ppm	------	0.1 ml	0.9 ml	99 ml
0.2 ppm	------	0.2 ml	0.8 ml	99 ml
0.5 ppm	------	0.5 ml	0.5 ml	99 ml
1.0 ppm	------	1.0 ml	------	99 ml
2.0 ppm	0.2 ml	------	0.8 ml	99 ml
3.0 ppm	0.3 ml	------	0.7 ml	99 ml
5.0 ppm	0.5 ml	------	0.5 ml	99 ml
7.0 ppm	0.7 ml	------	0.3 ml	99 ml
10.0 ppm	1.0 ml	------	------	99 ml

Total Volume = 100 ml in a Volumetric Flask

The analysis samples used for the interference study were prepared by adding the required volume of the 1000 µg/ml manganese stock solution, the desired volume of the standardized anion or cation solution and the required amount of water to prepare the solution of the desired concentration. The aqueous portion of the solution was 1% of the total volume. The solution was then diluted with anhydrous methanol, inverted forty times, and stored in plastic bottles.

An example of how these solutions were prepared is shown in Table 4.

VII. Sample Preparation For Determination By the Official AOAC Colorimetric Method

The standard potassium permanganate solution used for the colormetric method was prepared in a similar manner to that described in reference 12.

Preparation of the grain samples for the determination of manganese by the AOAC colorimetric method was as follows: The ground plant material was dried in a porcelain evaporation dish for 2 hours at 110°C prior to the weighing of the samples into the 30 ml Vicor crucibles. The plant material was allowed to cool in a desiccator for a period of 30 minutes before proceeding. The empty weight of the 30 ml Vicor crucibles and lid was recorded and a 5 gm sample of the grain was added. The combined weight of the crucible and sample were obtained. The difference of the two weights was taken as the weight of the sample. The samples were ashed by placing them in an electric muffle furnace at 500 to 510°C for 12 hours.

Table 4

EXAMPLE OF HOW THE INTERFERENCES SOLUTIONS WERE PREPARED

Concentration of Interfering Ions		Volume of 1000 µg/ml Manganese Stock Solution	Volume of Added Interference Solution	Volume of Water	Total Volume Aqueous	Total*1 Volume
$SO_4^{=}$	100 ppm	0.5 ml	0.3 ml	1.7 ml	2.5 ml	250 ml
	200	0.5	0.5	1.5	2.5	250
	500	0.5	1.5	0.5	2.5	250
		Concentration of $SO_4^{=}$ Stock Solution = 1.013 M				
$PO_4^{=}$	100 ppm	0.5 ml	0.5 ml	1.5 ml	2.5 ml	250 ml
	200	0.5	1.0	1.0	2.5	250
	500	0.5	2.0	0.0	2.5	250
		Concentration of $PO_4^{=}$ Stock Solution = 0.6780 M				
Cl^-	100 ppm	0.5 ml	0.3 ml	1.7 ml	2.5 ml	250 ml
	200	0.5	0.5	1.5	2.5	250
	500	0.5	1.2	0.8	2.5	250
		Concentration of Cl^- Stock Solution = 2.825 M				
Na^+	100 ppm	0.5 ml	0.25 ml	1.75 ml	2.5 ml	250 ml
	200	0.5	0.50	1.50	2.5	250
	500	0.5	1.25	0.75	2.5	250
		Concentration of Na^+ Stock Solution = 2.821 M				

*1 -- all solutions were diluted with methanol to 250 ml in a volumetric flask

The ashed residue was dissolved in approximately 10 ml of 12 M Hydrochloric acid and evaporated to near dryness on a hot plate. The wetting of the ashed residue with distilled-deionized water prior to addition of the concentrated Hydrochloric acid was found to be advantageous in avoiding "splattering" of the alkaline plant ash. This was found to be very important for the soybean ash, but less so with the wheat or clover residues. The dried residue is then redissolved in 5 ml of 12 M HCl and heated to a slow boil and diluted in the crucible to approximately 25 ml with water. This solution is then filtered through a Whatman #41 filter paper into a 250 ml beaker and rinsed thoroughly with distilled-deionized water.

The fifteen milliliters of 18 M H_2SO_4 is added to the filtrate. The beaker containing the sample is placed on a hot plate and the solution evaporated slowly to approximately 30 ml total volume. Eight milliliters of 12 M NHO_3 is added to this solution and heating continued for about three minutes.

Three tenths gram of KIO_4 is added and the solution boiled until the permanganate color develops to maximum intensity in about 8 to 10 minutes. The solution was then poured (hot) into a 100 ml volumetric flask and cooled in a water bath at room temperature. Once the solution has cooled it is brought to volume with distilled-deionized water and inverted a minimum of forty times to insure proper mixing.

VIII. Determination of Manganese Using the AOAC Method

The determination of Manganese in the plant materials by the colorimetric method was accomplished by comparison of the absorbance

of the sample solution against a water blank using matched one centimeter cuvettes on a GCA-McPherson spectrophotometer. The wavelengths commonly used for colorimetric methods of manganese determination are either the 525 nm resonance line or the 546 spectral line. The optimum wavelength used in this study was found to be the 525.25 nm line. A plot of Absorbance vs Wavelength is shown in Figure 2. The absorbance of the sample solutions was measured within a 15 minute period after the permanganate color first appeared. This was necessary due to a decrease in absorbance over a period of time. The absorbance was found to be inversely proportional to the time elapsed from the development of the permanganate color.

The absorbance values obtained from the sample solutions were compared against those obtained for a set of standards prepared in a similar manner to that of the samples. The analysis standards were prepared by using a volume of water equal to that of the sample prior to the addition of the concentrated H_2SO_4 and adding 15 ml of 18 M H_2SO_4, 0.3 gm of KIO_4 and boiling the solution for about three minutes. The hot solution was poured into a 250 ml volumetric flask and cooled to room temperature in a water bath. The solution was diluted to the mark with water, inverted forty times, and the absorbance compared against that of a water blank. The absorbance value of the analysis standard was recorded.

A plot of Absorbance vs the Concentration of Manganese in the standards in μg/ml was prepared with the aid of a Hewlett-Packard Model 9820 A Calculator-plotter. The concentration of the sample was found by using the least-squares slope from the average of three absorbance readings.

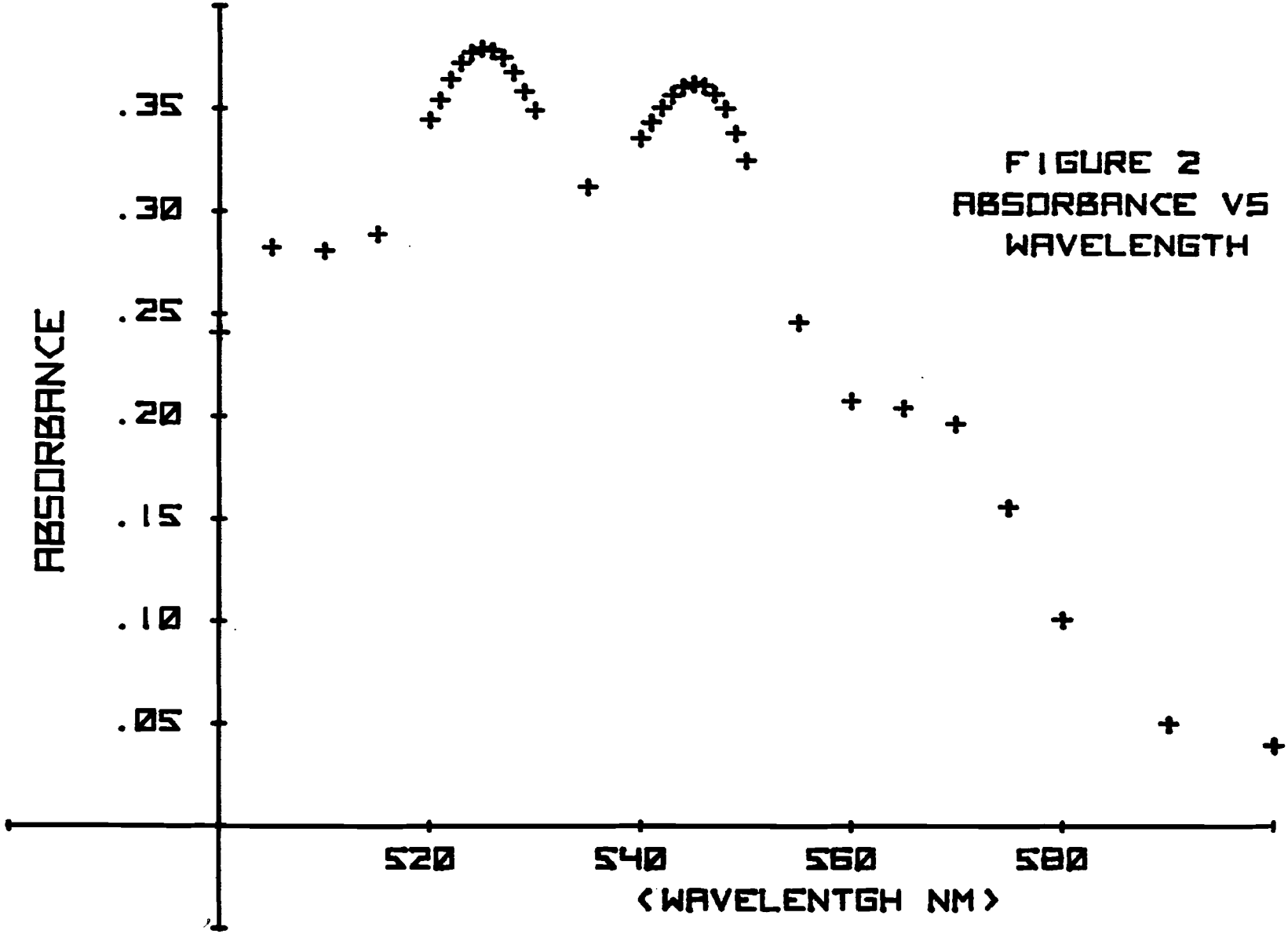


FIGURE 2
ABSORBANCE VS
WAVELENGTH

EXPERIMENTAL RESULTS AND DISCUSSION

The sensitivity of the Atomic Absorption method is defined as the maximum concentration of absorbing atoms at one percent of absorption. The sensitivity of the method is affected by the solvent type, and the instrument parameters; burner height, cathode current, and the fuel-to-oxidant ratio. Therefore the optimum instrument parameters were determined and used throughout this study.

Cathode Current

The hollow cathode current was determined by aspirating a 2 μg/ml manganese solution into the flame and recording the percentage absorption after allowing the hollow cathode to warm up for thirty minutes at each milliampere setting. The optimum hollow cathode current was determined by the setting that gave the highest percentage absorption by the 2 μg/ml manganese solution. A plot of Absorbance vs the Hollow Cathode Current is shown in Figure 3 and the results for each current setting appear in Table 5.

The plot shows two maximums one at 3.5 to 4.0 mA and another smaller peak at 5.5 mA. The optimum hollow cathode current was taken to be the 3.5 milliampere setting because the highest percentage absorption was found at that setting.

Fuel Flow Rate

The fuel used in this study was acetylene that was obtained from commercial sources. The fuel flow rate was determined by setting the optimum cathode current and adjusting the burner height and oxidant flow rate to their approximate optimum settings. The fuel flow rate

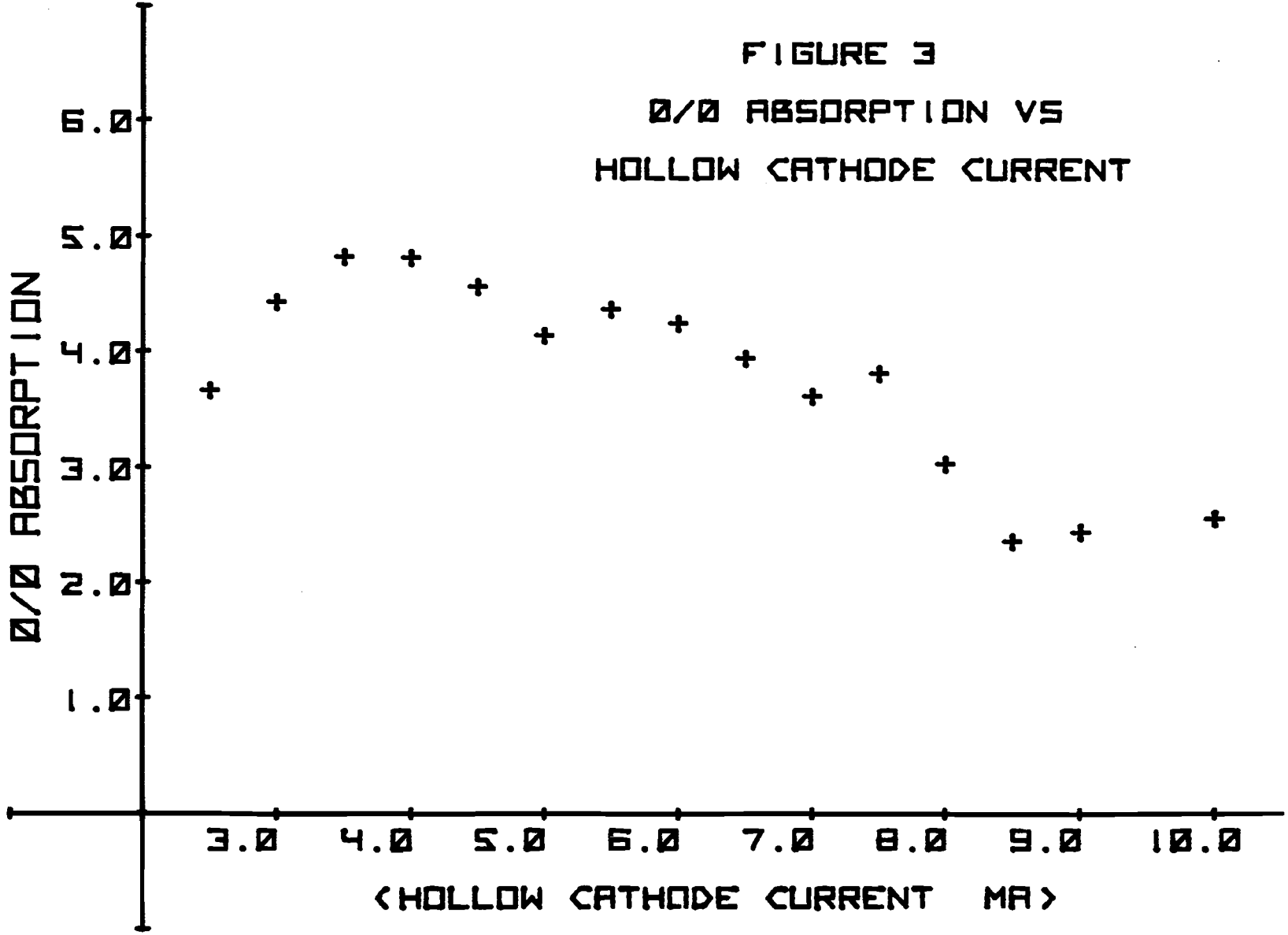


FIGURE 3
Ø/Ø ABSORPTION VS
HOLLOW CATHODE CURRENT
Ø/Ø ABSORPTION
6.Ø
5.Ø
4.Ø
3.Ø
2.Ø
1.Ø
3.Ø
4.Ø
5.Ø
6.Ø
7.Ø
8.Ø
9.Ø
1Ø.Ø
(HOLLOW CATHODE CURRENT MA)

Table 5

HOLLOW CATHODE CURRENT

Setting	% Absorption	Absorbance
2.5 mA	3.66	0.016
3.0	4.43	0.020
3.5	4.82	0.022
4.0	4.81	0.021
4.5	4.56	0.020
5.0	4.14	0.018
5.5	4.37	0.019
6.0	4.25	0.019
6.5	3.94	0.017
7.0	3.62	0.016
7.5	3.81	0.017
8.0	3.03	0.013
8.5	2.36	0.010
9.0	2.44	0.011
10.0	2.56	0.011

was then adjusted to produce a slight yellow tint in the flame. A 2 μg/ml manganese solution was aspirated into the flame and the percentage absorption recorded at that particular setting. The flow rate was then decreased, and the manganese solution was aspirated at each setting while keeping the oxidant flow rate constant. The optimum fuel flow rate was determined from the flow rate setting that produced the highest percentage absorption of the manganese solution.

In Figure 4 the plot of Absorbance vs the Fuel Flow Rate is shown for the absorbance of manganese in two different solvents, aqueous and Methanol-Aqueous (99%-1%). A tabulation of this data appears in Table 6. Inspection of this plot shows for the aqueous solvent the fuel flow rate is less critical than in the Methanol-Aqueous solvent. The Methanol-Aqueous solvent shows a broad peak between 1.2 l/min. and 1.4 l/min. with a maximum at 1.25 l/min. The latter flow rate was used as the optimum fuel flow rate used for the analysis of manganese in grains using a Methanol-Aqueous solvent.

In this figure, the relative differences in the two solvents becomes apparent from the magnitude of the absorbance values. When an organic solvent is used not only is the fuel flow rate different than aqueous solvents, the enhancement of using an organic solvent becomes apparent. The different fuel flow ratio is brought about from the oxidation of the solvent. The organic or methanol solvent is oxidized in the flame causing the flame to be more of a reducing flame thus elongating the flame. The elongated flame lenghthens the

FIGURE 4
Ø/Ø ABSORPTION VS FUEL FLOW RATE

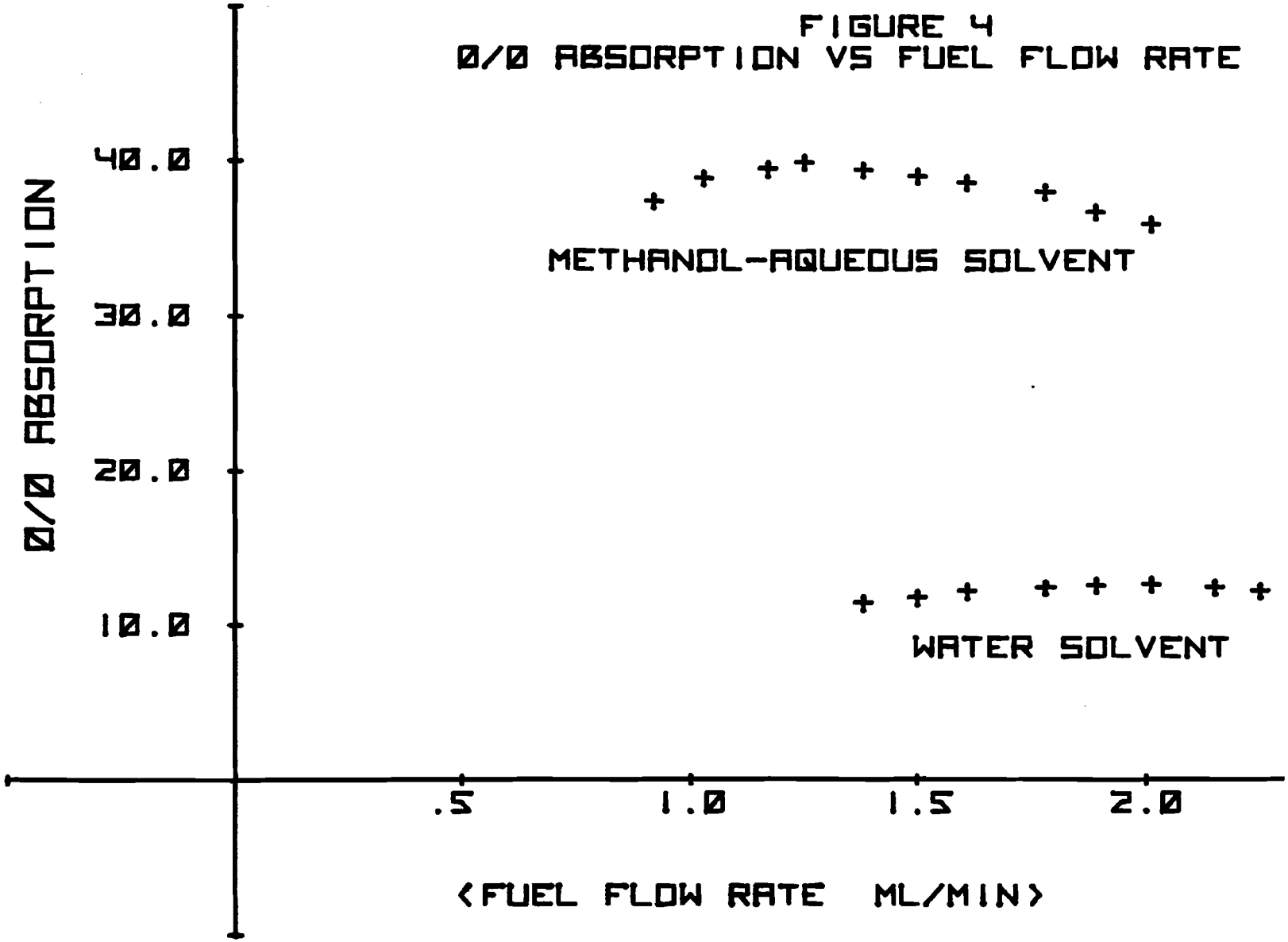


Table 6

FUEL FLOW RATES FOR METHANOL-AQUEOUS AND WATER SOLVENTS USING A 2 µg/ml MANGANESE SOLUTION

99% Methanol-1% Aqueous		Water	
Flow Rate	Absorbance	Flow Rate	Absorbance
0.92 l/min	0.203	1.38 l/min	0.053
1.03	0.214	1.50	0.054
1.17	0.217	1.61	0.056
1.25	0.221	1.78	0.058
1.38	0.217	1.89	0.058
1.50	0.214	2.01	0.059
1.61	0.212	2.15	0.058
1.78	0.208	2.25	0.057
1.89	0.199		
2.01	0.193		

zone where gaseous atoms exist. This enhances the sensitivity of the method. While the aqueous solvent shortens the atomic zone by creating more of an oxidizing flame, thus creating a greater tendency of oxide formation than the organic solvent. The final result is the aqueous solvent decreases the amount of time for gaseous atoms to exist in the flame from oxide formation.

Oxidant Flow Rate

The oxidant used in this study was compressed air obtained from an air compressor located in the building. The oxidant (or air) flow rate was determined by optimizing the hollow cathode current and fuel flow rate, and approximating the burner height to their appropriate setting. The oxidant flow rate was first set by adjusting the flow rate knob until a slight yellow tint appeared in the flame. The oxidant flow was then varied, maintaining a constant fuel flow rate, while aspirating a 2 μg/ml manganese solution and recording the percentage absorption at each flow rate setting. The optimum air flow rate was determined by the highest percentage absorption of the manganese solution at a particular setting of the flow meter. The procedure for determining the optimum fuel and oxidant rates was repeated until the combination yielding the maximum sensitivity for manganese was established. The optimum air flow rate was determined to be 3.41 l/min for the manganese methanol-aqueous solvent and 4.60 l/min for the aqueous-manganese solution. The results for both solvents are tabulated in Table 7.

Burner Height

The burner height is the distance expressed in millimeters from the top surface of the burner to the bottom of the beam of incident

Table 7

OXIDANT FLOW RATES FOR A 2 µg/ml MANGANESE SOLUTION IN A METHANOL-AQUEOUS AND WATER SOLVENT

99% Methanol-1% Aqueous		Water	
Flow Setting	Absorbance	Flow Setting	Absorbance
3.15 l/min	0.272	4.60 l/min	0.061
3.29	0.255	4.79	0.057
3.41	0.253	4.90	0.050
3.56	0.249	5.00	0.049
3.65	0.242	5.19	0.043
3.79	0.238	5.32	0.040
3.90	0.236		
4.05	0.233	6.01	0.030
4.20	0.230		
4.30	0.225	6.70	0.023
4.49	0.222		
4.60	0.213	7.39	0.018
4.79	0.205		
4.90	0.197	8.12	0.015
5.00	0.192		
5.19	0.181	8.81	0.012
5.32	0.171		

radiation.

The procedure by which the optimum burner height was determined was aspiration of a 2 μg/ml manganese solution into the flame and recording the percentage absorption at a selected height. This sequence was then repeated as the burner was lowered until a maximum percentage absorption was found. The height of the beam above the burner where the maximum percentage absorption was found is what is referred to as the optimum burner height.

Figures 5 and 6 show the absorbances obtained for a 2 μg/ml manganese solution vs the burner height using a 99% methanol-1% aqueous solvent and an aqueous solvent, respectively. Inspection of these two plots reveals that at different heights a definite maximum in the percentage absorption of the manganese solution occurs. The optimum burner height using the methanol-aqueous solvent was determined to be 5 millimeters, and for the aqueous solvent 2 millimeters is optimal. The differences in these heights can be justified by the differing characteristics of the solvents in the flame, as was discussed earlier in the fuel flow rate section.

Solvents

The affect of different organic solvents was investigated on the enhancement of manganese in relation to water. Five linear chain alcohols were investigated along with water and 4-Methyl-2-Pentanone (MIBK). The solutions were prepared by adding 0.5 ml of an 100 μg/ml manganese stock solution into a 25 ml volumetric flask, diluting to the mark with the solvent, inverting a minimum of 40 times, and storing the solutions in plastic bottles.

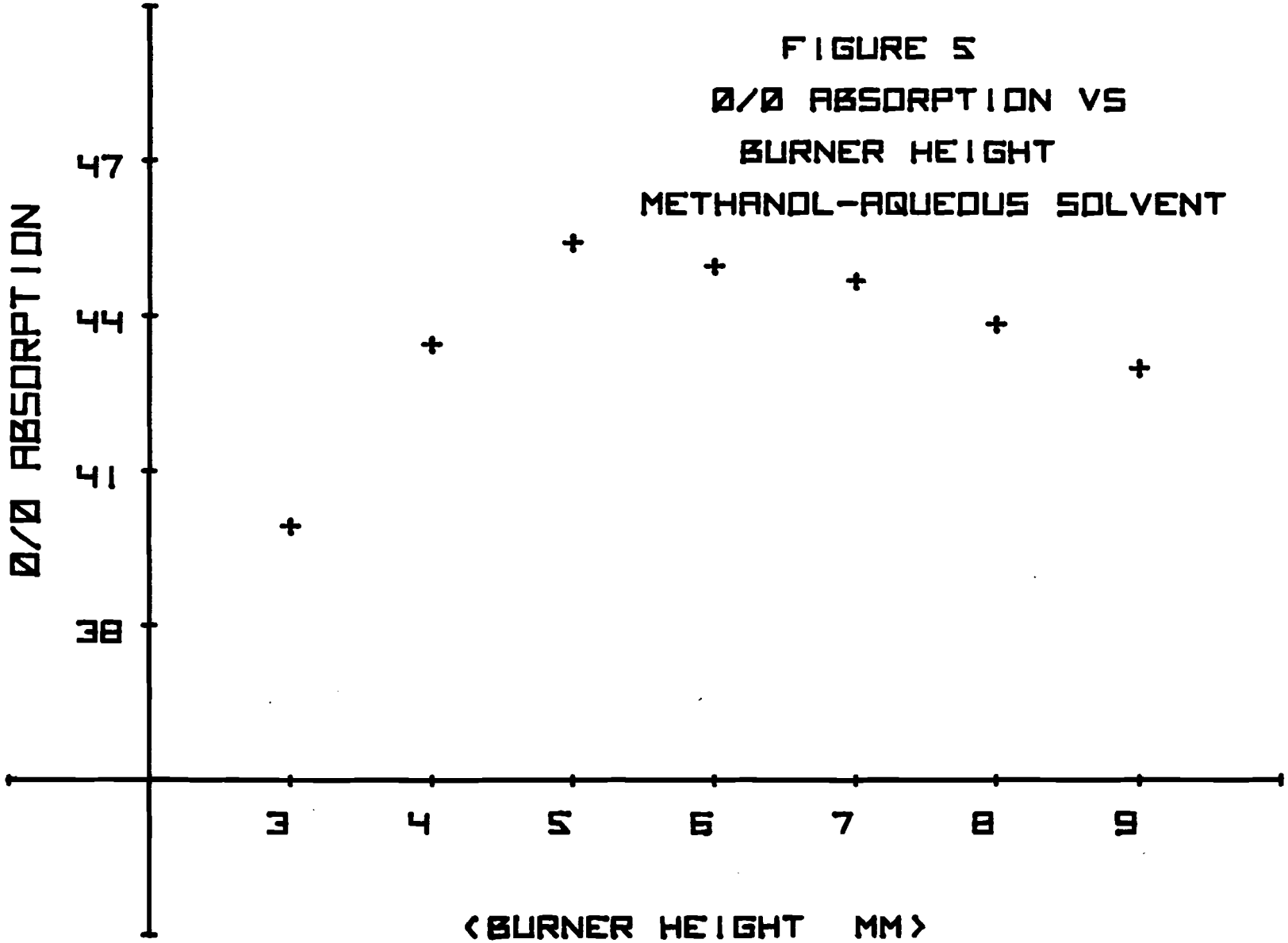


FIGURE 5
Ø/Ø ABSORPTION VS
BURNER HEIGHT
METHANOL-AQUEOUS SOLVENT
Ø/Ø ABSORPTION
47
44
41
38
3
4
5
6
7
8
9
(BURNER HEIGHT MM)

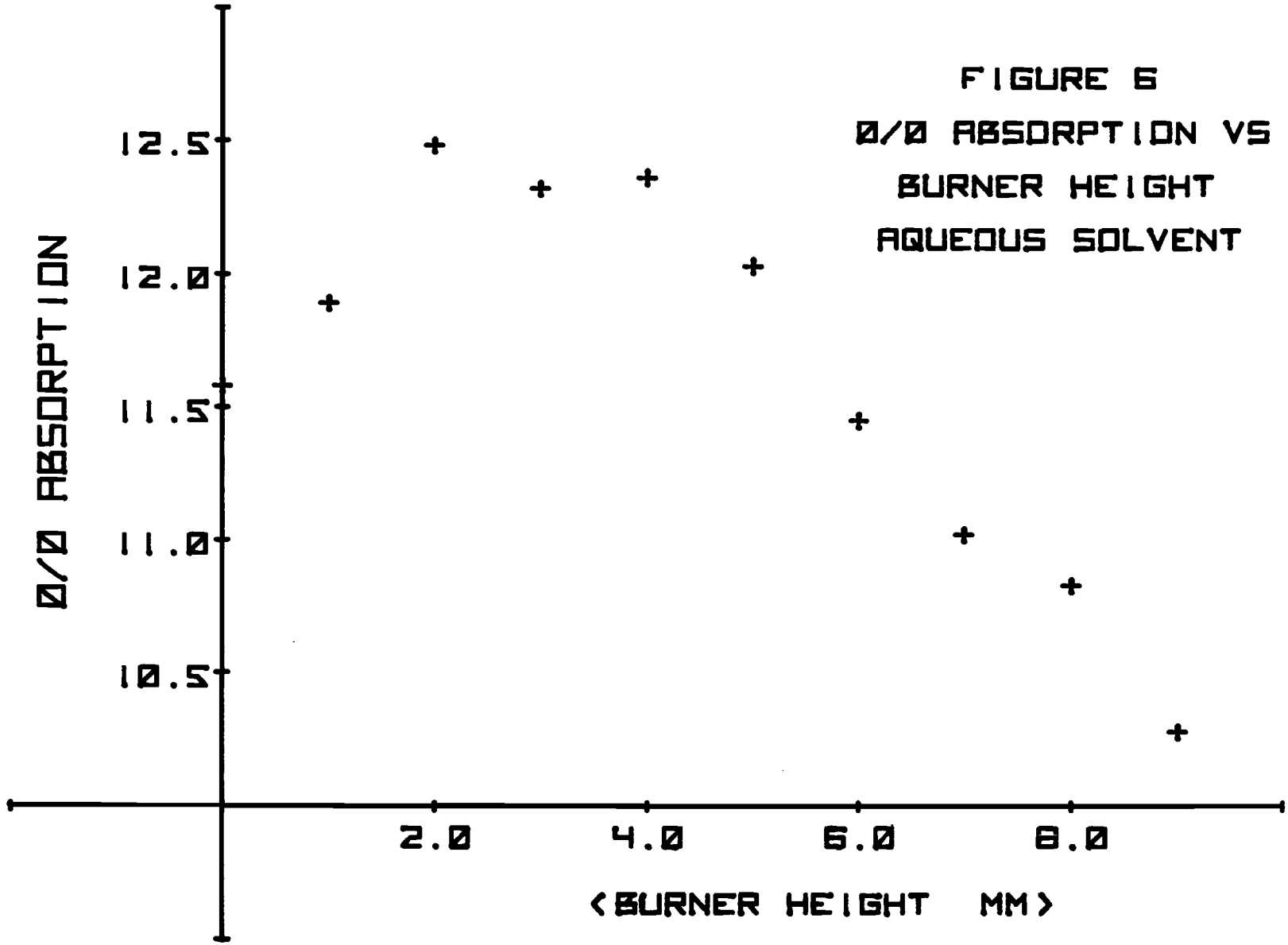


FIGURE 6
Ø/Ø ABSORPTION VS
BURNER HEIGHT
AQUEOUS SOLVENT
Ø/Ø ABSORPTION
12.5
12.Ø
11.5
11.Ø
1Ø.5
2.Ø
4.Ø
6.Ø
8.Ø
<BURNER HEIGHT MM>

In Figure 7 the results for the percentage absorption of manganese in various solvents are shown. The solvent Methanol as shown in this figure and in Table 8, was found to give the highest absorbance of all the solvents analyzed. The solvent Ethanol was found to give the second highest with 1-Propanol the third highest and etc. in order of increasing length of the hydrocarbon chain.

The solvent's physical properties affect the rate at which the solution is introduced into the flame. This property is called the aspiration rate of the solvent. In Figure 8 the absorbances of manganese contained in each of the solvents are plotted against the aspiration rate of the solvents. Methanol showed a higher aspiration rate than any of the other alcohols or water. MIBK showed a higher aspiration rate than methanol, but it also showed a lower absorbance value for manganese. The absorbance value obtained in the MIBK solvent is believed to be that of the aqueous solvent which contained the manganese ions, and the high aspiration rate due to the hydrophobic nature of the solvent.

In order to determine the reasons behind why the methanol solvent enhanced the absorbance of manganese higher than other solvents, a study was done to determine what physical properties enhance the absorption of manganese. Seven physical properties were selected for study: the Dielectric Constant at 25°C; the Viscosity at 25°C; the Surface Tension at 20°C; the Heat of Combustion; the Vapor Pressure, the Density and the Boiling Point of the pure solvent. To analyze the relationship between absorbance and the physical properties a series of plots were prepared as Absorbance vs X, X^2, $X^{1/2}$, 1/X, log X, and others where X represents the physical property of the pure solvent.

Table 8

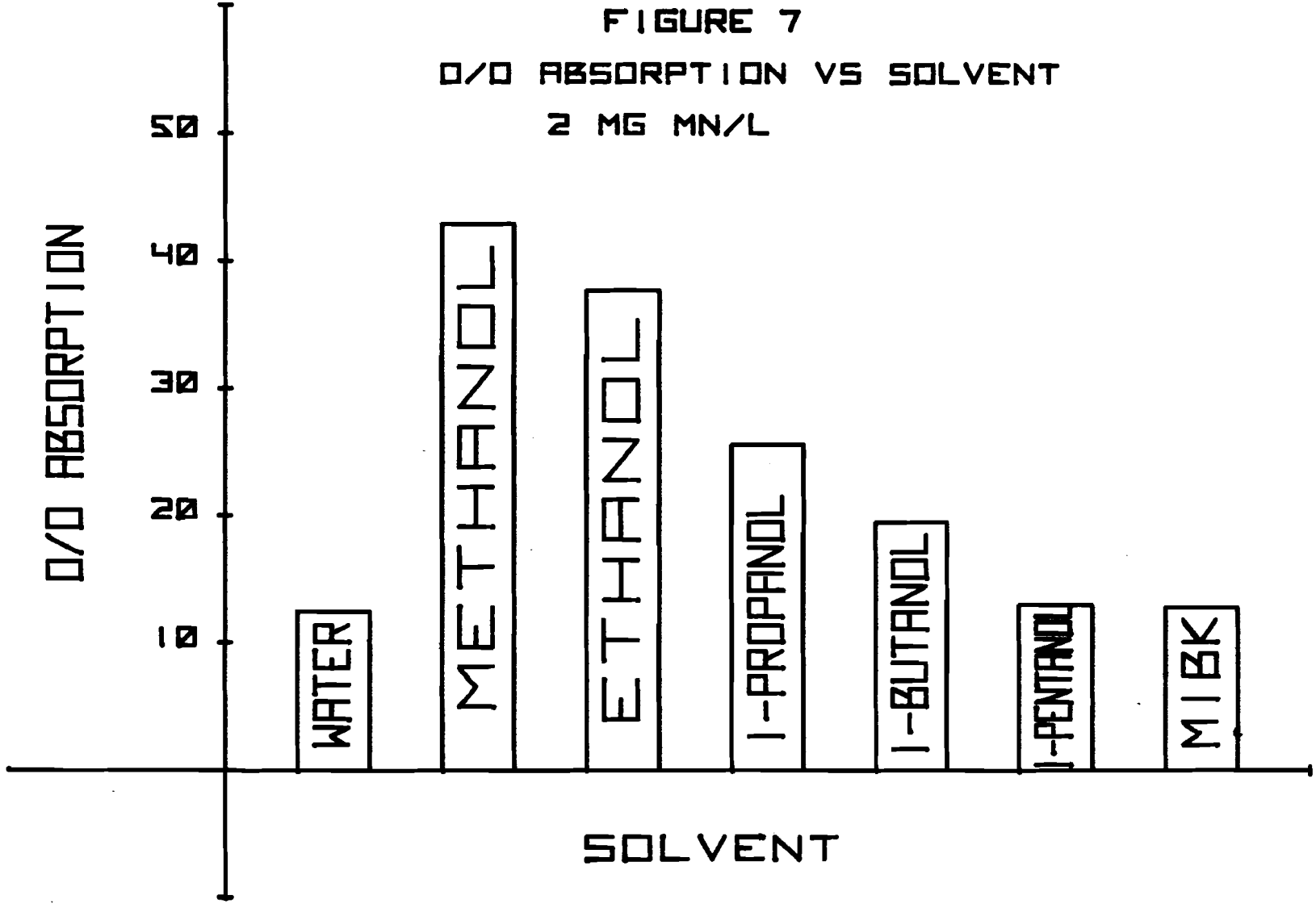
COMPARISON OF SOLVENTS ON THE ABSORPTION OF MANGANESE

	% Absorption	Absorbance	Aspiration Rate
Methanol	42.88	0.243	4.80 l/min
Ethanol	37.68	0.205	3.18
1-Propanol	25.60	0.129	2.09
1-Butanol	19.47	0.094	1.78
1-Pentanol	13.01	0.061	1.30
4-Methyl-2-Pentanone	12.77	0.059	5.07
Water	12.46	0.058	4.40

FIGURE 7

O/O ABSORPTION VS SOLVENT

2 MG MN/L



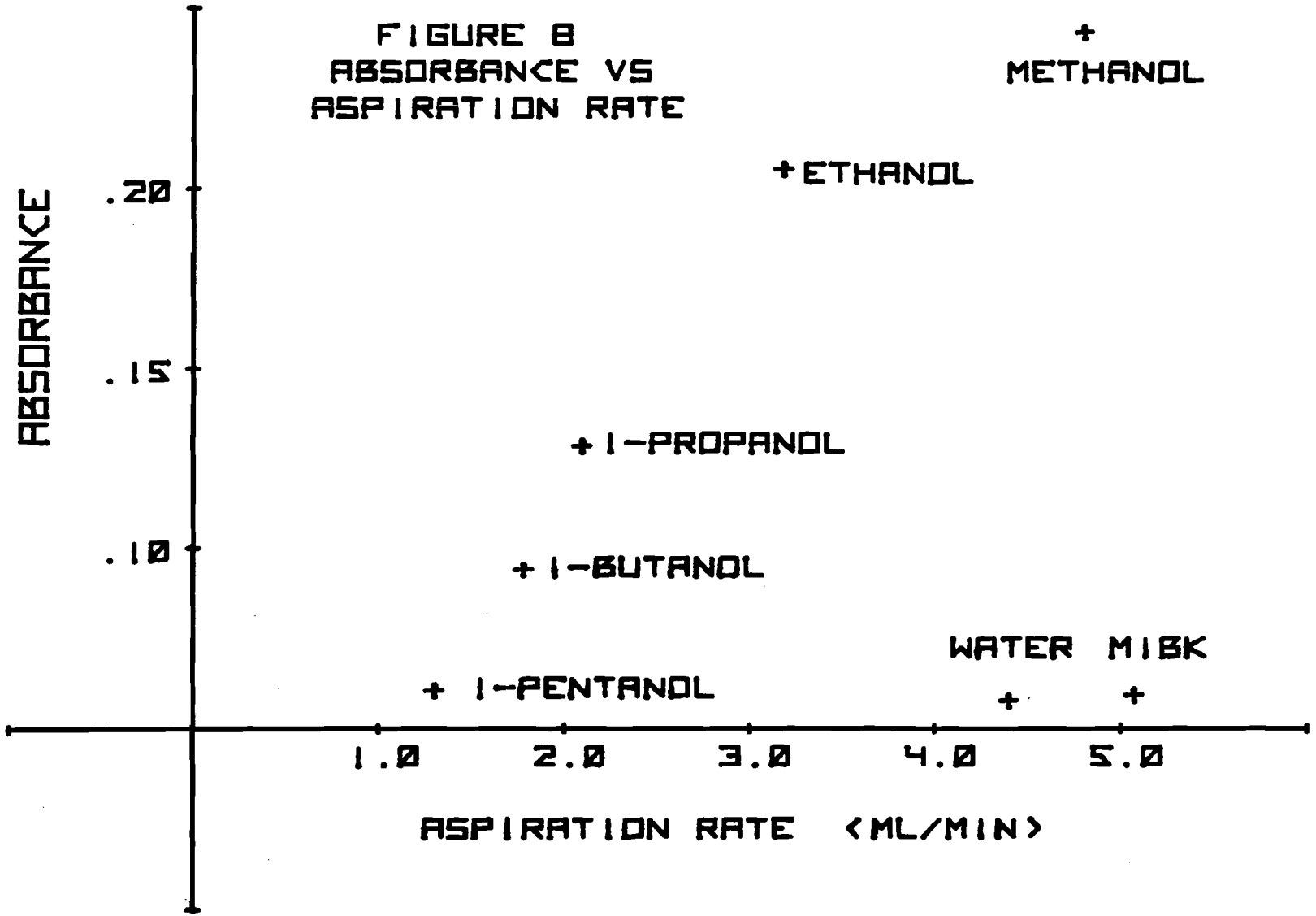


FIGURE 8
ABSORBANCE VS
ASPIRATION RATE
METHANOL
ETHANOL
1-PROPANOL
1-BUTANOL
WATER
MIBK
1-PENTANOL
ABSORBANCE
.20
.15
.10
1.0
2.0
3.0
4.0
5.0
ASPIRATION RATE (ML/MIN)

A number of different plots were prepared that showed a linear relationship with the absorbance of manganese. These plots are shown in figures 9 through 29. Other variables were tried and were found not to give a linear relationship and are presented in Table 9. The physical properties of the solvents are listed in Table 10.

Feldman, Bosshart, and Christian[23] reported a linear relationship could be obtained by plotting the absorbance of a 100 ppb manganese solution in various organic solvents against the product of the density and viscosity of the solvent. This plot is shown in Figure 10 for the solvents used in this study. The plot showed a linear relationship up to 1-Pentanol where it deviated from the other alcohols slightly. This deviation was taken to be a result of the solubility of the compound in the water.

Calibration Curves for Manganese by AAS and AOAC Methods

The calibration curve obtained for the Atomic Absorption Spectroscopy determination of manganese is shown in Figure 30. This plot shows two calibration curves one for manganese in a methanol-aqueous solvent (99%-1%) and the other using water as a solvent. The calibration curve using the methanol-aqueous solvent was found linear over a 100-fold concentration range and the one obtained using the water solvent was linear over a 10-fold concentration range. The enhancement of absorbance in the methanol-aqueous solvent by manganese was approximately 6 to 1 over the aqueous solvent. The sensitivity in the methanol-aqueous solution was 30 μg/ml and that obtained using water was 220 μg/ml. The concentration range for this

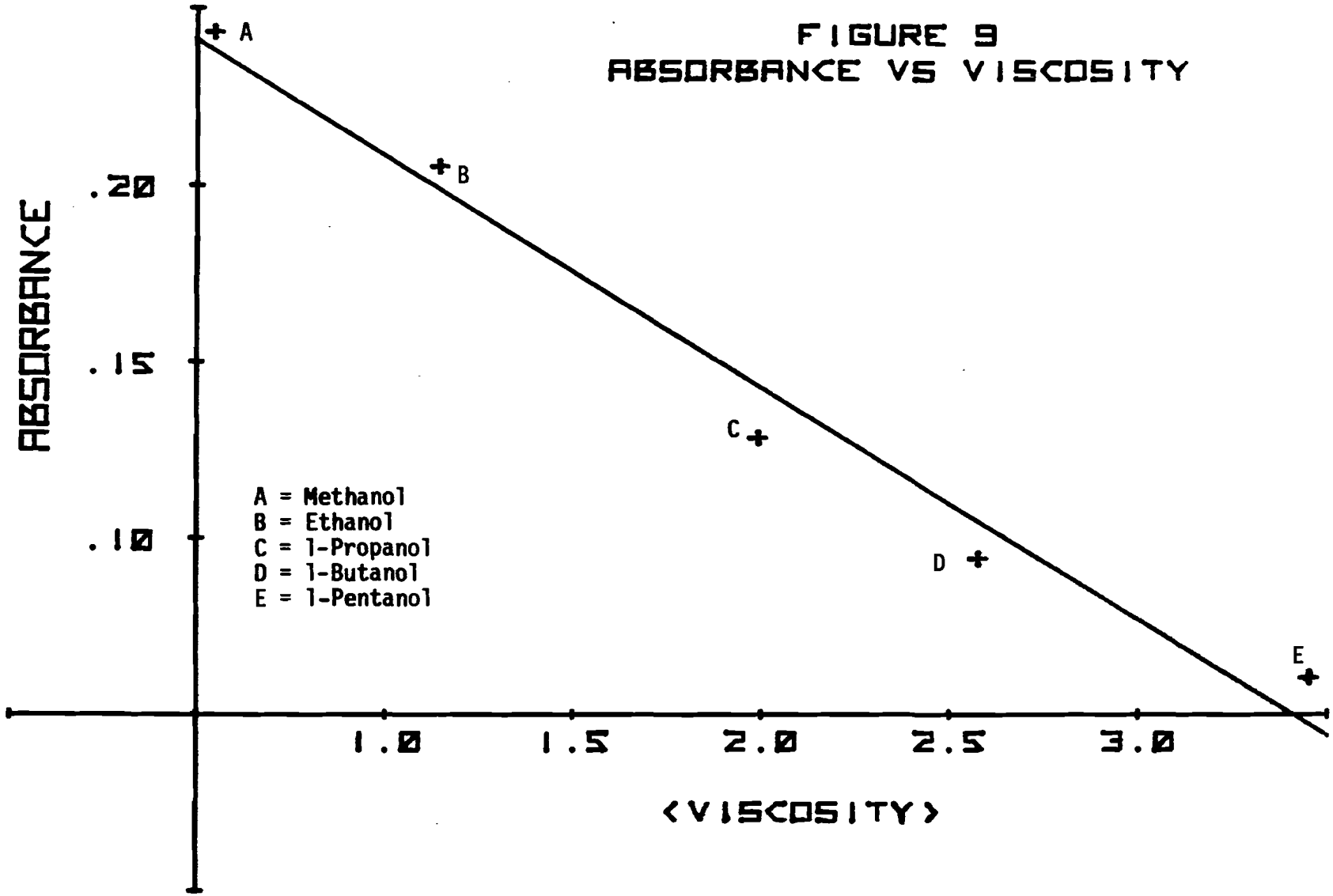


FIGURE 9
ABSORBANCE VS VISCOSITY
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
1.0
1.5
2.0
2.5
3.0
<VISCOSITY>

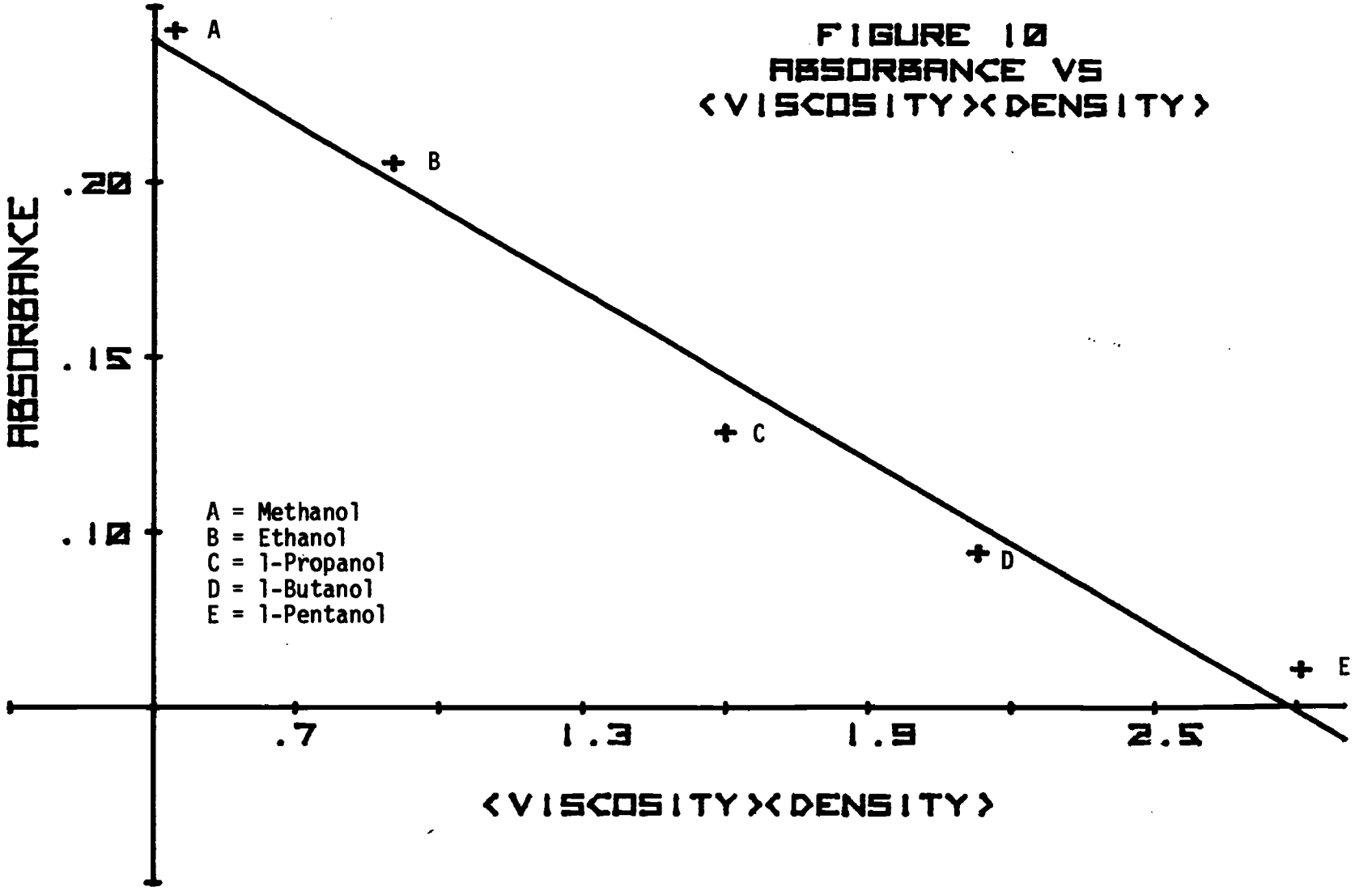


FIGURE 10
ABSORBANCE VS
<VISCOSITY><DENSITY>
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
.7
1.3
1.9
2.5
<VISCOSITY><DENSITY>

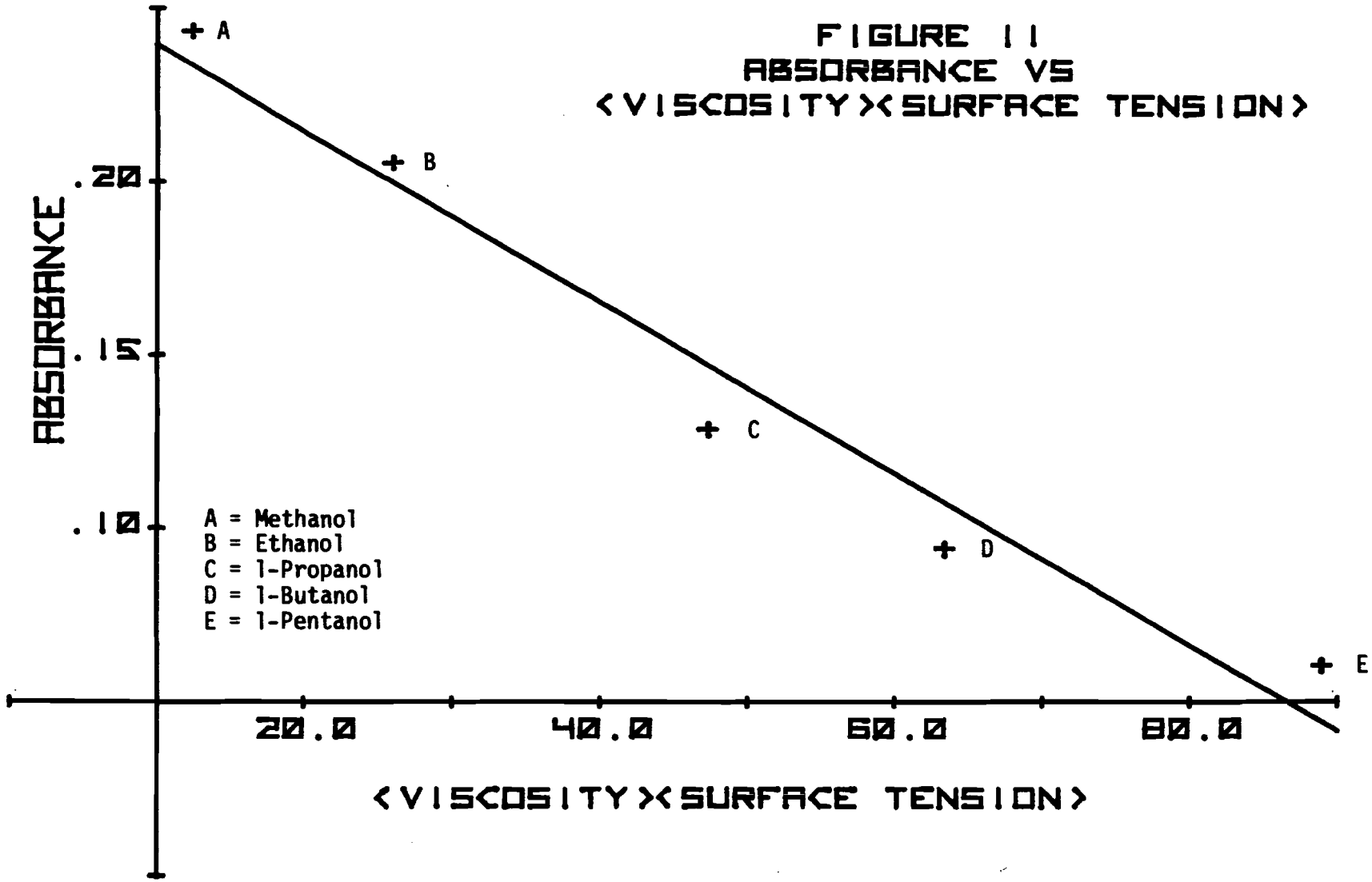


FIGURE 11
ABSORBANCE VS
<VISCOSITY><SURFACE TENSION>
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
20.0
40.0
60.0
80.0
<VISCOSITY><SURFACE TENSION>

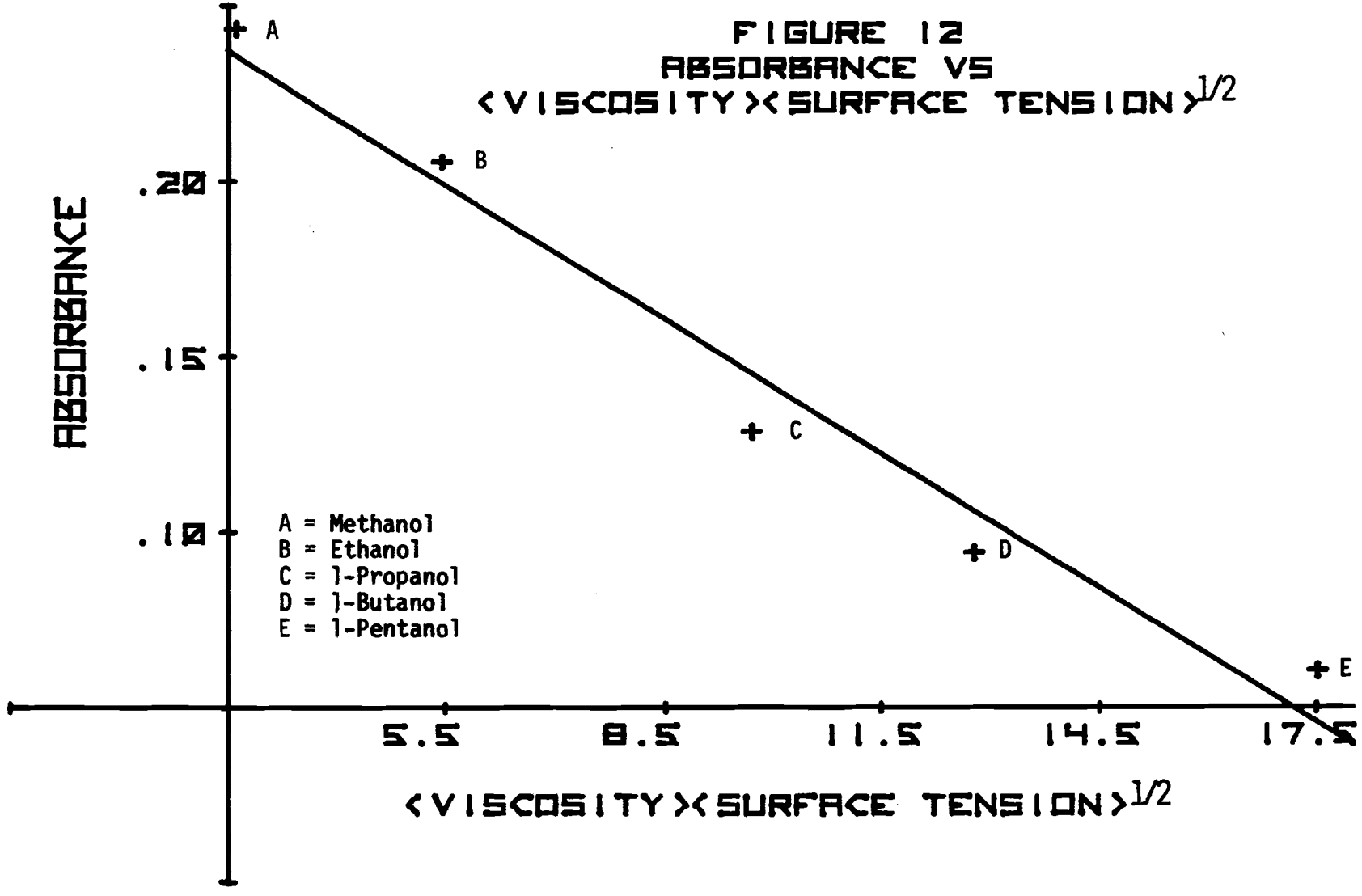


FIGURE 12
ABSORBANCE VS
<VISCOSITY><SURFACE TENSION>1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
5.5
8.5
11.5
14.5
17.5
<VISCOSITY><SURFACE TENSION>1/2

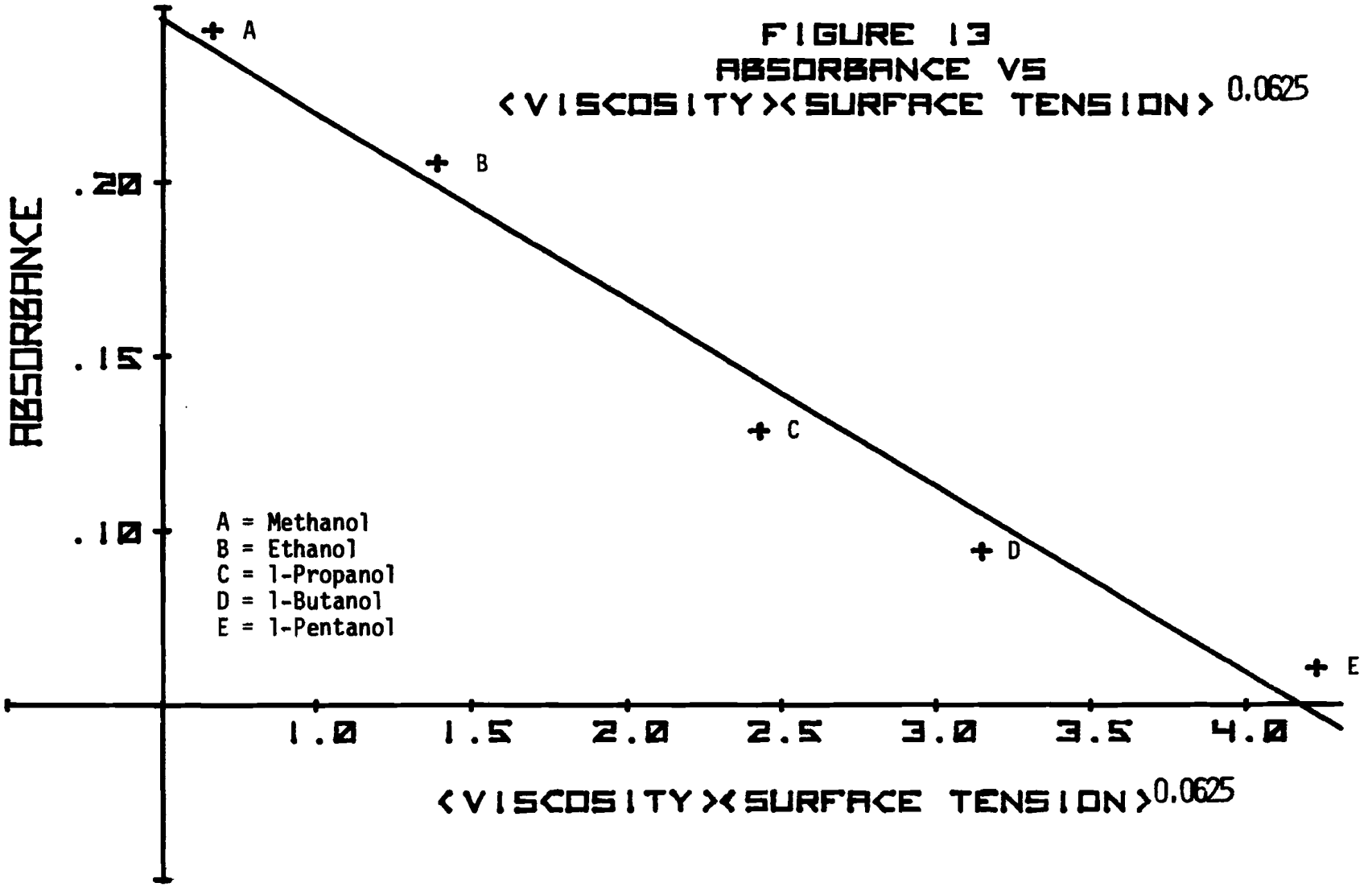


FIGURE 13
ABSORBANCE VS
<VISCOSITY><SURFACE TENSION>0.0625
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
1.0
1.5
2.0
2.5
3.0
3.5
4.0
<VISCOSITY><SURFACE TENSION>0.0625

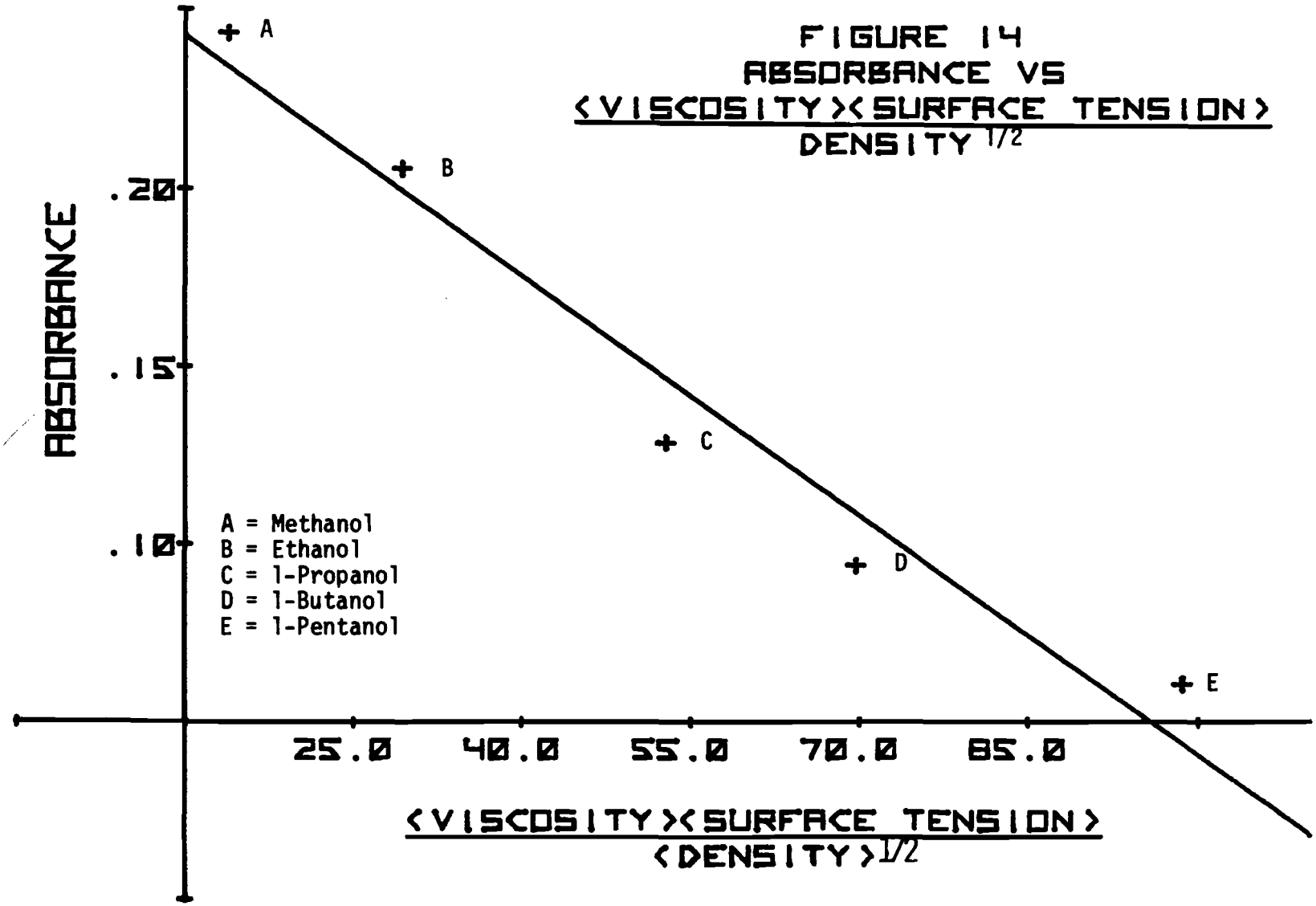


FIGURE 14
ABSORBANCE VS
⟨VISCOSITY⟩⟨SURFACE TENSION⟩ / DENSITY 1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
25.0
40.0
55.0
70.0
85.0
⟨VISCOSITY⟩⟨SURFACE TENSION⟩ / ⟨DENSITY⟩1/2

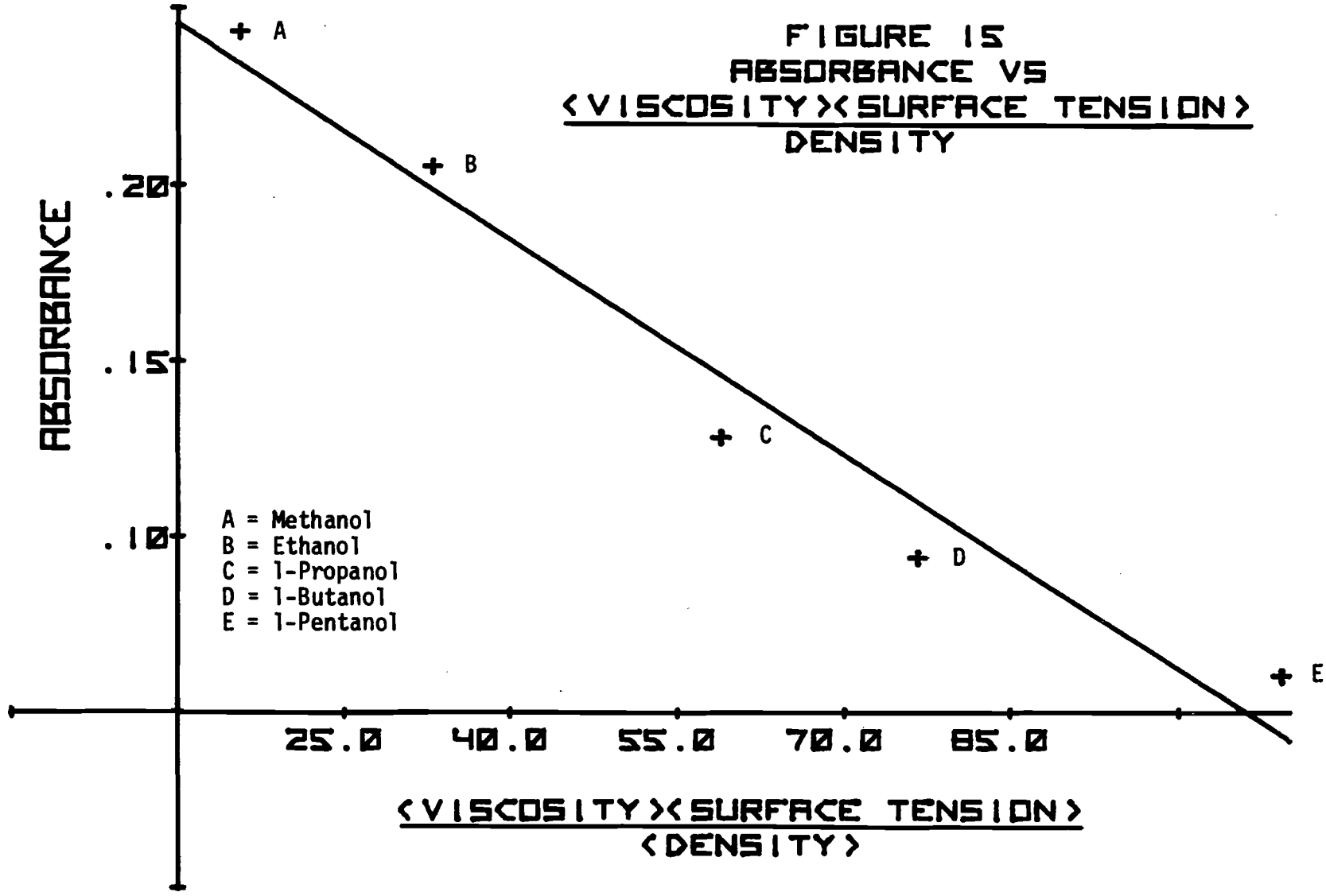


FIGURE 15
ABSORBANCE VS
<VISCOSITY><SURFACE TENSION>
DENSITY
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
25.0
40.0
55.0
70.0
85.0
<VISCOSITY><SURFACE TENSION>
<DENSITY>

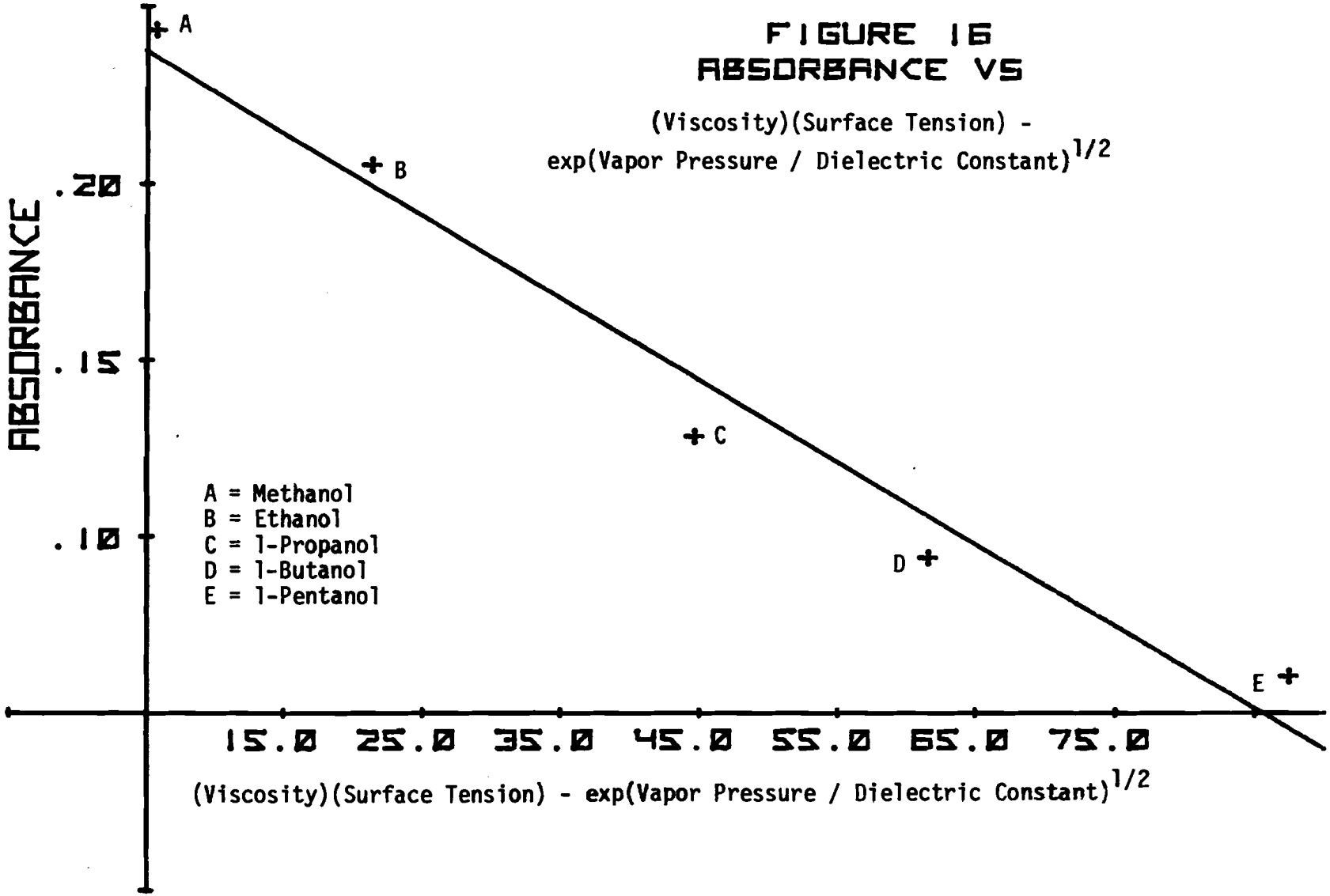


FIGURE 16
ABSORBANCE VS
(Viscosity)(Surface Tension) -
exp(Vapor Pressure / Dielectric Constant)^1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
15.0 25.0 35.0 45.0 55.0 65.0 75.0
(Viscosity)(Surface Tension) - exp(Vapor Pressure / Dielectric Constant)^1/2

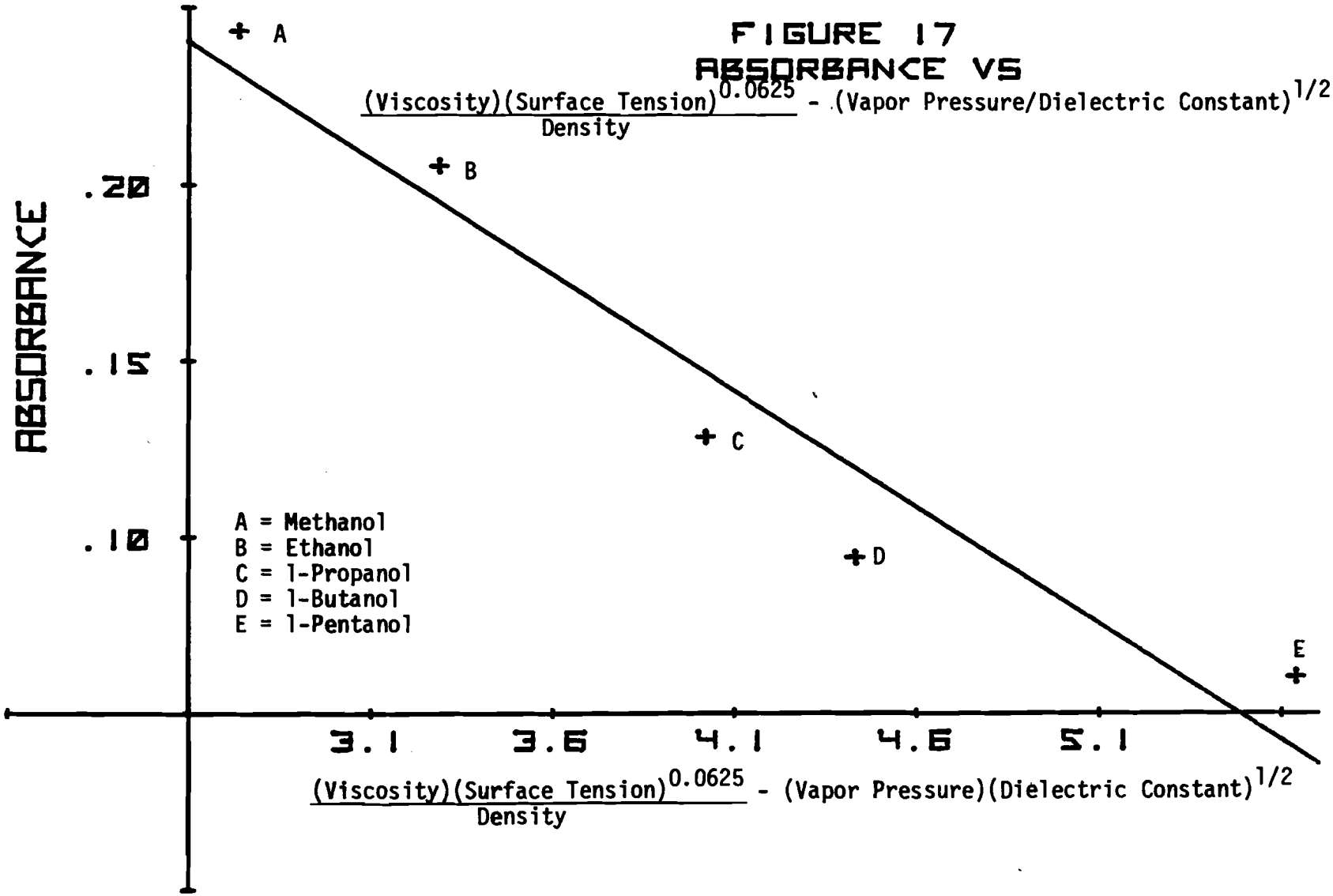


FIGURE 17
ABSORBANCE VS
(Viscosity)(Surface Tension)^0.0625 / Density - (Vapor Pressure/Dielectric Constant)^1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
3.1
3.6
4.1
4.6
5.1
(Viscosity)(Surface Tension)^0.0625 / Density - (Vapor Pressure)(Dielectric Constant)^1/2

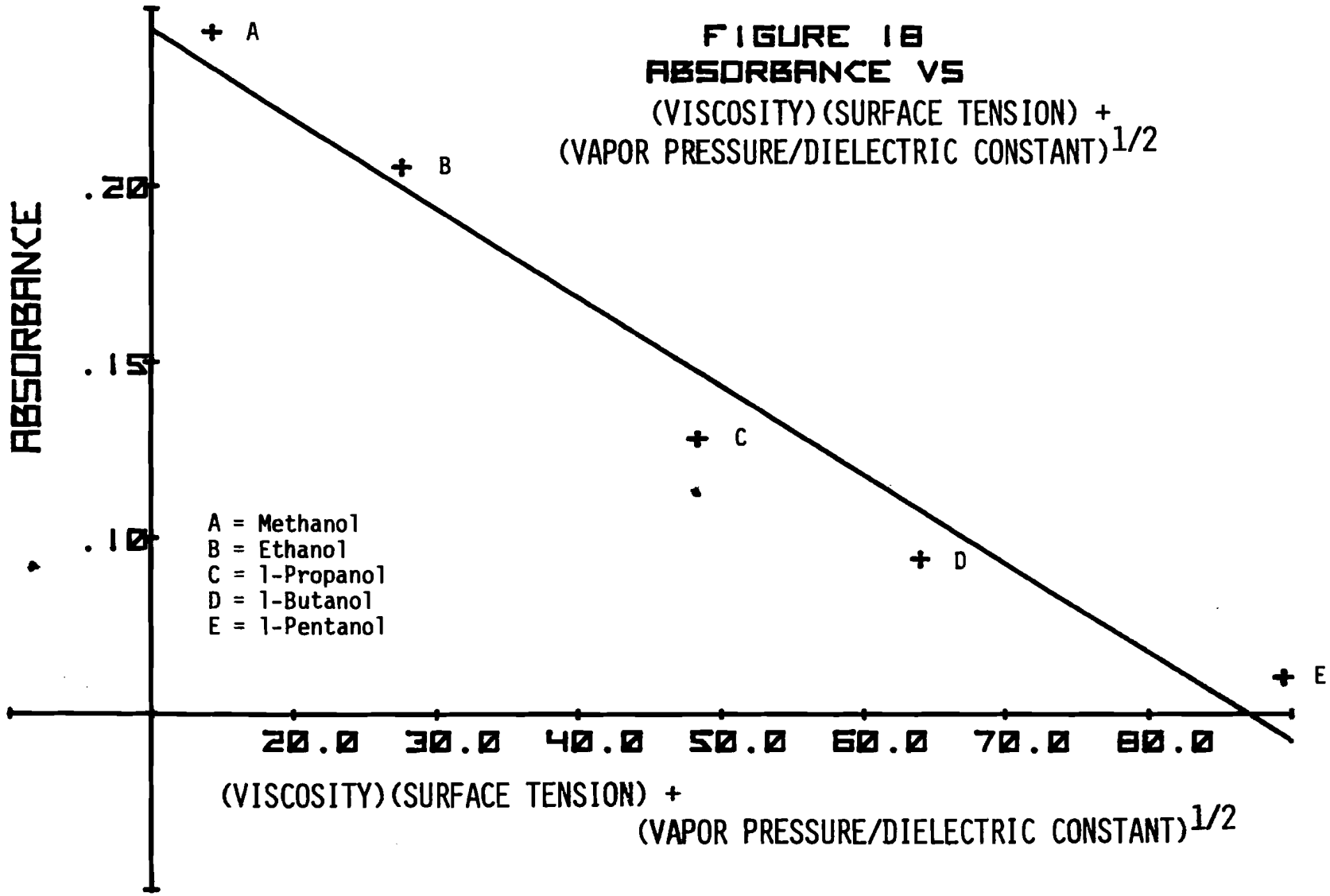


FIGURE 18
ABSORBANCE VS
(VISCOSITY)(SURFACE TENSION) +
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
20.0
30.0
40.0
50.0
60.0
70.0
80.0
(VISCOSITY)(SURFACE TENSION) +
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2

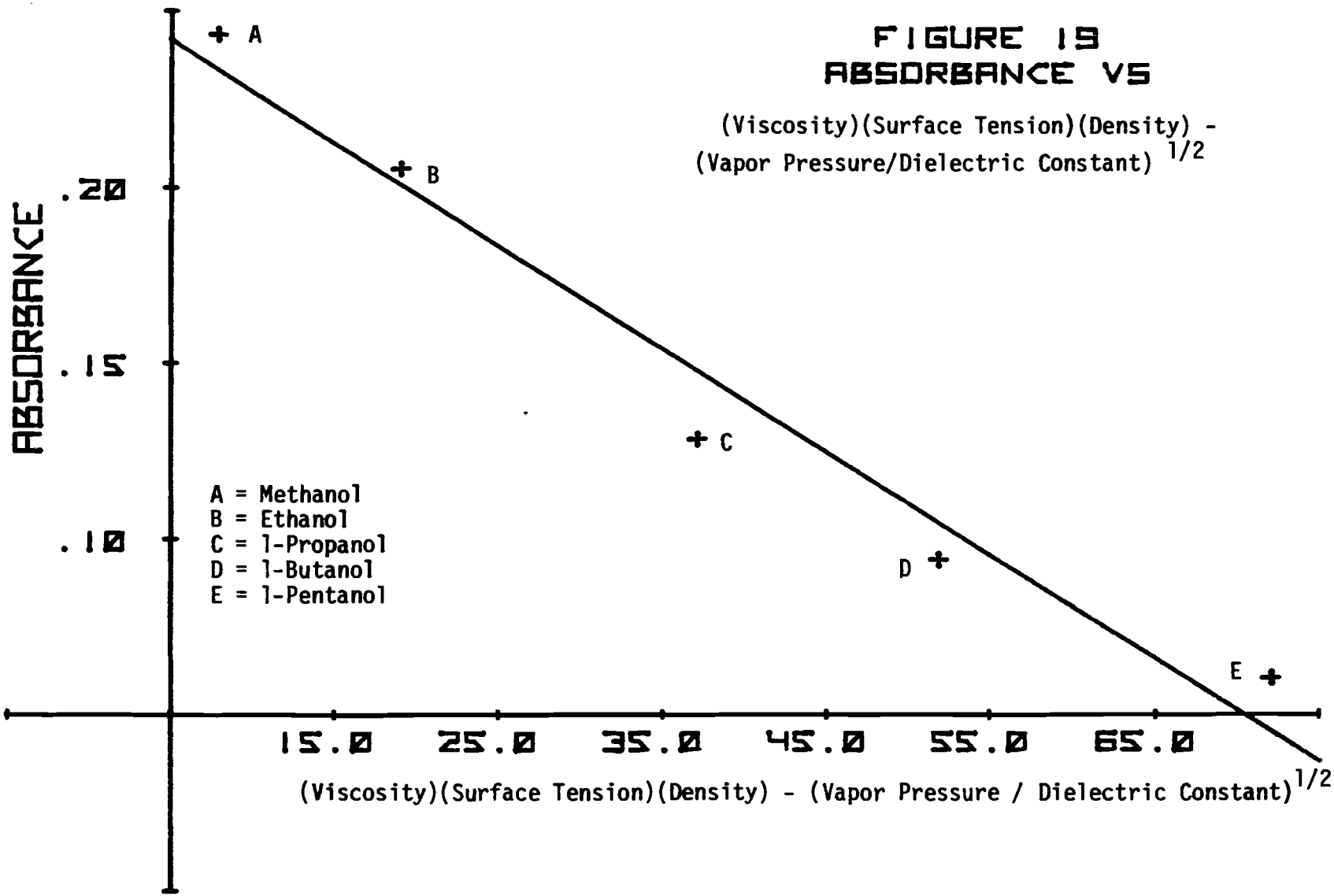


FIGURE 19
ABSORBANCE VS
(Viscosity)(Surface Tension)(Density) -
(Vapor Pressure/Dielectric Constant) 1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
15.0
25.0
35.0
45.0
55.0
65.0
(Viscosity)(Surface Tension)(Density) - (Vapor Pressure / Dielectric Constant)1/2

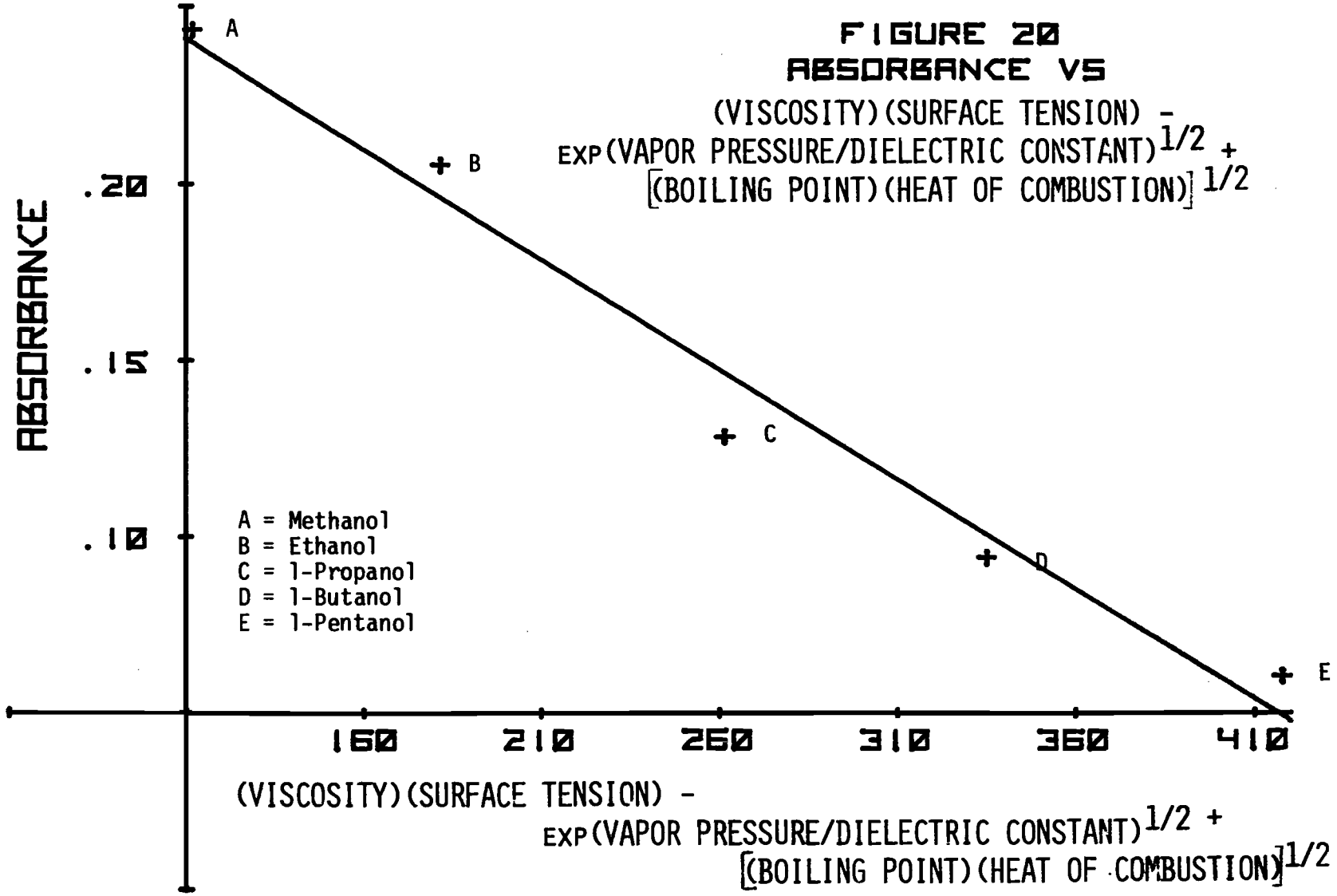


FIGURE 20
ABSORBANCE VS
(VISCOSITY)(SURFACE TENSION) -
EXP(VAPOR PRESSURE/DIELECTRIC CONSTANT)^1/2 +
[(BOILING POINT)(HEAT OF COMBUSTION)]^1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
160
210
260
310
360
410
(VISCOSITY)(SURFACE TENSION) -
EXP(VAPOR PRESSURE/DIELECTRIC CONSTANT)^1/2 +
[(BOILING POINT)(HEAT OF COMBUSTION)]^1/2

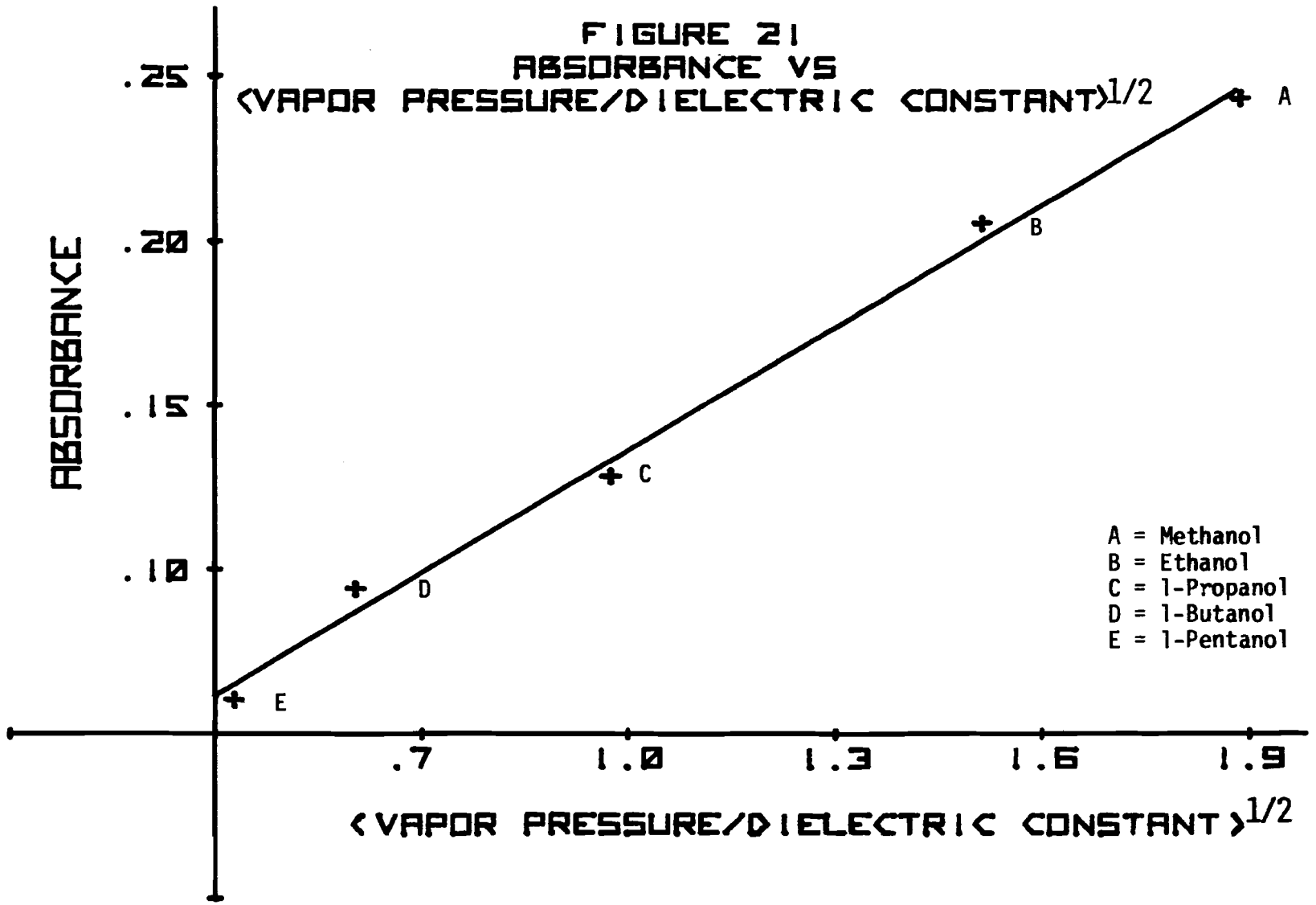


FIGURE 21
ABSORBANCE VS
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2
ABSORBANCE
.25
.20
.15
.10
.7
1.0
1.3
1.6
1.9
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2

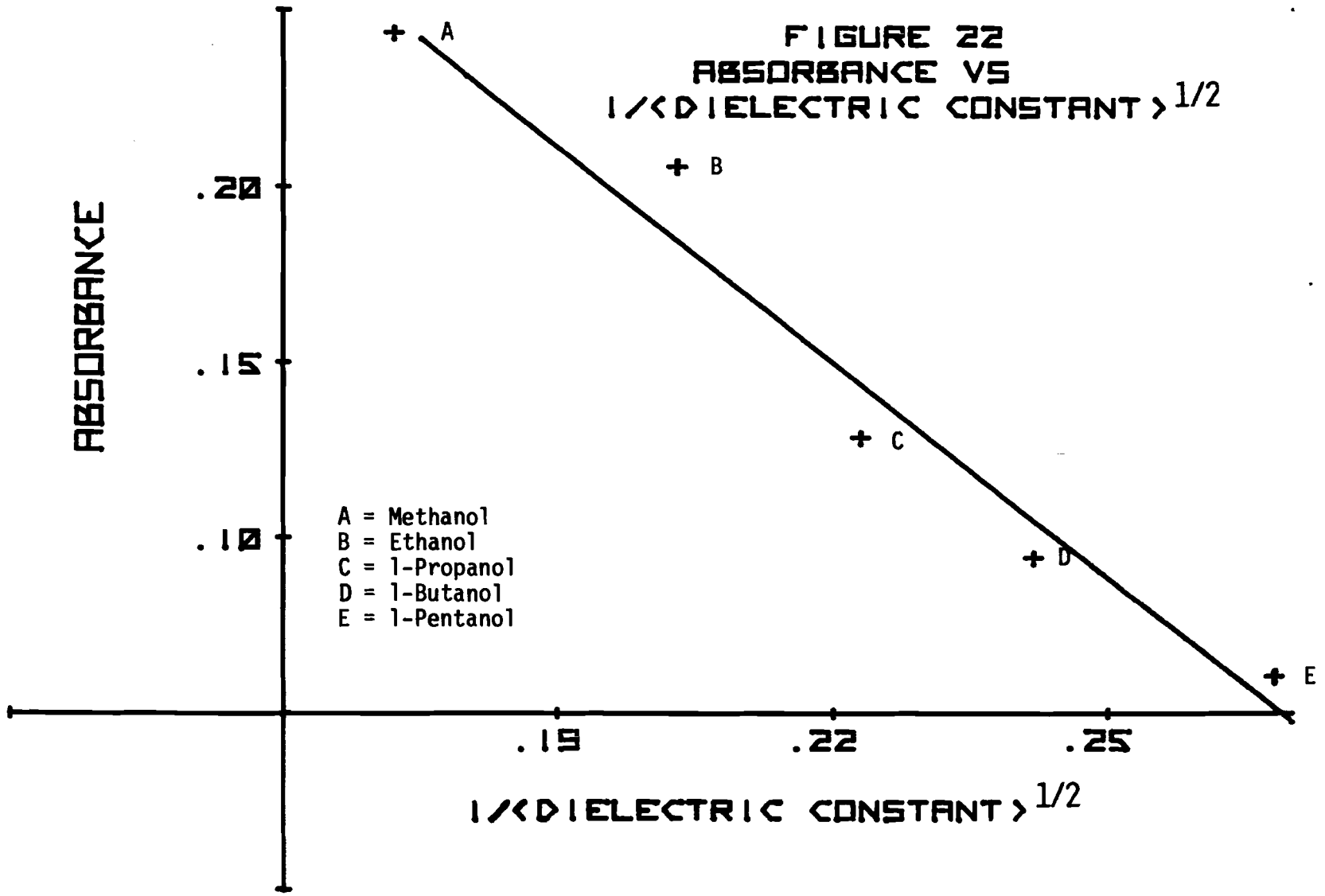


FIGURE 22
ABSORBANCE VS
1/<DIELECTRIC CONSTANT>1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
.19
.22
.25
1/<DIELECTRIC CONSTANT>1/2

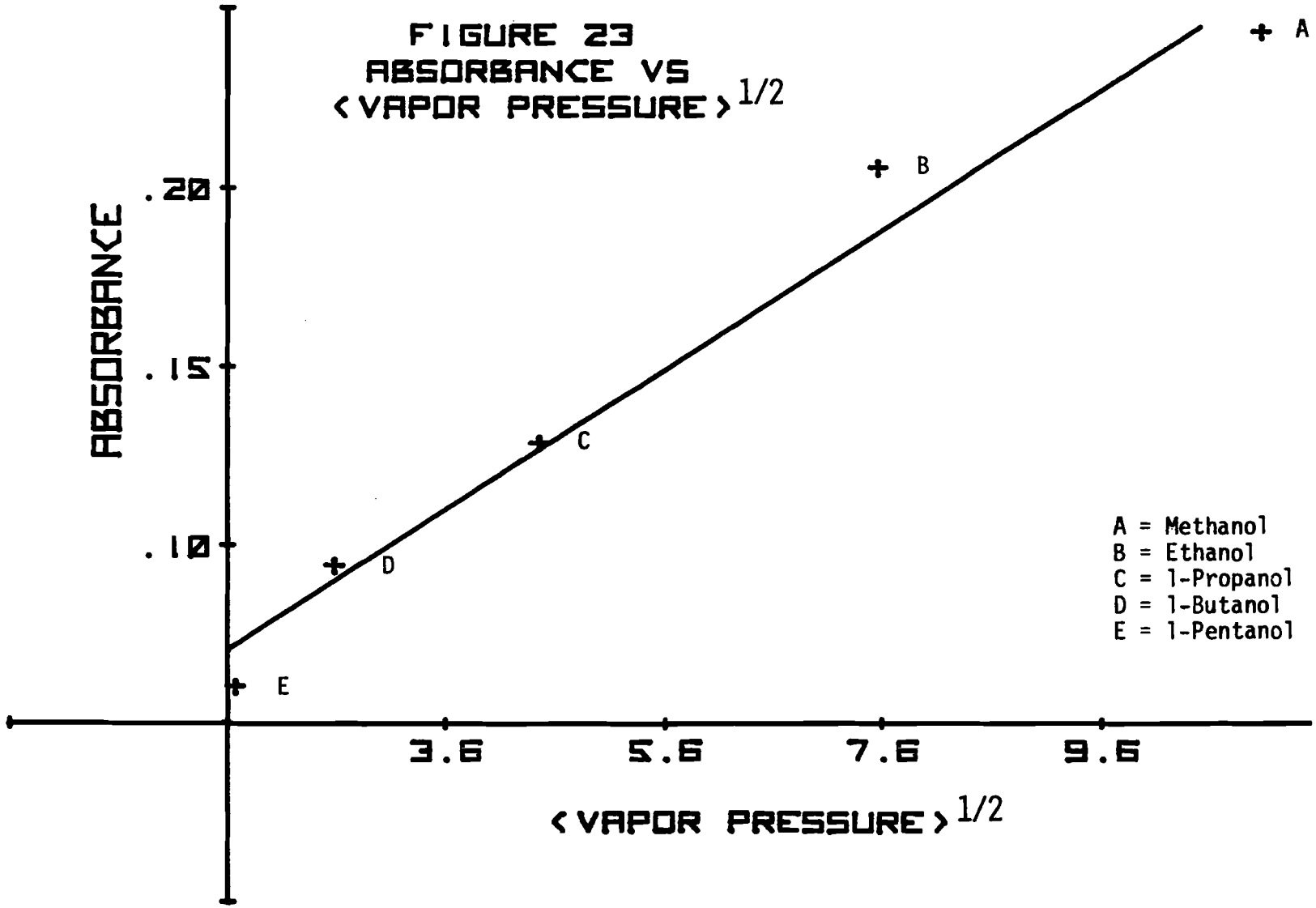


FIGURE 23
ABSORBANCE VS
<VAPOR PRESSURE>1/2
ABSORBANCE
.20
.15
.10
A
B
C
D
E
3.6
5.6
7.6
9.6
<VAPOR PRESSURE>1/2
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol

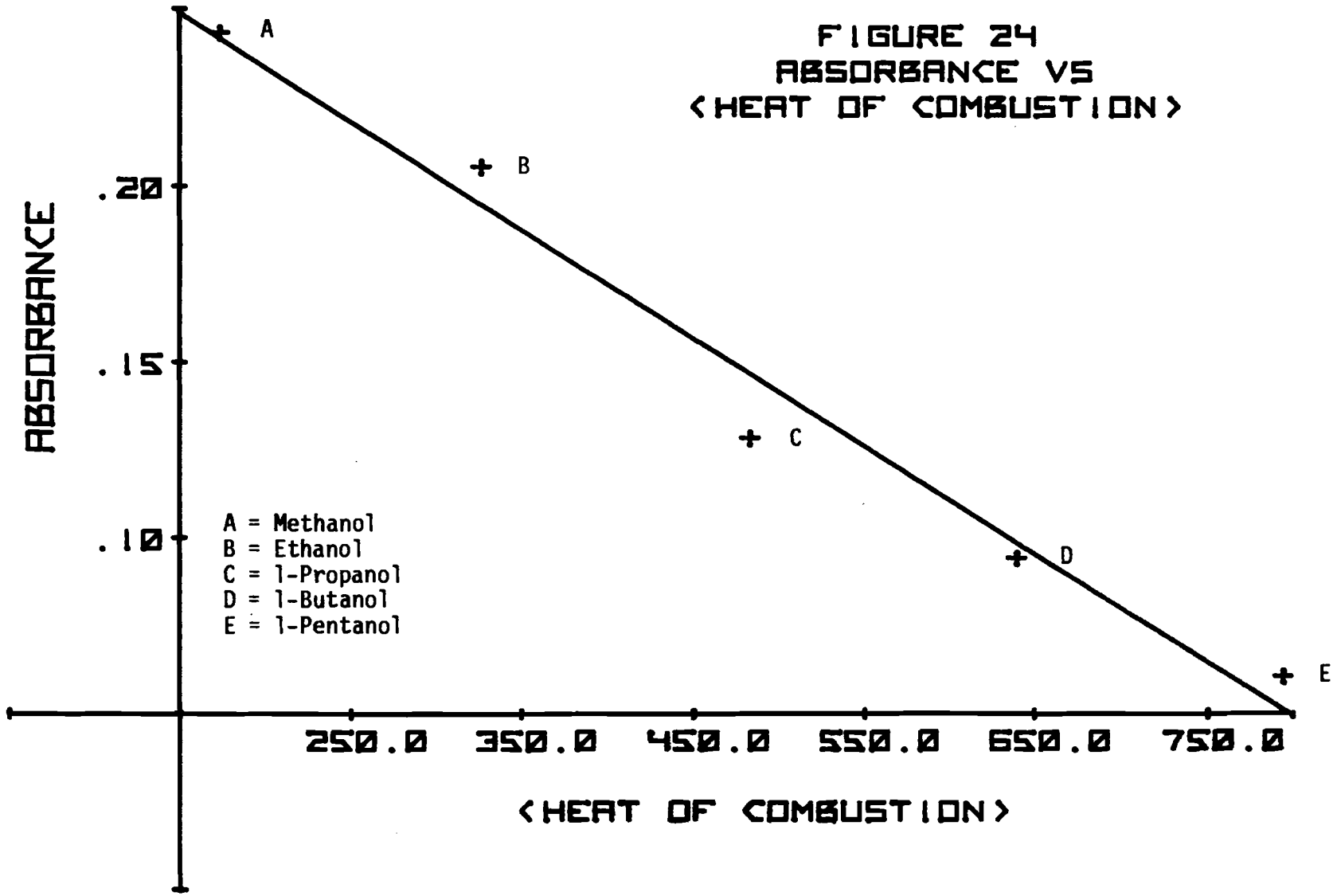


FIGURE 24
ABSORBANCE VS
<HEAT OF COMBUSTION>
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
250.0
350.0
450.0
550.0
650.0
750.0
<HEAT OF COMBUSTION>

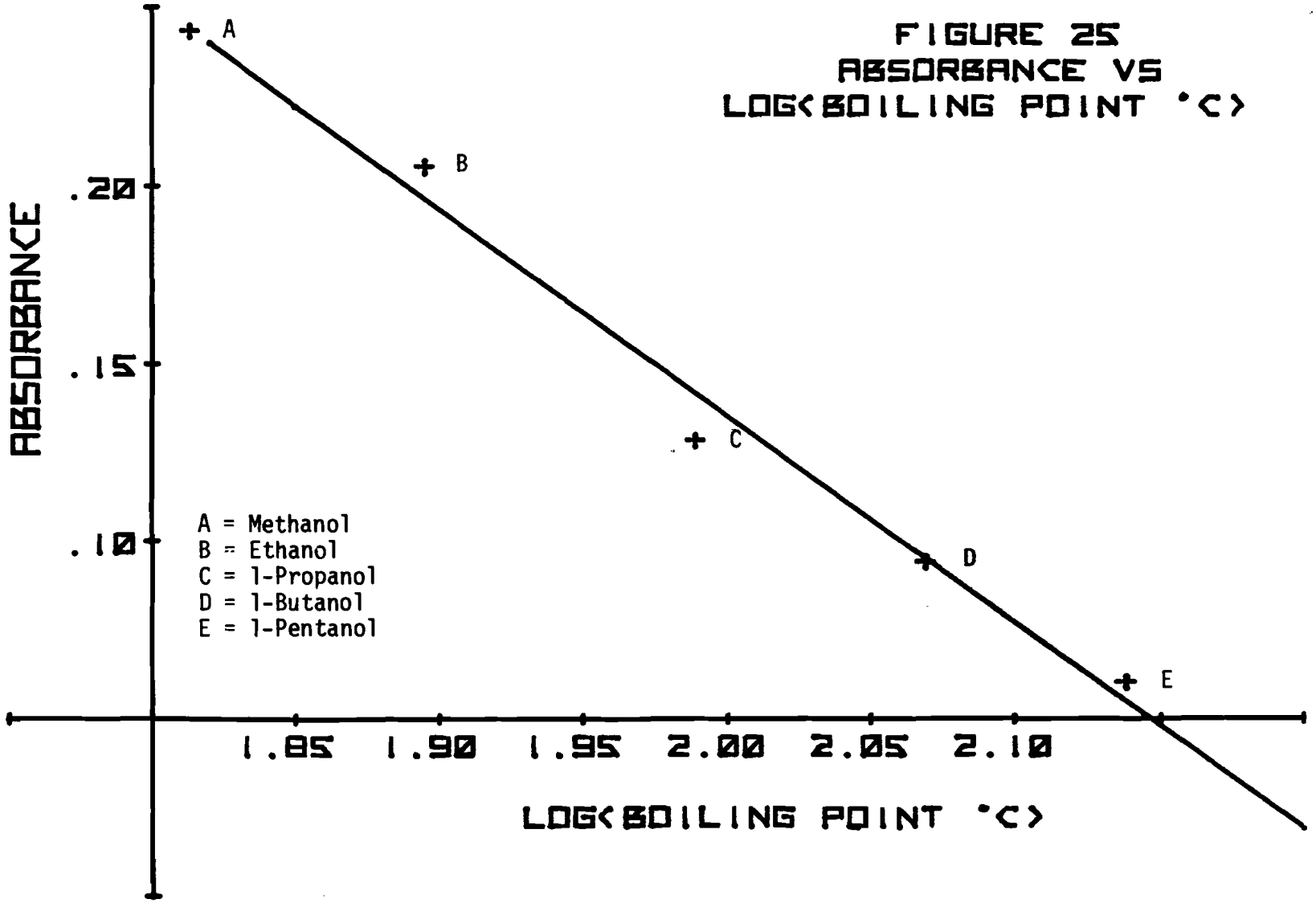


FIGURE 25
ABSORBANCE VS
LOG(BOILING POINT °C)
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
1.85
1.90
1.95
2.00
2.05
2.10
LOG(BOILING POINT °C)

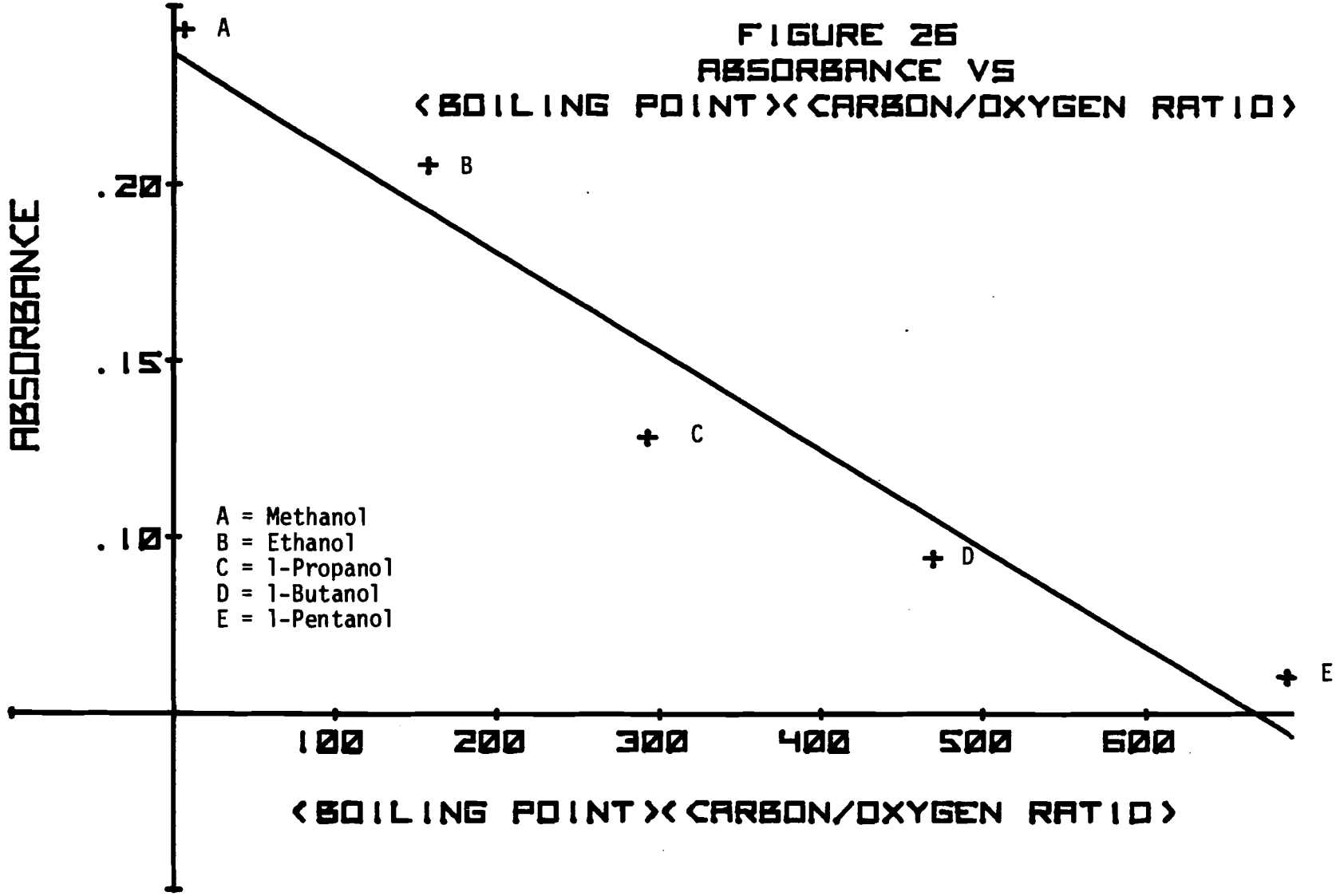


FIGURE 26
ABSORBANCE VS
(BOILING POINT)(CARBON/OXYGEN RATIO)
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
100
200
300
400
500
600
(BOILING POINT)(CARBON/OXYGEN RATIO)

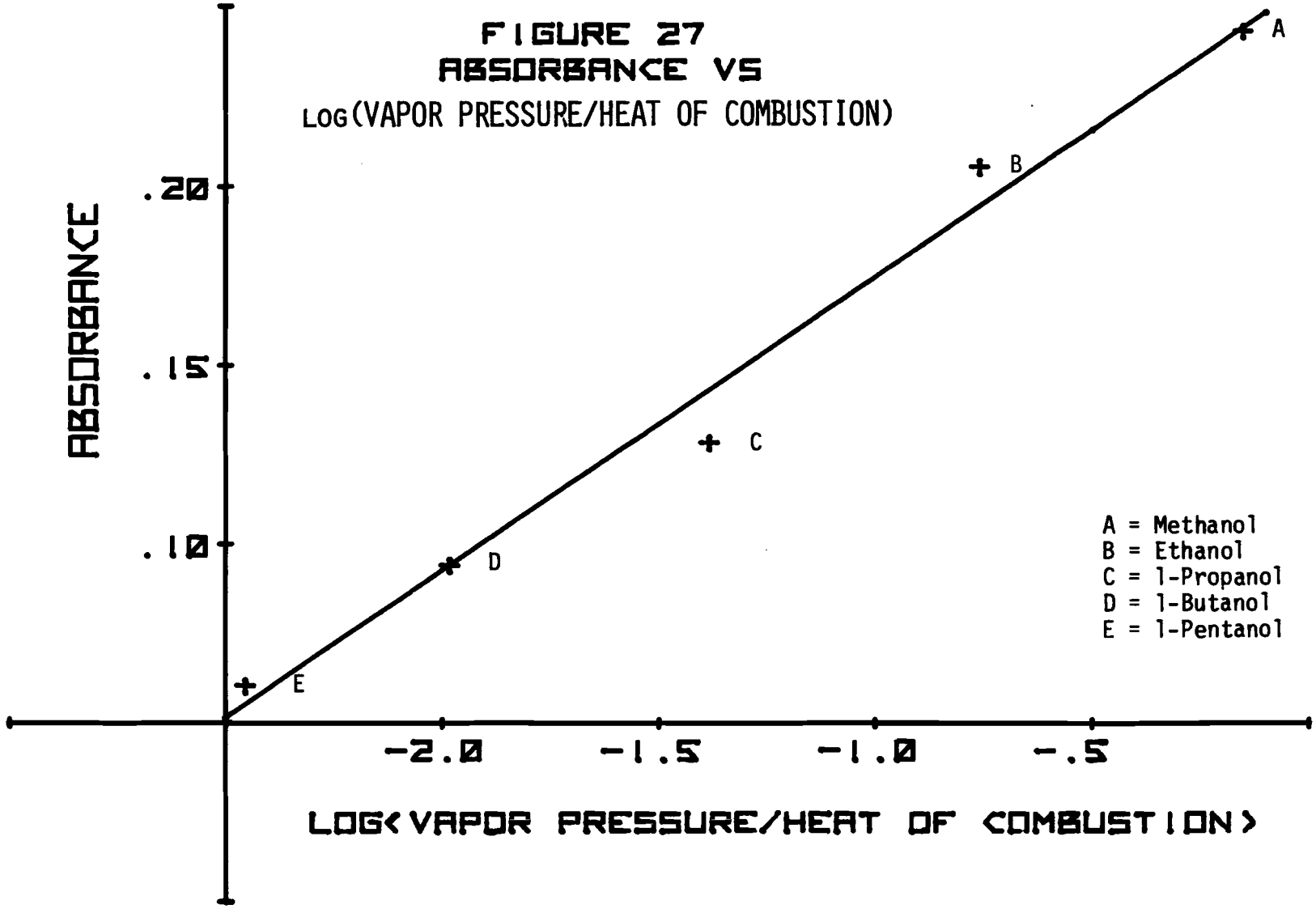


FIGURE 27
ABSORBANCE VS
LOG(VAPOR PRESSURE/HEAT OF COMBUSTION)
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
-2.0
-1.5
-1.0
-.5
LOG< VAPOR PRESSURE/HEAT OF COMBUSTION>

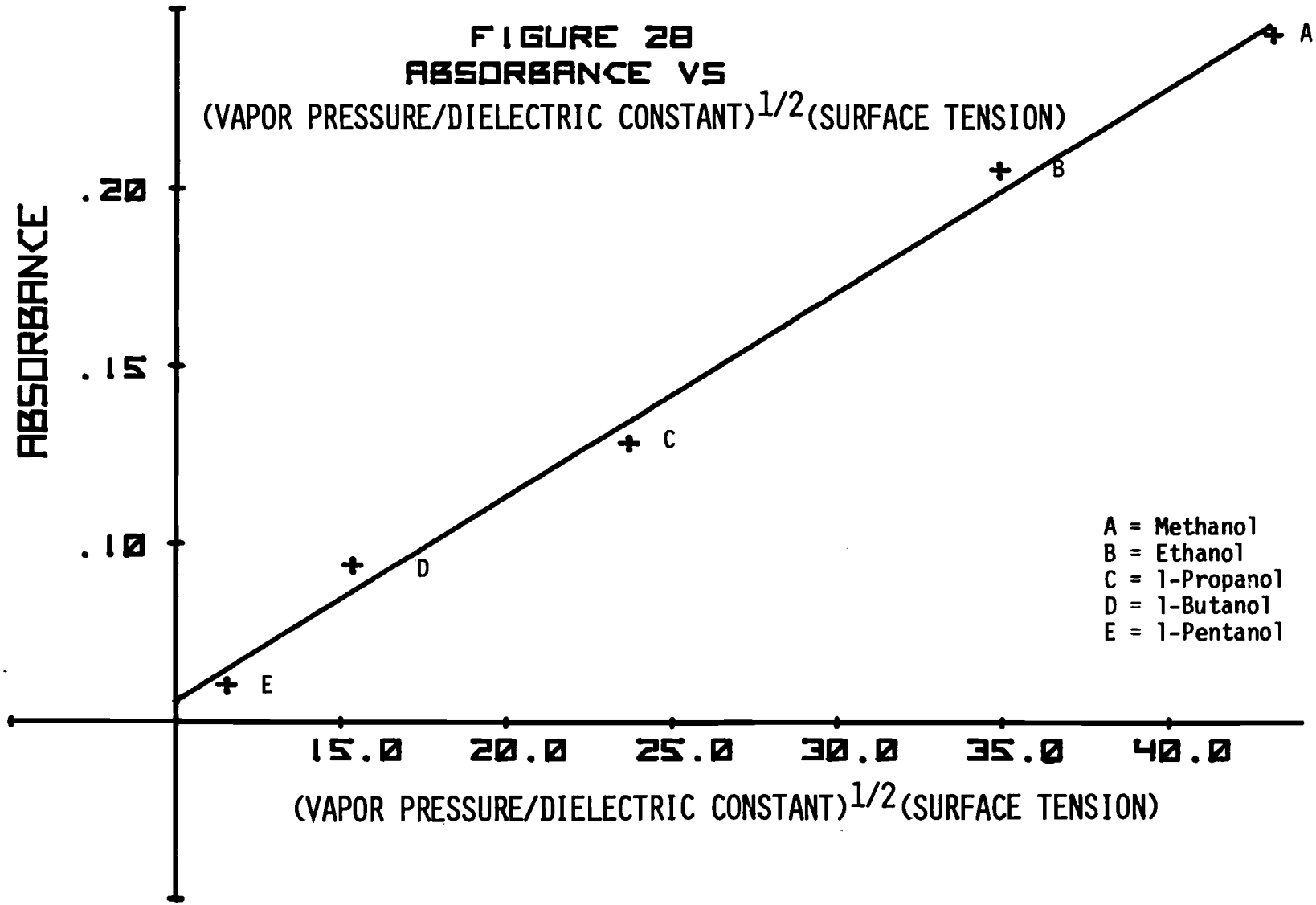


FIGURE 28
ABSORBANCE VS
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2(SURFACE TENSION)
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
15.0
20.0
25.0
30.0
35.0
40.0
(VAPOR PRESSURE/DIELECTRIC CONSTANT)1/2(SURFACE TENSION)

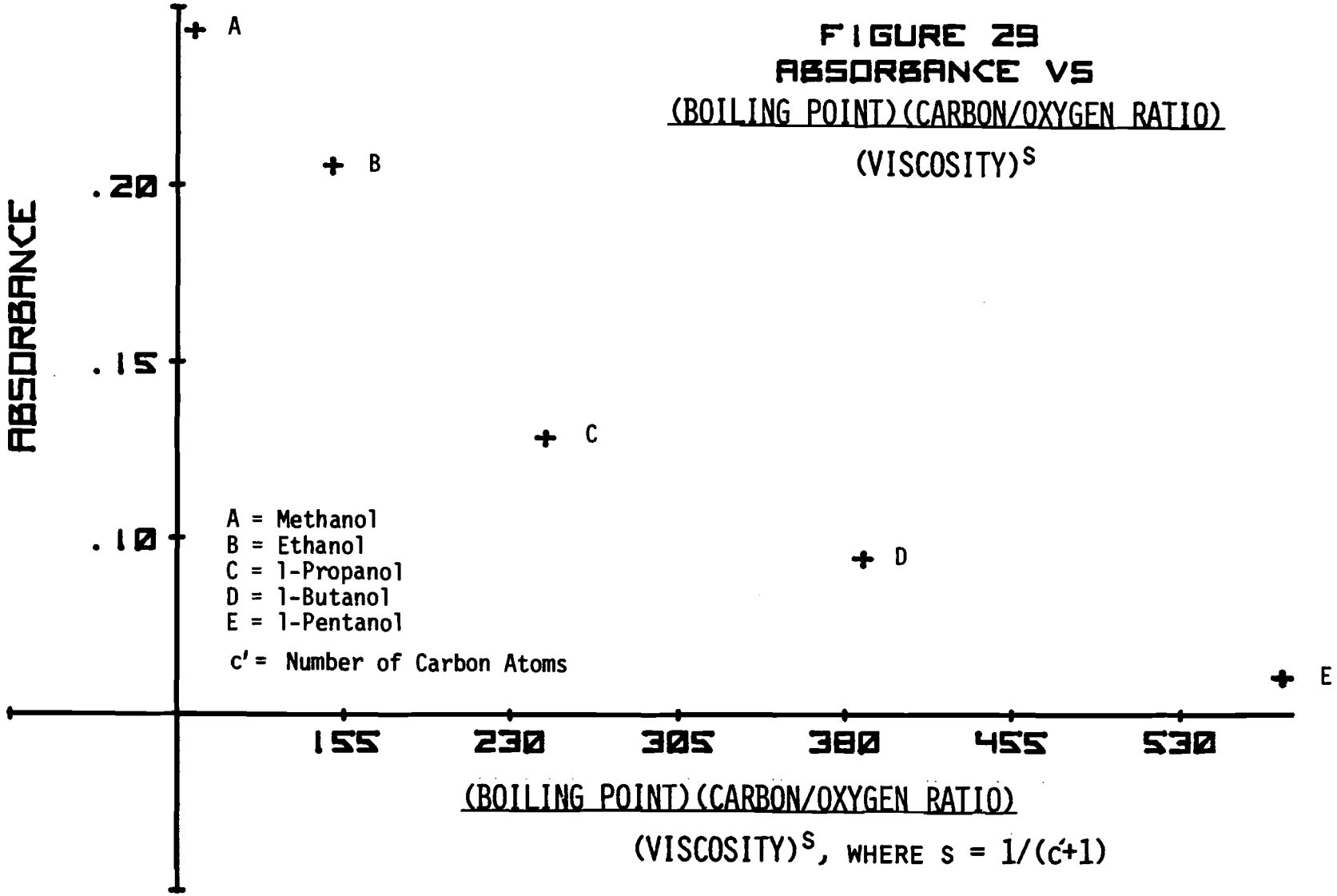


FIGURE 29
ABSORBANCE VS
(BOILING POINT)(CARBON/OXYGEN RATIO)
(VISCOSITY)^S
ABSORBANCE
.20
.15
.10
A
B
C
D
E
A = Methanol
B = Ethanol
C = 1-Propanol
D = 1-Butanol
E = 1-Pentanol
c' = Number of Carbon Atoms
155
230
305
380
455
530
(BOILING POINT)(CARBON/OXYGEN RATIO)
(VISCOSITY)^S, WHERE s = 1/(c'+1)

Table 9

SUMMARY OF PHYSICAL PARAMETERS INVESTIGATED HAVING NO LINEAR RELATIONSHIP WITH THE ABSORBANCE OF MANGANESE

p°	= Vapor Pressure	ρ	= Density
ε	= Dielectric Constant	ΔH_{comb}	= Heat of Combustion
η	= Viscosity	C	= Number of Carbon Atoms
γ	= Surface Tension	X	= Carbon to Oxygen Ratio
T_{bp}	= Boiling Point	s	= (1/C+1)

Absorbance vs Physical Properties

ε	p°	$(p^\circ/\varepsilon)^{1/2} / \sqrt[s]{\eta}$
ε^2	$1/p^\circ$	$(p^\circ/\varepsilon)^{1/2} + \sqrt[s]{\eta}$
$\varepsilon^{1/2}$	log p°	$(p^\circ/\varepsilon)^{1/2} + \eta^s$
$1/\varepsilon$	$(p^\circ)^2$	$(p^\circ/\varepsilon)^{1/2} - \eta^s$
log ε	T_{bp}	$(p^\circ/\varepsilon)^{1/2} / \eta^s$
$\eta^{1/2}$	$(T_{bp})^2$	$(p^\circ/\varepsilon)^{1/2} - (1/\eta)^2$
η^2	$(T_{bp})^{1/2}$	$1/\eta^s$
log η	$[\eta\gamma - (p^\circ/\varepsilon)^{1/2}]/ \Delta H_{comb}$	$\eta\gamma - (p^\circ/\varepsilon)^{1/2}$
$1/\eta$	$(p^\circ/\varepsilon)^{1/2}/(\eta\gamma)$	$(p^\circ/\varepsilon)^{1/2}\eta$
η^s	$(\eta\gamma^{0.0625})/[\rho - \exp(p^\circ/\varepsilon)^{1/2}]$	$[(\Delta H_{comb})(p^\circ/\varepsilon)^{1/2}]^{1/2}$
γ	$(\eta\gamma^{0.0625})/[\rho + \exp(p^\circ/\varepsilon)^{1/2}]$	$1/[\Delta H_{comb})(p^\circ)(\varepsilon)]^{1/2}$
$1/\gamma$	$(p^\circ/\varepsilon)^{1/2}/(\eta\gamma)$	$(\varepsilon)(\Delta H_{comb})$
$\gamma^{1/2}$	$(\eta\gamma)/\rho^{1/2}$	$(p^\circ/\varepsilon)^{1/2}(\Delta H_{comb})$
γ^2	$\exp(p^\circ/\varepsilon)^{1/2}$	$[(p^\circ)(\gamma)]/\varepsilon$
log γ	$\eta\gamma - \exp(p^\circ/\varepsilon)^{1/2} + \Delta H_{comb}$	$(p^\circ/\varepsilon)^{1/2}(\gamma)(\eta)$
$1/\Delta H_{comb}$	$(p^\circ/\varepsilon)^{1/2}/\eta$	$[(p^\circ/\varepsilon)^{1/2}\gamma]/\eta$
$(\Delta H_{comb})^{1/2}$	$(p^\circ/\varepsilon)^{1/2} + \Delta H_{comb}$	$[\eta\gamma - (p^\circ/\varepsilon)^{1/2}]/T_{bp}$
$[(\Delta H_{comb})(p^\circ)]^{1/2}$		$[(\Delta H_{comb})(p^\circ)(\varepsilon)]^{1/2}$

Table 10

PHYSICAL PROPERTIES OF THE SOLVENTS

Solvent	Dielectric Constant[28]	Viscosity[33] (Centipoise)	Surface Tension[28] (dyne/cm)	ΔH comb.[28] $\frac{KgCal}{gm\ mol.\ wt.}$	Vapor Pressure[29] @ 760 mm Hg	Boiling Point[28] °C	Density[28] g/ml
Methanol	33.62	0.547[28]	22.61	173.64	122.203	64.96	0.7914
Ethanol	24.30	1.145[28]	22.75	326.68	57.176	78.5	0.7893
1-Propanol	20.1	1.9911	23.78	482.75	19.936	97.4	0.8035
1-Butanol	17.1	2.5772	24.60	639.53	6.652	117.25	0.8270
1-Pentanol	13.9	3.450	25.79[35]	793.7	2.784	137.3	0.8144
Water	78.54	0.8904[28]	78.05	-----	760	100	1.000

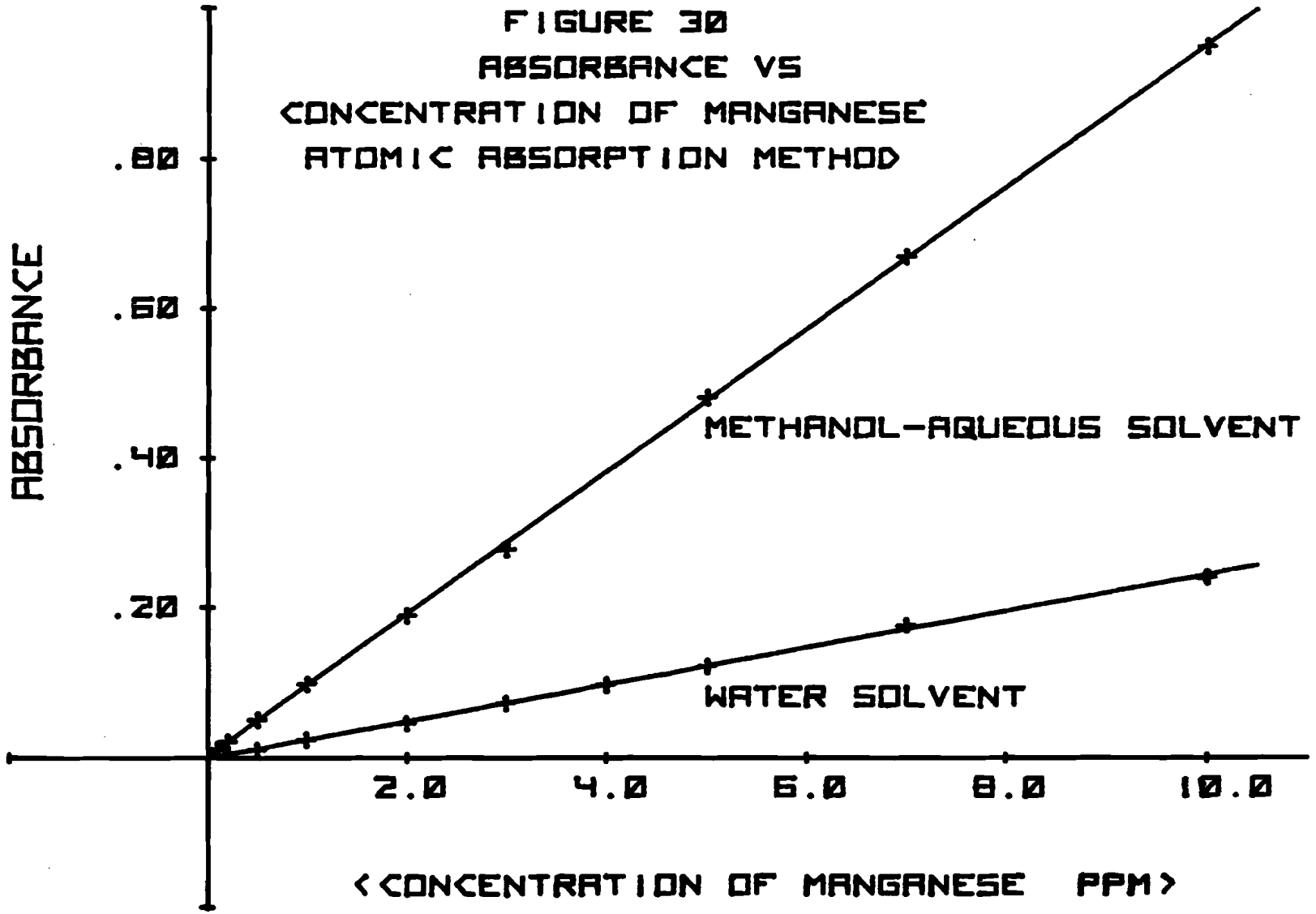


FIGURE 30
ABSORBANCE VS
CONCENTRATION OF MANGANESE
ATOMIC ABSORPTION METHOD
ABSORBANCE
.80
.60
.40
.20
METHANOL-AQUEOUS SOLVENT
WATER SOLVENT
2.0
4.0
6.0
8.0
10.0
<CONCENTRATION OF MANGANESE PPM>

method, at a five percent relative analysis error per one percent photometric error, was 0.75 to 7.7 µg/ml for the Methanol-Aqueous and 0.77 to 6.9 µg/ml for the aqueous solvent.

The calibration curve obtained for the AOAC colorimetric determination of manganese is shown in Figure 31 this curve was found to be linear throughout the concentration range of manganese found in the plant materials used in this study. The sensitivity of this method varied from one set of standards to another. The sensitivity obtained for the standards prepared for the determination of manganese in wheat, soybeans, and clover were 156, 83 and 107 ng/ml, respectively. The concentration range for this method to have a five percent relative analysis error per one percent photometric error was 1.95 to 28 µg/ml. Thus the error in the various sensitivities resulted from the low concentrations of manganese in the samples used and the increasing error introduced when working below the analytical region.

Time Dependency for AOAC Colorimetric Methods of Plant Materials

When the AOAC colorimetric method of analysis for manganese was used for the determination of the metal in wheat, soybeans, and clover a problem was observed. This problem resulted from the decrease in intensity of the permanganate color in the latter samples with time before they could be analyzed. Therefore a study was undertaken to determine an optimum time of analysis after the development of the permanganate color. An optimum time was not found. The samples were analyzed within a controlled time period of 15 to 20 minutes after the development of the permanganate color. A typical result

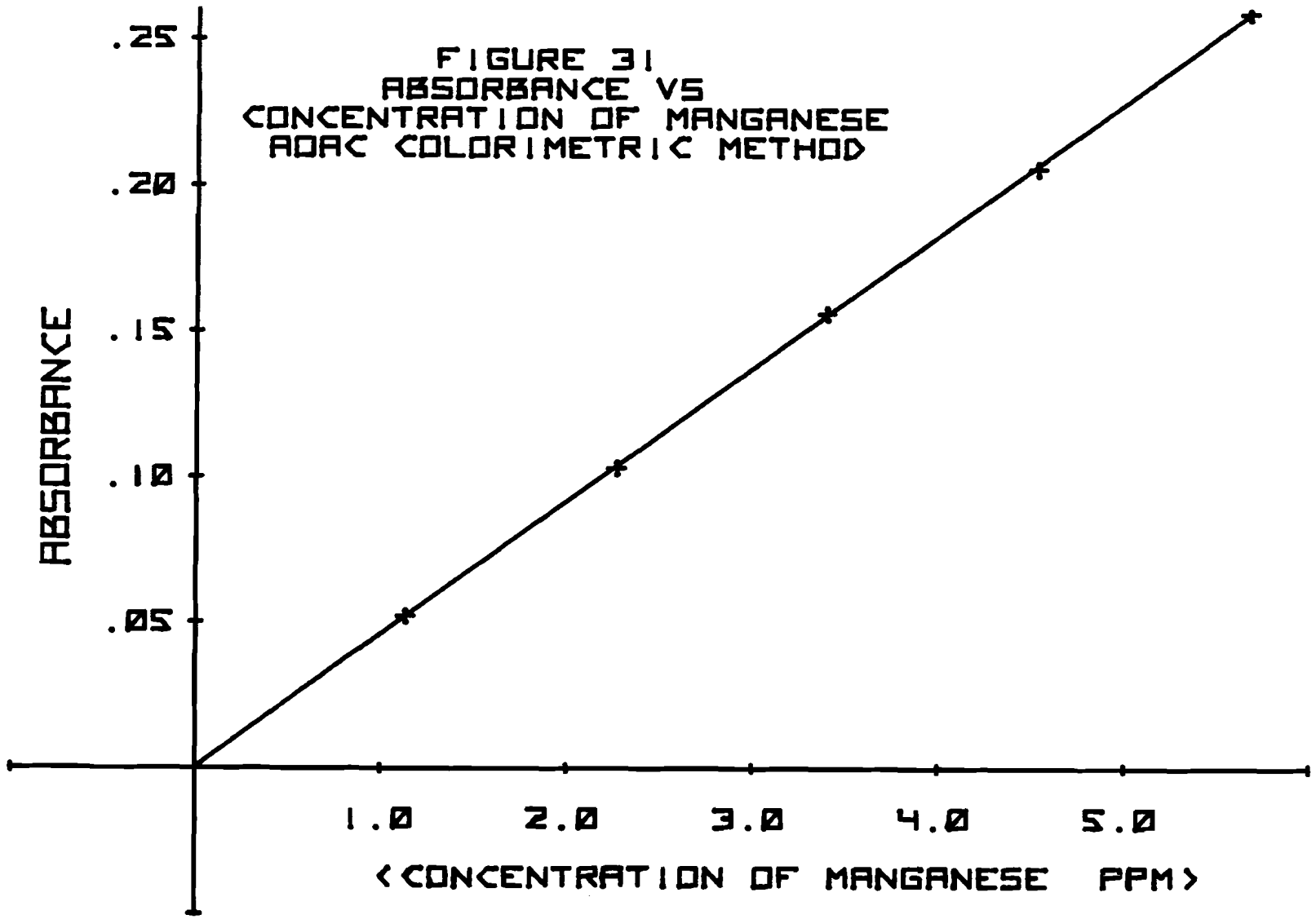


FIGURE 31
ABSORBANCE VS
CONCENTRATION OF MANGANESE
AOAC COLORIMETRIC METHOD
ABSORBANCE
.25
.20
.15
.10
.05
1.0
2.0
3.0
4.0
5.0
(CONCENTRATION OF MANGANESE PPM)

of the decrease of intensity with time is shown in Figure 32. At time zero, the sample solutions were analyzed after the 15 to 20 minute preparation time. The absorbance was then measured as a function of elapsed time.

Results Obtained by Atomic Absorption and AOAC Methods for Manganese

The results obtained by both methods appear in Table 11. The mean values for the manganese content found in each grain sample are shown along with the standard deviations, σ, the percentage coefficient of variance, and the number of analysis. The percentage coefficient of variance was found by dividing the standard deviation by the mean and multiplying by 100.

The mean values obtained for the amount of manganese found in the grain samples are comparable by both methods. In all three of the grains the standard deviation and percent coefficient of variance are lower in the atomic absorption method than in the AOAC method. The relative error is explained by the heterogeneity of the solid samples, the time dependency of the colorimetric method, and the relative analysis error brought about by the low concentration of standards used in the colorimetric method.

Recovery Study

A recovery study was performed by adding a known amount of manganese stock solution into the weighed grain samples and drying at 110°C for two hours. The samples were then treated in a similar manner as the sample preparation previously described. The percent recovery was calculated by the equation:

$$\% \text{ Recovery} = \frac{A * 25 \text{ ml}}{(B*C) + D} \times 100$$

where A is the concentration of the manganese contained in the sample

FIGURE 32
ABSORBANCE VS TIME
AOAC COLORIMETRIC METHOD

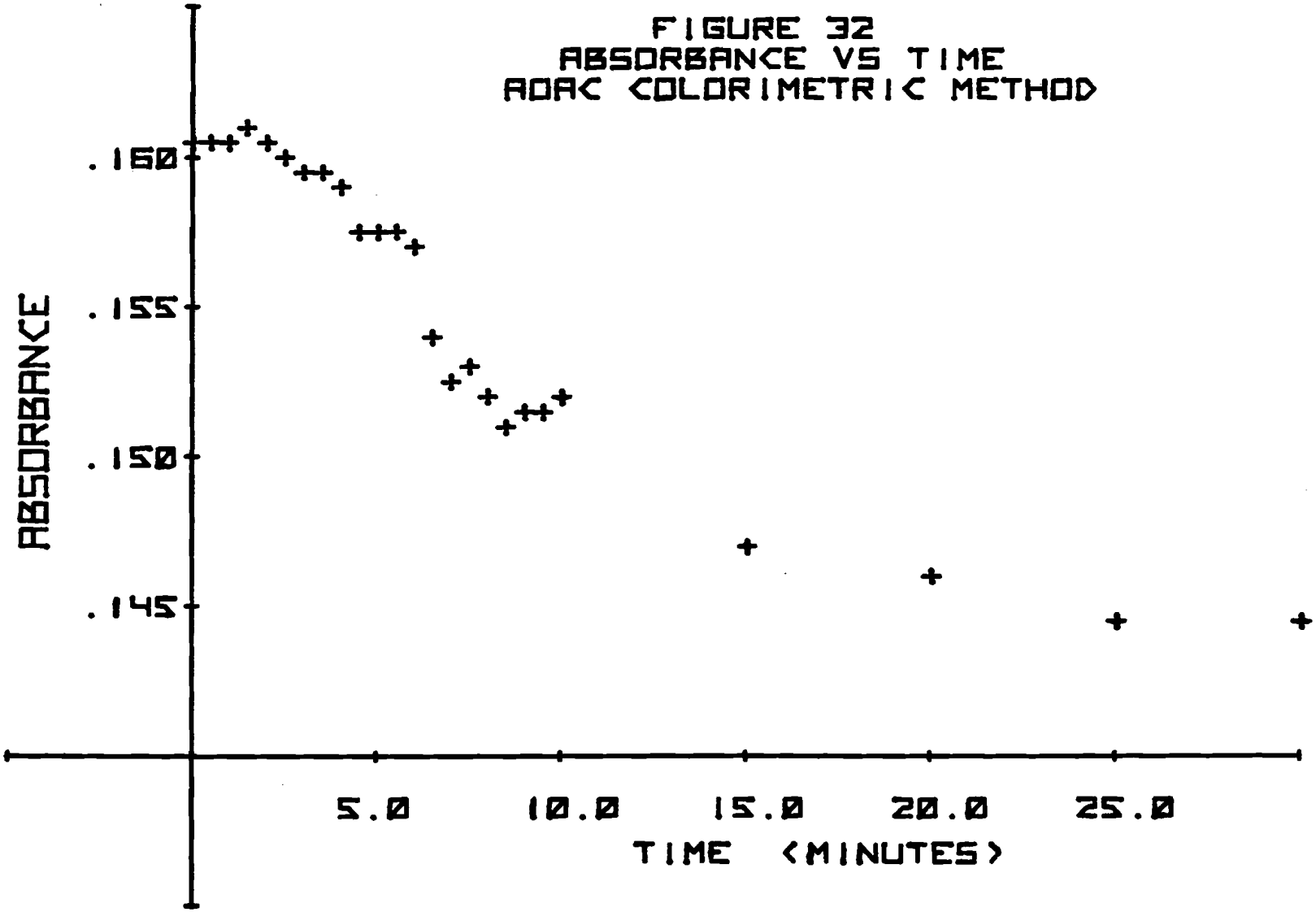


Table 11

ATOMIC ABSORPTION AND AOAC METHODS FOR MANGANESE

	Soybeans		Wheat		Clover	
	AAS	AOAC	AAS	AOAC	AAS	AOAC
Mean µg/gm	24.5	29.9	65.9	67.9	12.6	12.9
σ	0.4	3.1	3.8	6.1	0.6	1.2
% Coeff. of Var.	1.6	10.2	5.8	9.0	5.0	9.2
No. of Analysis	12	11	23	37	10	12

from the calibration curve expressed in μg/ml, B is the average concentration of manganese found in the grain sample expressed as μg/gm, C is the weight of the sample in grams, and D is the concentration of manganese added into the sample expressed in μg.

The results obtained for the recovery studies for both methods are shown in Table 12. The percentage recoveries are within the experimental error of the methods.

Interference Study

The results for the interference study appear in Table 13. The relative percent absorption was found by dividing the percentage absorption of an average of five trials containing the interfering ions by the percentage absorption of a solution containing no interfering ions and multiplying by 100. The concentrations of interfering ions were 100, 200, and 500 parts per million. These concentrations were selected because they are not normally found in plant materials at this high of concentration. Thus, any affect produced at the high concentrations would ultimately lead to affects at lower concentrations. The affect of added interferring ions on the absorption of manganese in a methanol-aqueous solvent was found to be negligible at the concentrations studied.

Precision Study

The reproducibility of a method is a process in which a desired signal from a particular solution is reproduced from one analysis to the next. In Table 14, the reproducibility of the atomic absorption analysis standards are shown. The mean absorbance was determined by aspirating the standards twenty consecutive times. The coefficient

Table 12

COMPARISON OF METHODS OF

RECOVERY OF MANGANESE

Atomic Absorption Method	Added Manganese, µgm	% Recovery
Wheat	50	97.2 (12)
Clover	10	94.6 (12)
Soybean	--	---------
AOAC Colorimetric Method	**Added Manganese, µgm**	**% Recovery**
Wheat	100	106.3 (11)

Table 13

INTERFERENCE STUDY

ADDED ION	RELATIVE PERCENT ABSORPTION		
Conc. (µg/ml)	100	200	500
Cl^- as HCl	100.6	100.1	99.0
SO_4^{-2} as H_2SO_4	98.9	97.1	96.0
PO_4^{-3} as H_3PO_4	-----	95.3	96.6
Na^+ as NaCl	102.5	96.8	95.5

Table 14

REPRODUCIBILITY OF ANALYSIS STANDARDS BY AAS

	0.1 ppm	0.2 ppm	0.5 ppm	1.0 ppm	2.0 ppm	5.0 ppm
Mean Absorbance	0.010	0.021	0.055	0.159	0.329	0.500
σ	0.001	0.001	0.001	0.002	0.004	0.005
% Coeff. of Var.	9.5	5.4	2.4	1.4	1.3	1.0

No. Of Analysis = 20

of variance expressed in percent is seen to decrease as the concentration of manganese is increased.

Stability of the Methanol-Aqueous Standards

When storing standards prepared in the part-per-million range problems can occur due to an adsorption-desorption of metal ions with the walls of the container over a period of time. When volatile organic solvents are stored, a second problem may arise, such as volatilization of the solvent. This results in the prepared standards becoming more concentrated over a period of time. In this study the problem of volatilization of the solvent was restrained by wrapping the threads of the plastic bottles used to store the standards with a Teflon pipe wrap until a tight seal was formed between the bottle and the cap.

In Table 15 the stability of the analysis standards are shown over a 60 day period. The values for the mean absorbance are shown for each standard, along with the standard deviation, percent coefficient of variance, and the number of analysis. The mean absorbance was determined from a number of trials at intervals whenever the grain samples were analyzed, not at any regular time interval. The absorbance values obtained for the standards were not used in the determination of the manganese content in the grain samples but were compared to the analysis standards prepared at regular intervals. In most cases, the absorbance values obtained for the standards analyzed over a period of time were comparable with those prepared on a regular basis.

To adequately determine whether the methanol-aqueous standards were stable over an extended period of time, Tables 14 and 15 must

Table 15

ANALYSIS OF ATOMIC ABSORPTION STANDARDS

OVER A PERIOD OF 60 DAYS

	0.1 ppm	0.2 ppm	0.5 ppm	1.0 ppm	2.0 ppm	3.0 ppm	5.0 ppm	7.0 ppm	10.0 ppm
Mean Absorbance	0.012	0.021	0.051	0.100	0.194	0.288	0.498	0.705	1.008
σ	0.002	0.002	0.002	0.005	0.009	0.014	0.021	0.035	0.069
% Coeff. of Var.	13.6	8.6	4.7	5.0	4.4	4.8	4.2	5.0	6.9
No. of Analysis	21	21	21	25	25	25	25	21	21

be compared. Comparison of the standard deviations between the two sets of data the standards analyzed over an extended period of time shows a slight increase over the standards prepared at regular intervals. This increase could probably be attributed to the evaporation and adsorption-desorption process. This effect is small in comparison to the number of times the instrument parameters were optimized. The instrument parameters as stated previously influence the signal delivered to the detector. Thus, the major source of error would appear to be the precision of the operator in optimizing the instrument parameters over an extended period of time.

CONCLUSION

The analysis of manganese in plant materials by the atomic absorption method using a 99% Methanol-1% Aqueous solvent was found to be comparable to results found for the official AOAC colorimetric method. The analysis standards prepared for use with the atomic absorption method were found to be stable in concentration over an extended period of time e.g. 60 days. The AOAC colorimetric standard and sample solutions were found to decrease in intensity within a period of hours. The recovery study performed by both methods showed that the recoveries of added metal was within experimental error for the two methods.

The effects of added ions on the absorption of manganese as analyzed by atomic absorption were found to be negligible at the concentration of added ions studied. The enhancement of the absorption of manganese using the Methanol-Aqueous solvent over the aqueous solvent was found to be 6 to 1. The sensitivities for manganese 30 μg/ml and 220 μg/ml for the methanol-aqueous and aqueous solvents respectively. The sensitivity of the AOAC colorimetric method was found to vary from one plant sample to another due to the various standards used.

A Ringbom plot of percentage absorption vs concentration of manganese showed that the concentration range for a five percent relative analysis error per one percent photometric error was 0.75 to 0.77 μg/ml for the atomic absorption method and 1.95 to 28 μg/ml for the AOAC method. Thus at the concentrations of manganese found in the prepared solution of plant materials i.e.

0.5 to 3 µg/ml the relative analysis error per 1% photometric error on the AOAC method is slightly higher than 5 percent.

The physical properties of the organic solvents on the absorbance of manganese was studied. The findings suggested a linear relationship could be obtained from the absorbance of manganese if plotted against the square root of the quantity vapor pressure divided by the dielectric constant. The vapor pressure of the pure solvent could be related to the size of droplet production while the dielectric constant can be related to the degree of association between the metal and solvent. This quantity when multiplied by the surface tension also produced a linear relationship. The surface tension could be related to the degree of association between the aqueous and Methanol solvents.

The atomic absorption method was found to be easier, less time consuming, not affected by changes in concentration with time, and as precise as the AOAC colorimetric method.

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APPENDIX

TABLE A-1

Analysis of Plant Material by Atomic Absorption Spectroscopy

Wheat

Sample Number	µg/gm Found
WH-1	61.2
WH-2	60.5
WH-3	63.2
WH-4	64.5
WH-5	60.4
WH-6	60.2
WH-7	61.6
WH-8	64.4
WH-9	64.3
WH-10	63.9
WH-11	63.3
WH-12	69.3
WH-13	66.7
WH-14	66.2
WH-15	73.6
WH-16	70.6
WH-17	68.0
WH-18	68.2
WH-19	67.4
WH-20	70.2
WH-21	71.4
WH-22	69.5
WH-23	66.7
Mean	= 65.9
σ	= 3.8
% Coeff. of Var.	= 5.8%

Soybean

Sample Number	µg/gm Found
SH-1	24.8
SH-2	24.9
SH-3	24.0
SH-4	25.1
SH-5	24.8
SH-6	24.2
SH-7	24.6
SH-8	24.0
SH-9	23.9
SH-10	24.5
SH-11	24.6
SH-12	24.2
Mean	= 24.5
σ	= 0.39
% Coeff. of Var.	= 1.6%

Clover

Sample Number	µg/gm Found
CH-1	13.6
CH-2	12.4
CH-3	12.7
CH-4	12.5
CH-5	12.9
CH-6	13.0
CH-7	12.9
CH-8	12.1
CH-9	12.6
CH-10	11.2
Mean	= 12.6
σ	= 0.6
% Coeff. of Var.	= 5.0%

TABLE A-2

Analysis of Plant Material by the Official AOAC Method

Wheat

Sample Number	µg/gm Found
WH-1	56.1
WH-2	68.9
WH-3	67.7
WH-4	74.4
WH-5	55.7
WH-6	79.2
WH-7	75.6
WH-8	76.5
WH-9	71.9
WH-10	63.7
WH-11	60.4
WH-12	64.6
WH-13	54.4
WH-14	70.1
WH-15	69.2
WH-16	63.7
WH-17	71.1
WH-18	56.3
WH-19	72.6
WH-20	70.6
WH-21	74.9
WH-22	69.2
WH-23	69.5
WH-24	66.0
WH-25	66.6
WH-26	70.0
WH-27	72.3
WH-28	68.2
WH-29	70.9
WH-30	69.2
WH-31	75.2
WH-32	71.2
WH-33	62.7
WH-34	61.2
WH-35	62.1
WH-36	70.7
WH-37	70.4

Mean = 67.9
σ = 6.1
% Coeff. Of Var. = 9.0%

Soybean

Sample Number	µg/gm Found
SH-1	32.6
SH-2	27.9
SH-3	33.1
SH-4	35.5
SH-5	32.6
SH-6	28.2
SH-7	27.6
SH-8	25.6
SH-9	28.1
SH-10	28.4
SH-11	29.6

Mean = 29.9
σ = 1:2
% Coeff. of Var. = 10.1%

Clover

Sample Number	µg/gm Found
CH-1	10.7
CH-2	12.6
CH-3	12.9
CH-4	14.9
CH-5	13.5
CH-6	14.6
CH-7	13.7
CH-8	13.0
CH-9	12.7
CH-10	11.3
CH-11	12.9
CH-12	12.5

Mean = 12.9
σ = 1.2
% Coeff. of Var. = 9.2%

TABLE B-1

Recovery Data for Atomic Absorption Analysis of Manganese

Sample, Wheat	µg Added	Total µg Present Plus Addition	Total µg Found	% Recovery
WCH-1	50	195.2	184.8	94.6
WCH-2	50	210.9	203.2	96.3
WCH-3	50	206.8	198.2	95.9
WCH-4	50	206.1	206.1	100.0
WCH-5	50	188.8	186.1	98.6
WCH-6	50	212.3	207.4	97.7
WCH-7	50	213.8	208.3	97.4
WCH-8	50	202.1	199.8	98.9
WCH-9	50	213.8	211.0	98.7
WCH-10	50	196.0	187.9	95.7
WCH-11	50	197.3	189.0	95.8
WCH-12	50	198.1	191.7	96.8
				Mean = 97.2

Sample, Clover	µg Added	Total µg Present Plus Addition	Total µg Found	% Recovery
CCH-1	10	41.6	40.2	97.6
CCH-2	10	35.2	33.2	94.3
CCH-3	10	35.2	33.9	95.4
CCH-4	10	40.5	37.8	93.4
CCH-5	10	39.5	37.6	95.2
CCH-6	10	36.9	34.8	94.2
CCH-7	10	36.9	34.7	94.2
CCH-8	10	37.7	35.6	94.4
CCH-9	10	37.9	35.5	93.7
CCH-10	10	36.4	33.7	92.6
CCH-11	10	39.2	37.7	96.4
CCH-12	10	42.1	39.9	94.6
				Mean = 94.6

TABLE B-2

Recovery Data For AOAC Colorimetric Analysis of Manganese

Sample, Wheat	µg Added	Total µg Present Plus Addition	Total µg Found	% Recovery
WCH-1	100	412.8	434.9	105.4
WCH-2	100	425.4	450.5	105.9
WCH-3	100	463.0	448.3	94.8
WCH-4	100	457.2	432.7	94.6
WCH-5	100	425.2	430.5	101.6
WCH-6	100	412.5	452.7	109.7
WCH-7	100	492.9	517.7	105.0
WCH-8	100	470.8	530.6	112.2
WCH-9	100	476.2	517.3	108.6
WCH-10	100	445.9	506.2	113.5
WCH-11	100	475.1	550.7	115.9
				Mean = 106.3