Determination of Organic Compounds by Atomic Absorption Spectrophotometry(AAS) with Special Reference to Compounds Encountered in Environmental Problems

A thesis submitted for the degree of

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by

El-SAYED A. K. YACOUB

at

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- TO -

All the members of my family
who have given me endless
encouragement and support to
produce this Thesis

DETERMINATION OF ORGANIC COMPOUNDS BY ATOMIC ABSORPTION SPECTROPHOTOMETRY (AAS) WITH SPECIAL REFERENCE TO COMPOUNDS ENCOUNTERED IN ENVIRONMENTAL PROBLEMS

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SUMMARY

A comprehensive review of the indirect methods for the determination of organic compounds by atomic absorption spectroscopy is given.

The various techniques used are summarised, two examples chosen and discussed in detail in order to demonstrate the feasibility of the method. Saccharin, an important artificial sweetener, and common food additive, may be determined by atomic absorption spectroscopy via complexation as its tris-(1,10-phenanthroline)iron(II) cation. The other subject chosen for study was the possible determination of certain N-nitrosamines. These are carcinogenic substances formed by the interaction of organic secondary amines with nitrite salts.

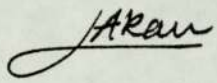
In the present work, the spectrophotometric determination of saccharin by selective solvent extraction from aqueous solutions containing an excess of tris-(1,10-phenanthroline)-iron(II) cation into nitrobenzene as an ion-association system, has been thoroughly investigated in order to determine the optimum conditions. This method has been adapted to give an atomic absorption spectroscopy finish using both flame and flameless techniques, in which both the organic and aqueous phases have been analysed.

Furthermore, a new simple, rapid and accurate spectrophotometric procedure for the determination of saccharin has been devised utilising the fact that "ferroin" is strongly adsorbed on silica gel. The procedure is based on the quantitative formation of the ion-association complex $(Fe\ phen_3)^{2+}$. $(saccharin)_2$ when an acetone (70%) or methanolic (80%) or ethanolic (60%) solutions of saccharin is shaken with ferroin-impregnated silica gel matrix. Desorption of the ion-association complex is then carried out and the absorbance is measured at 510nm. A further development of the adsorption method is described in which the desorbed complex is analysed by atomic absorption spectroscopy instead of a spectrophotometric procedure.

A brief review of the spectrophotometric methods available for the determination of N-nitrosamines is given. Attempts to determine N-nitrosamines by atomic absorption spectroscopy via formation of some of their metal complexes is also reported.

KEY WORDS: Saccharin determination; Indirect method; Atomic absorption spectrophotometry; Silica gel adsorption; Tris-(1,10-phenanthroline)-iron(II).

The work described in this thesis was carried out between 1977 and 1981. It has been done independently by undersigned and has not been submitted for any other degree.



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CHAPTER ONE

INTRODUCTION

1.1 Indirect Determinations in Analytical Chemistry

Indirect analytical methods are those which do not involve the direct measurement of the analyte but rather a species equivalent in concentration to it and this relationship forms the basis of the final assay. The method of 'indirect determinations' is not an entirely new analytical technique. In fact, most of spectrophotometric and gravimetric methods are indirect in the sense that the final absorbance or weight measurements are made on a new species. For example, a metal complex may be formed with an organic ligand in which metal/ligand is in stoichiometric proportion enabling the original analyte to be determined. Thus, according to this definition only a few determinations can be called 'direct' . For example, the colorimetric determination of permanganate, the uv-spectrophotometric determination of some organic compounds (hormones etc..) and atomic absorption, emission or fluorescence methods for metals would be classified as direct methods. Also, a method may be direct in one technique and indirect in another. For example, the determination of a metal by solvent extraction with organic ligands is a common analytical procedure. If the absorbance of extracted complex is measured by spectrophotometry it would be an indirect method but if the metal concentration is determined by atomic absorption it could be described as a direct method.

Indirect methods offer three advantages; they furnish a means of analysis when an alternative method may not be available; they sometimes offer a more sensitive and better selectivity of the determination coupled with the avoidance of interferences.

As mentioned above, indirect methods have wide application

in the field of analytical chemistry and one of the most common techniques employed is spectrophotometry. The indirect method may be explained in several ways:

i) a coloured species may be developed between the constituent and a reagent and the absorbance of coloured species is then measured.

ii) a colourless species is formed by a reaction taking place between the constituent and a coloured reagent; the fading in colour (or decrease in absorbance) is the measure of the concentration of the species to be determined.

iii) a slightly soluble coloured precipitate is formed between the constituent and a suitable reagent; and the colour intensity of the solution is measured.

iv) a slightly soluble colourless precipitate is produced; a component in this precipitate other than the constituent of interest reacts with another reagent developing a colour to be measured, or the excess of reagent is determined by its own colour.

v) catalytic reactions are also available, in which the species of interest catalyze a certain reaction or alter a reaction between two components; and determination can be made by measuring the colour intensity of one of the coloured components as a function of time.

vi) chemical amplification procedures 'multiplication reactions' , in which, the normal equivalence is altered or amplified in order to make the measurement possible.

1.2 The Analytical Role of 1,10-Phenanthroline in the Indirect Methods

1,10-phenanthroline and related compounds play an important

role in the indirect spectrophotometric and atomic absorption spectrophotometry methods. They serve as important colorimetric reagents for the determination of trace metals particularly iron and copper (some of its features are mentioned in chapter three). These reagents are widely used for determination of various anions, such as iodide, thiocyanate, perchlorate, and many other anions, by solvent extraction and colorimetry of tris-(1,10-phenanthroline)-iron(II) salts which are insoluble or only slightly soluble in aqueous solutions.

Trace amounts of 'reductants' , such as ascorbic acid (vitamin C), tannin, hydrazine, vitamin E, etc.. could be simply and conveniently determined because these substances are capable of reducing iron(III) to iron(II) ions. The iron(II) complexes with 1,10-phenanthroline and the absorbance of the resulting orange colour is measured. In the same way, oxidizing substances such as hydrogen peroxide and potassium permanganate may be determined using indirect techniques based on oxidation of iron(II) to iron(III), followed by addition of the 1,10-phenanthroline reagent to react with the excess iron(II).

The analytical applications of 1,10-phenanthroline and related compounds using the indirect atomic absorption methods are discussed in chapter two.

Since the present study is concerned with the development of indirect determination of certain organic compounds by atomic absorption spectrophotometry, it is important to understand the theoretical and the practical background of the technique of atomic absorption.

1.3 Atomic Absorption Spectrophotometry (A.A.S)

1.3.1 Historical Developments of Flame-AAS

The basic principles of atomic absorption spectrophotometry (AAS) has been well known for a long time. The first atomic absorption observations were made as early as in 1802 by Wollaston (1) , when he examined the daylight by means of a prism. He reported seeing darklines in the sun's spectrum. When Wollaston looked at candlelight he noted a bright yellow line as well as a series of five broad, distinct images of different colours. This was the first signs of recognition of the possible implications of spectral differences. Although, Wollaston gave no satisfactory explanation for those lines, and although the significance of the findings was not appreciated, both flame emission and atomic absorption studies can be considered to have started by 1802.

In 1814, Fraunhofer (2) extended the study of these lines when he noticed them using better equipment and technique. Fraunhofer made a map for the dark lines of the solar spectrum, designating the most dark ones by letters of the alphabet such as the D lines for the yellow sodium doublet which we use to this day.

No significant progress in flame spectroscopy came until Bunsen, in 1855, who designed an efficient burner for the new fuel at that time (coal gas) instead of the unsatisfactory alcohol burner or oil lamp which used before. Emission chemical spectroscopy was born, when Bunsen viewed the flame through a spectroscope, he noted that the colours were linked to the element, not the compound in which the element is bound. It had been realized by then that the bright lines seen with the

spectroscope were characteristic of specific elements and that the method could be an extremely sensitive and simple method of qualitative analysis. Bunsen soon applied the method to discover and isolate two unknown elements in mineral water, cesium and rubidium, soon other elements have been discovered using this new technique.

The basic principles underlying atomic absorption spectrometry were established by Kirchoff in 1860 who was first to demonstrate that atomic spectra could form the basis of a new and highly specific method of analysis. He re-measured the wavelength of some of the Fraunhofer lines (solar spectra) , compared them to the lines obtained in the laboratory. It was thus established that they were from the same elements. He explained the dark lines of the solar spectrum (the Fraunhofer lines) as due to a process of absorption by certain elements present in the cooler outer atmosphere of the sun. It had been concluded then, that the absorption spectrum is just as characteristic of a specific element as its emission spectrum and absorption occurs only at the same wavelength as emission. This became known as the so-called 'Resonance' phenomenon. At this period in time, the fundamental process of absorption was well understood and the foundation of analytical spectroscopy was established.

The analytical potentialities of atomic absorption spectroscopy for the determination of metallic elements in chemical analysis was first realized in 1955 by Walsh (3) and Alkemade and Milatz (4) . They introduced and developed the technique. They showed that this atomic absorption phenomenon could be applied to a wide range of analytical problems. A solution containning the analyte element was injected into a flame; was scanned with a lamp or other source emitting the spectrum

of the same element, and they then measured the absorption as a ratio of the intensity of the scanning beam to that of the unabsorbed beam. They concluded from their experiments that this absorption could be used as a measure of the atomic concentration in the flame gas, which is a function of the concentration of the element in the solution sample. A comparison of the absorption had to be made with standard solutions. Walsh discussed thoroughly the physical basis of the method and realized its applicability to all elements which can be vaporized. The analytical development of AAS has been due almost entirely to Walsh and his colleagues. In 1957, Russel, Shelton and Walsh (5) described a practical atomic absorption spectrophotometer. They used modulated hollow cathode radiation to enable absorption in the flame to be measured without interference from flame emission, and a double-beam system with ratio-recorder to enable percent absorption to be recorded directly.

The first commercial equipment became available about 1960, and since then the use of the technique has become widespread. Improved instrumentation, more reliable sources of resonance radiation, hotter flames and non-flame atomizers have enabled the technique to be extended to nearly every metallic element in the periodic table (see table 1.1). Moreover, the analyst has extended the analytical methods and the techniques used in order to increase the sensitivity of some determinations and to demonstrate the applicability of AAS to organic compounds determinations.

It is only very recently that the use of alternative atomization sources to the flame has been explored. The electrothermal atomizer and the Inductively-Coupled Plasma (ICP) are the two most successful alternative techniques appear to be at the present time.

Electrothermal atomizers provide a considerable improvement in sensitivity for the majority of the elements determined by AAS.

1.3.2 Historical Developments of Electrothermal Atomization of AAS

The electrothermal atomizers have been developed in the period 1959 to 1976. The first graphite tube furnace used for spectroscopic measurements was reported by King (6,7) , in 1905 and 1908, who used it mainly to investigate the emission spectra of many elements. He used electrically heated carbon furnaces in which a temperature of 2200°C was obtained. In 1956, the principle of electrothermal atomization for quantitative analytical measurements was utilized , when Mandel'shtam et.al (8) in U.S.S.R designed an electrothermal vaporizer to separate and preconcentrate trace elements onto a graphite electrode prior to emission spectrochemical analysis. At the same time, another group of workers, Zaidel et.al (9) used similar technique with an enclosed system, under vacuum.

The use of the high-temperature furnace was first suggested and described by L'vov (10) in 1959, who realized that non-flame electrothermal atomization may offer the possibility of very high absolute sensitivities, performing analyses on samples of small volume and of handling many complex samples without pretreatment. L'vov in his early work used, a graphite furnace similar in principle to the arc atomizer used by King. The sample to be analyzed was placed on an electrode which was introduced into the horizontal graphite tube through a hole in the under side. This furnace was 5-10 cm in length and 3 mm in diameter lined with tantalum foil, the atomization temperature was

obtained by striking an arc between the sample electrode and the graphite furnace tube.

Woodriff (11,12), and Massmann (13,14), in 1968, introduced the biggest step forward in the development of an electrothermal atomizer. The most popular modifications have been made to L'vov's furnace was Massmann's furnace. In this design, a tube (5 cm length and 6.5 mm diameter) with a hole (2 mm) is placed half way along for the introduction of liquid samples of 2-200 μl volume and is supported between water-cooled steel end-cones for electrical connections. The device is placed within a metal casting contains an inert atmosphere (Argon gas), to prevent oxidation of the tube during the atomization process. Three principal steps are operating in order to provide the atomic population for the absorption measurements. The first step in this programme is the drying step. This is included in order to remove the solvent. The second is the ashing or pyrolysis step at a higher temperature and this included in order to remove the organic matter. The final step is the atomization step at a temperature sufficiently high enough to convert the element to be determined to an atomic vapour. The Woodriff furnace has not received the same wide acceptance as the smaller Massmann furnace because its large size.

The development of the flameless techniques have been increased in 1969 when West and Williams (15) demonstrated their work on a carbon filament atomizer for atomic absorption measurements. Electrically heated tantalum boat (16) , flexible graphite braid filament (17) , a modified design of the Massmann type furnace (18,19) and a compact T-shaped electrothermal carbon furnace (20) are reported.

1.3.3 Theoretical Considerations

When an atomic vapour (a population of atoms free from any molecular bonding forces) is formed by a suitable energy source (atomization) such as in flame, the analyte existing in samples and standards is transformed into active entities. Some of the atoms are excited to higher energy levels by vibrationally excited flame gas molecules.

e.g. $$\overset{*}{F} + A = F + \overset{*}{A} \qquad (1)$$

$$\overset{*}{A} \longrightarrow A + h\nu \qquad (2)$$

where :

A = atom produced from the nebulized solution.

F = flame gas molecule.

$\overset{*}{A}$ = electronically excited atom.

$\overset{*}{F}$ = vibrationally excited flame gas molecule.

However, the life time of the activated energy levels is limited and after a certain time (n sec. range) the activated electrons return eventually to the lower energy levels 'ground state' emitting the energy acquired from the thermal source. The radiation emitted corresponds to a frequency ν given by the well-known equation :

$$E_2 - E_1 = \Delta E_\lambda = h\nu \qquad (3)$$

in which the emitted radiation is equivalent to the difference in energy between the ground state (E_1) and the excited state (E_2) , where h is Planck's constant. The radiation can be located at a wavelength λ

given by the relationship $\lambda = C/\nu$. The quantitative measurement of this emitted radiation which is characteristic for every elements, is the basis for 'Flame Emission Spectrophotometry' (FES).

According to the energy supplied to the atom population, a portion (N_E) of the total atoms present (N) will occupy higher energy levels. The ratio of the number of atoms in the excited state (N_E) to the number of atoms in the ground state (N_o) is very small and (N_E) is negligible compared with (N_o) , (N_o) will thus be equal to the total number of atoms, (N) . The ratio N_E/N_o is given by Boltzmann distribution :

$$\frac{N_E}{N_o} = \left(\frac{g_a}{g_o}\right) \exp\left(\frac{\Delta E_\lambda}{KT}\right) \qquad (4)$$

where g_a and g_o are the statistical weights of the excited and ground states, T is the absolute temperature of the vapour and K is the Boltzmann constant. From the relationship (4) ; while N_E varies exponentially with temperature, N_o remains almost constant, and at temperature up to 3,000°, the majority of atoms are in the ground state.

If the atoms in the ground state are irradiated with the emission line of the same element of interest, the resonance absorption of the specific element is obtained in the atomic vapour. The absorbed energy ΔE_λ causes a corresponding excitation of orbital electrons of the same energy. Thus, a weakening occurs in the line intensity of the irradiated atomic vapour of energy difference ΔE_λ from $I_{o\lambda}$ to I_λ . This weakening is measured as the absorbancy E_λ , where :

$$E_\lambda = \text{Log}\left(I_{o\lambda}/I_\lambda\right) \qquad (5)$$

The absorbancy E_λ is a measure of the concentration of the atomic vapour. This is the principle of AAS.

If we now consider a parallel beam of radiation of intensity $I_{o\lambda}$, at frequency ν, incident on an atomic vapour of thickness L cm, I_λ is the intensity of the transmitted radiation (see Fig. 1.1).

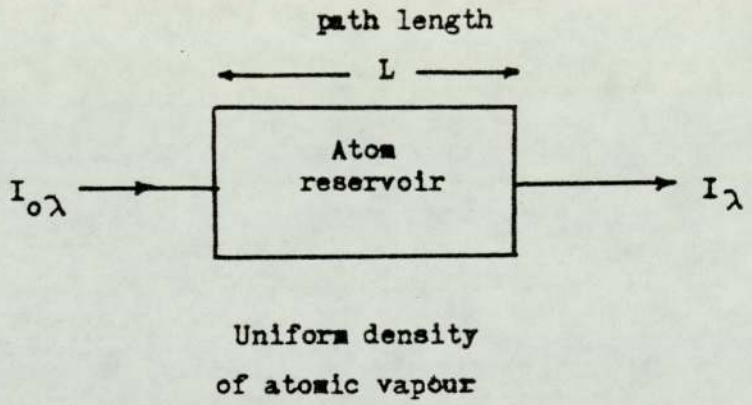


Fig.(1.1) A representation of AAS.

The absorption coefficient, K_ν, of uniform atomic vapour at frequency ν could be defined by the relation (5) where :

$$I_\lambda = I_{o\lambda} \exp - (K_\nu L) \qquad (6)$$

The value of K_ν will vary with ν, since the absorption line has a finite width. According to the classical dispersion theory, the integrated

absorption is given by the relation :

$$\int K_\nu \, d\nu = \frac{\pi e^2}{mc} N_\nu f \qquad (7)$$

where :

e = electronic charge, m = electronic mass, c = velocity of light, N_ν = number of atoms per cm^3 capable of absorbing energy in the range ν to $\nu + d\nu$. The f-value is the oscillator strength, i.e. the effective number of free electron oscillators per atom of the element of interest responsible for the absorption effect produced by the incident radiation. The value N, the total number of atoms of the element present can be substituted for N_ν . The energy absorption is a direct function of the number of free atoms in the absorbing path i.e. the population density of atoms in the flame.

There is a specific line of absorption for every element present in the atomic vapour in the reservoir. The natural half width of these lies at 10^{-5} nm (10^{-4} Å). A typical absorption line profile of an atomic vapour in a conventional flame and a molecular absorption band in solution is shown in Fig. 1.2.

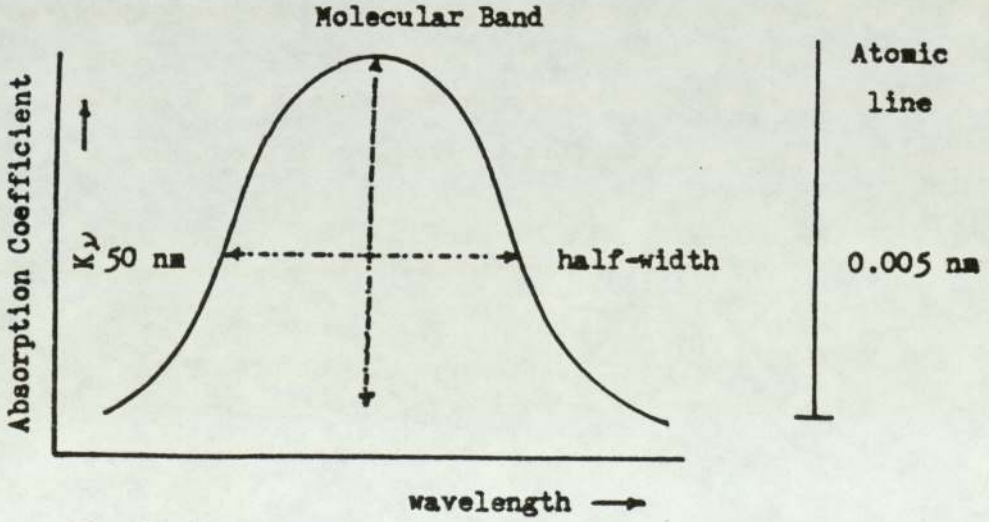


Fig.(1.2) A typical molecular absorption (in solution) and atomic absorption line (gas phase).

The overall width of an atomic spectral line is determined by different types of broadening processes which cause an increase in the effective halfwidth of an atomic line to 10^{-3}- 10^{-2} nm, which is still very narrow. Doppler broadening, D, which arises because the absorbing or emitting atoms having different components of velocities along the line of observation; is given by the equation :

$$D = (1.7\lambda/C) (2RT/M)^{\frac{1}{2}} \qquad (8)$$

where R is the universal gas constant, C is the velocity of light, M is the atomic weight of the absorber and T is the absolute temperature. This equation shows that the Doppler half-width is proportional to the square root of the absolute temperature, and is independent of the pressure. Collisional broadening (known as pressure or Lorentz broadening) of the resonance line is due to the concentration of foreign gas atoms in the environment of the emitting or absorbing atoms. It is difficult to calculate but is on the same order as the Doppler broadening. Resonance broadening is of the same type as collisional broadening but occurs from the concentration of the same kind atoms. This type of broadening process can be considered negligible in flames at pressure below 0.01 mm Hg. Natural broadening is of another type, is due to the finite lifetime of the atom in the excited state. It is of the order of 10^{-5} nm which is also negligible. There is also the Stark and Zeeman broadening which are due to electric and magnetic fields respectively. This can cause lines to become diffuse in the type of emission spectra, but it has negligible effect here. Hyperfine structure can be attributed to a non-zero value of the nuclear spin and/or the presence of several

isotopes. It is not an actual broadening process, but it is one of the major factors influencing atomic-line profile.

1.3.4 Practical Considerations

1.3.4.1 The Basic Atomic Absorption System

The general picture of the instrumentation required for AAS is given in the following diagram (Fig. 1.3).

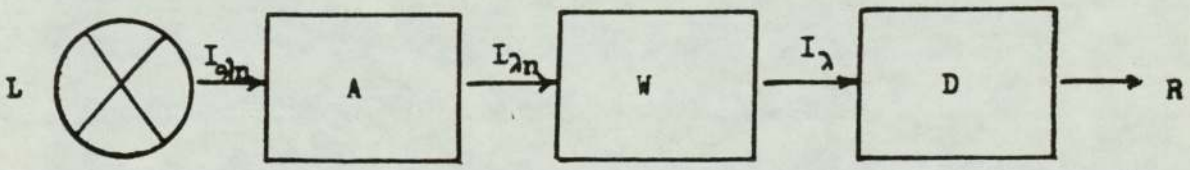


Fig.(1.3) Blockdiagram for AAS.

Light source (L), emitting a sharp line spectrum characteristic of the element of interest and of intensity of $I_{o\lambda n}$ passes through an atomic reservoir (A), in which the atomization process takes place. On emerging through (A) a line of intensity $I_{\lambda n}$ is focussed by the monochromator which serves as the selector of the desired wavelength from the polychromatic line radiation coming out of the atomic reservoir. The isolated resonance line falls on to the detector (D), a photomultiplier that detects the intensity of light energy I_{λ}. The

output is amplified and the measurements is taken using a readout device, e.g. a meter, strip chart recorder or, through data processing, to a digital display unit or printer.

The atomic absorption system shown in Fig. 1.3 is a typical of a single-beam instrument, in which, the light falling on the detector is proportional to the transmission of the 'sample' in the optical path, and it is thus necessary to make a reading with and without the sample in order to obtain the 'absorbance' which is a linear function of the concentration. The effect of source variation in this system can be overcome to great extent by employing double beam system which is shown in Fig. 1.4. The beam falls onto a rotating sector mirror before passing through the flame, which divides the beam. The beams are recombined, and the output signals corresponding to each beam are divided, amplified separately and compared in a bridge circuit. The out-of-balance signal is then compensated electronically and converted to absorbance.

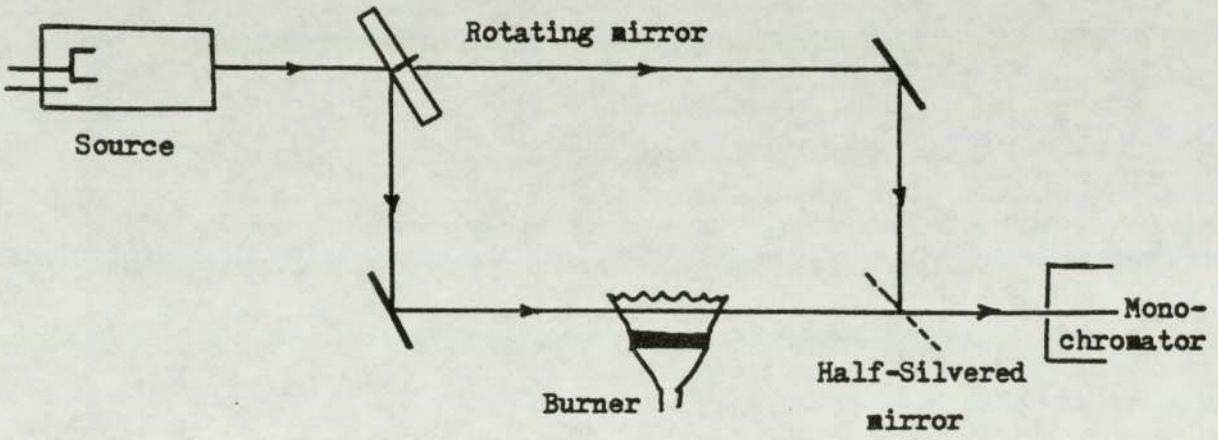


Fig. (1.4) Basic double beam optical system

Light Sources

It is essential in the atomic absorption assembly to use a sharp-line source which emits lines of smaller half-width than the absorption line itself. Idealy a half-width of 0.001 nm should be provided. The hollow cathode lamps have been known for many years in spectroscopy as a fine line source. It is undoubtedly the most convenient and widely used source. Vapour discharge tubes have been previously used, they consist of a glass or silica tube containing an inert gas at certain pressure and the metal of interest. This type of lamp has the advantage of much higher intensity than the hollow cathode lamps, and this gives better detection limits. On the other hand, these lamps are more unstable. Modern hollow cathode lamps are more stable and consist of a glass tube through which the electrodes are sealed, they are provided with an optical window of u.v. glass or silica. A cathode, hollow in shape, with an internal diameter of down 2 mm is made of the metal of interest and sealed in the tube together with an anode made of tungsten wire. The pressure inside the tube is reduced to about 10 torr. The tube within the cathode and anode are energized with voltage of about 300 v and currents of 4 mA up to 50 mA. Neon and argon gases are usually used. Multi-element lamps may be made in which the cathode is fabricated from more than one element of interest. Microwave and radio frequency excited electrode less discharges have been produced more recently.

The Flame Atomizer

The flame is the best known atomic reservoirs for AAS where the sample solution reaches the flame via the burner at a steady rate. It is the most convenient, stable and economic source of atomic vapours.

Fuel-oxidant mixtures are now commonly used for the combustion of the flame which provide a range of temperatures from about 2,000 to 3,000 K. Fuel gases include propane, hydrogen and acetylene, and oxidants include air and nitrous oxide.

Three mixtures of fuel-oxidant may be named as stoichiometric, lean and rich which contain a stoichiometric quantity of fuel gas, less than and more than this quantity, respectively. Air-acetylene is the most widely used of these fuel-oxidant mixtures and it could be used for the analysis of about 30 of the common metals (see table 1.1). The metals are the ones which do not form highly refractory oxides, such as calcium, chromium, iron, cobalt, nickel, magnesium, molybdenum, strontium and the noble metals. The use of nitrous oxide as oxidant is recommended for those metals which they form refractory oxides, such as aluminium, titanium and zirconium. A mixture of acetylene and nitrous oxide provides a higher temperature due mainly to the exothermic nature of its decomposition reactions (temperature of about 3000 K).

The 'Nebulizer-Burner' system is considered to be the heart of the atomic absorption assembly. It is responsible for conversion the sample solution into the atomic vapour. Many processes occur in this system; nebulization (solution to mist or aerosol), selection of mist droplets of the right size, mixing of the selected mist with the flame gases and finally introduction to the burner. Fuel mixtures, fuel speed and maximum temperatures, are most important parameters in the atomization process. The degree of atomization increases with increasing flame temperature, detection limits are improved and chemical interference is decreased.

Non-Flame Atomizer

Non-flame methods fall into two main categories, resistively heated devices and electrically induced plasmas. The electrothermal atomizer is one of many devices fall into the first group. This new technique of flameless AAS using an electrically heated open graphite tube enables the determination of trace metals to, as low as 10^{-12} gm to be analysed.

After a number of years development, the Perkin-Elmer Heated Graphite Atomizer (HGA-70) appeared in 1970 as the first commercial instrument on the market capable of determining trace metals in the range 10^{-9} gm to 10^{-12} gm. The design is based mainly on the work of Massmann. The furnace consists of a graphite cylinder, 51 mm long by 8.6 mm internal diameter, centralized and support at each end by two graphite cones serve as carrier for the current supply and also serve as an optical apertures. The tube and the cones are placed in the water-cooled metal housing in order to maintain the temperature outside the atomizer below 60°C. The graphite tube is heated to temperatures of 2500°C within 5 seconds by cables, in which electrical power, 10 v and 500 A maximum, is fed to the atomizer unit. To prevent the incineration of the graphite tube at red hot temperatures, an inert gas (Argon or Nitrogen) is passed through to surround the inside and outside the tube. The inert gas also flushes out fumes formed during the charring step. The atomizer unit is placed in the atomic absorption assembly in the path of the light beam from the hollow-cathode lamp in the position of the flame-atomizer system.

1.3.4.2 The Atomization Process in a Flame

The element being determined by AAS must be converted into 'free atoms' , capable of absorbing light. The success of analytical procedure depends upon the production of these 'free atoms' .

When a solution is nebulized into a flame, evaporation of the solvent takes place, leaving small solid particles (clotlets) which melt and vaporize. Equilibria are set up between the sample and the flame gases resulting mostly in the formation of oxide species (in a few cases hydroxides species are also formed, e.g. calcium, cesium and potassium). Those metals which give refractory oxides such as aluminium, molybdenum, uranium, etc. This means that these oxide species have low vapour pressures preventing evaporation.

Within the flame, the next step is a dissociation reaction of the metal oxide :

$$MO_{vap} \rightleftharpoons M_{vap} + [O] \qquad (9)$$

The equilibrium constant (K_p) of the above reaction can be defined as :

$$K_p = \frac{[M][O]}{[MO]} \quad , \text{ then}$$

$$\frac{[M]}{[MO]} = \frac{K_p}{[O]}$$

The ratio $[M]/[MO]$ is controlled by the value of the equilibrium constant (K_p) and the oxygen concentration $[O]$. The degree of atomization of the elements depends upon this ratio, in other words it depends on the value of the equilibrium constant (K_p) and the oxygen

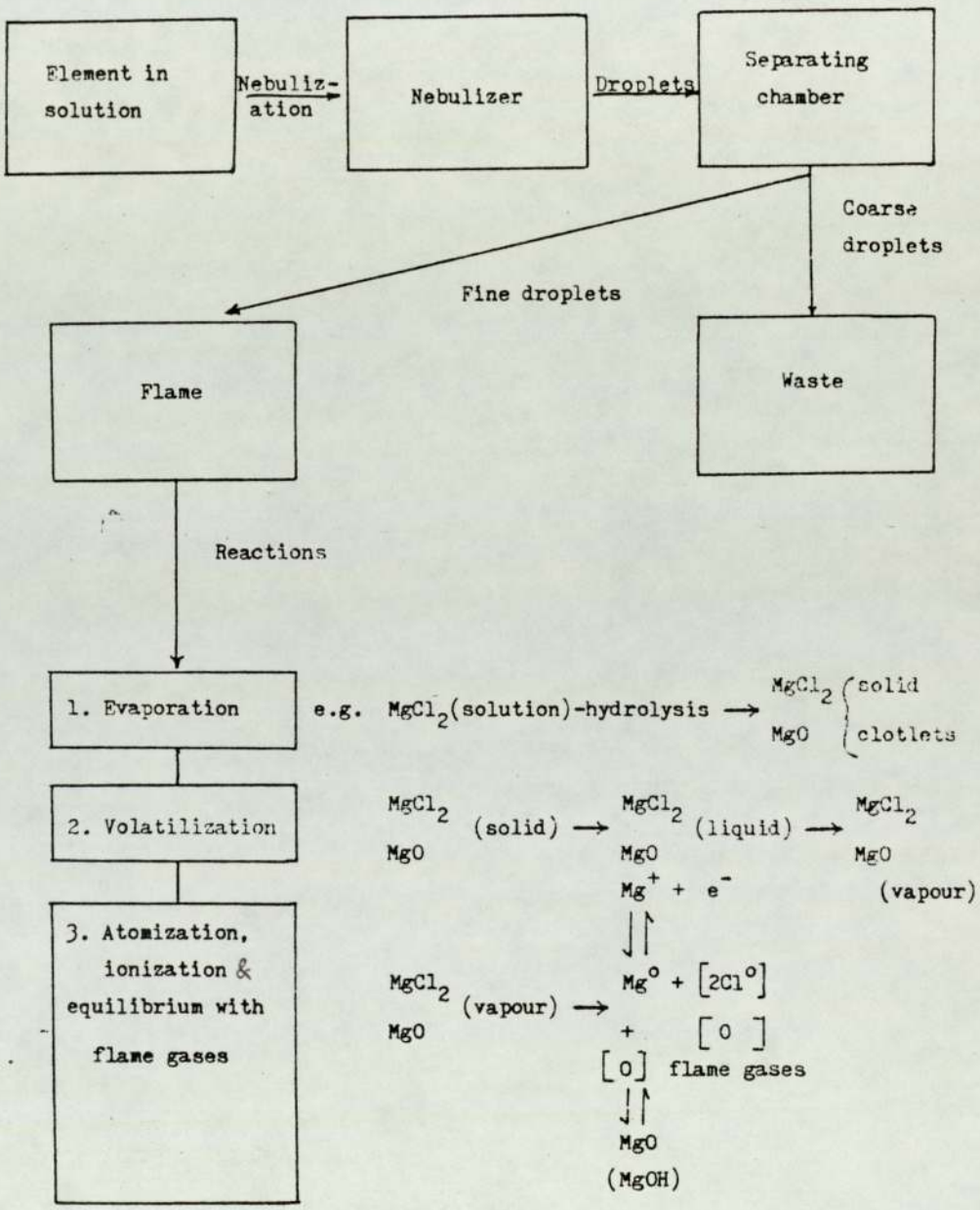


Fig.(1.5) Idealized processes of atomization in flame

concentration [O]. This fact has been utilized in order to determine some elements by indirect methods using the interference effect occuring from the change in degree of atomization of those elements when added to some other elements.

The idealized processes of atomization may be representing by the diagram shown in Fig. 1.5.

1.3.4.3 Atomization Process in an Electrothermal Device

The sensitivity of atomic absorption techniques can be increased considerably by using a graphite tube atomizer, graphite rod atomizer, or similar device as an atom reservoir in place of the flame. This great sensitivity arises from the modified technique's ability to retain a substantial proportion of the atomized analyte element in the observation zone for a finite period of time. Using these techniques, a small volume of sample can be completely atomized in a relatively small volume. This has the advantage that the large dilution factor associated with flames is avoided and very good limits of detection are obtained.

The basic condition for achieving high sensitivity in this process is that the rate of formation of the free atoms must be equal to or greater than their rate of removal from the optical path. The cylindrical furnace types of atomizer fulfil this condition better than other atomizers.

The overall furnace reaction can be regarded as occurring in several stages. Assuming that the metal salts obtained after drying the sample solution decompose to their metal oxides prior to atomization. The possible reactions can be summarized as follows :

i) evaporation of the metal oxide (MO) prior to atomization.

$$MO\ (s/l) \longrightarrow MO\ (g) \qquad (10)$$

ii) thermal dissociation of metal oxide is another possible reaction. The degrees of dissociation at various temperatures can be calculated from the thermodynamic data. The equilibrium constant for the dissociation of metal oxide can be calculated from the equation below :

$$MO \rightleftharpoons M + \tfrac{1}{2}O_2 \qquad (11)$$

For the carbon atomizers, there are another factors to be considered, the equilibria between oxygen and carbon.

$$2C + O_2 \rightleftharpoons 2CO \qquad (12)$$

$$2CO + O_2 \rightleftharpoons 2CO_2 \qquad (13)$$

iii) reduction of the metal oxide takes place by the furnace carbon according to the equation (14).

$$MO\ (s/l) + C\ (s) \rightleftharpoons M\ (g) + CO\ (g) \qquad (14)$$

iv) a stable carbide compound may formed from the oxide of some elements and graphite tube material.

$$MO + 2C \rightleftharpoons MC + CO \qquad (15)$$

1.3.5 Applicability of Atomic Absorption Spectrophotometry

Atomic absorption spectrophotometry is now one of the most widely used and well established instruments techniques of chemical analyses. Standard commercial instrumental have reached a very high level of performance. The extension of AAS to the determination of trace constituents and of major components is due not only to the developments of the commercial equipment and the improvement of its performance, but also to the resourcefulness of users to apply the technique to an ever wider number of applications. The technique has been applied in many fields : e.g. industrial, biological (including foods and animal feeds), geological, metallurgical, and environmental (air, water and food contamination). The applications are outlined in the diagram shown in Fig. 1.6.

Atomic absorption has proved to be a valuable tool in agricultural, biological and food analyses. In these fields, it offers a most rapid, convenient and reliable procedure for the estimations of toxic and nutritional metals in foodstuffs and natural products. Metals such as, zinc, copper, lead, lithium, arsenic, manganese, calcium, magnesium, selenium, antimony, cadmium, cobalt and iron, have been determined in foodstuff samples. Little work has been reported on the determination of organic species in foodstuffs using indirect AAS methods. Some applications are reviewed in chapter two.

Developments of AAS have been made in order to overcome the difficulties encountered with certain non-metallic elements e.g. halogens and gases which have their resonance lines in the vacuum ultra-violet region. Such developments include; special optical materials, special

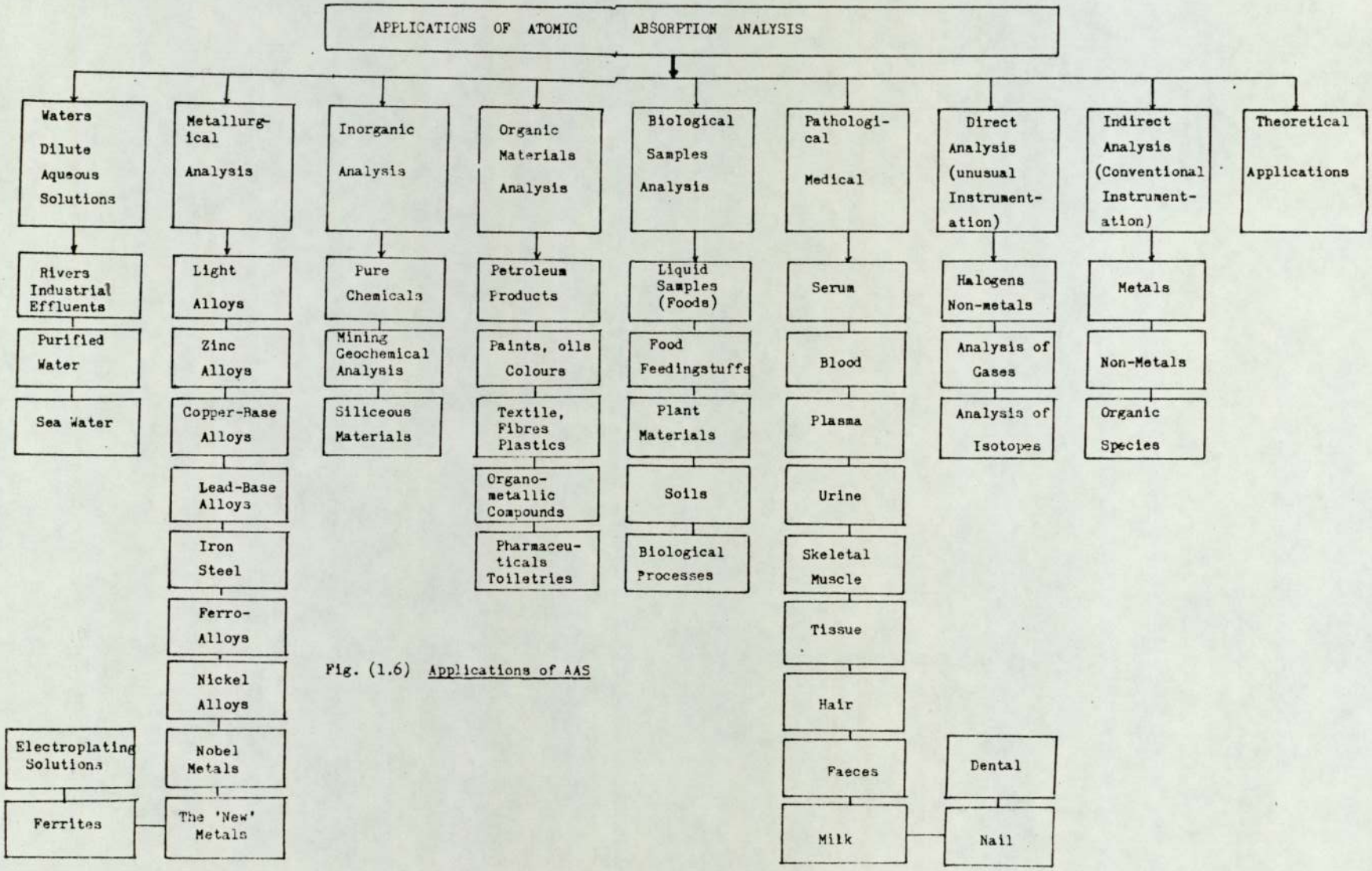


Fig. (1.6) Applications of AAS

photomultiplier tubes, graphite cuvette and sputtering techniques. This has extended the applicability of AAS to cover the analyses for halogens and non-metals such as ; F, Cl, Br, I, C, P, S, As, Se, Hg. It also covers the analyses of gases He, Ne, Ar, Kr, Xe, Rn, H, N, O, and isotopes of Hg, Li, U, H, He, and B. However, these analyses are not conveniently carried out with the available commercial equipments. They have not found wide applications as have the indirect methods which are alternative techniques in which the conventional equipment could be used. Indirect methods are in fact a recent extension of the applicability of AAS technique which have resulted in improved sensitivity for some metals and has enabled non-metals and organic species to be determined.

Some theoretical parameters have been measured using the electrothermal atomizer in which the experimental variables can be controlled, e.g. vapour-pressure measurements, dissociation energy, vacuum-u.v-spectroscopic studies, and atomic diffusion coefficient.

The potential of AAS for the determination of elements in general may be summarised in table 1.1 (27). This table may be divided into three groups of elements;

i) those elements which are directly determinable using air-acetylene flame AAS. This is successfully applied for most elements providing they do not form highly refractory oxides. Calcium, chromium, iron, cobalt, nickel, magnesium, molybdinum, strontium and noble metals are among this group.

ii) those elements which require a higher temperature because they form refractory oxides and are thus difficult to atomize or alternatively may give a very low sensitivity in air-acetylene flame. For these elements, the dissociation energies for the M-O bond are greater than about 5 eV.

Table (1.1) Elements determinable by AAS.

I	IIa	IIIa	IVa	Va	VIa	VIIa	VIII			Ib	IIb	IIIb	IVb	Vb	VIb	VIIb	0
· H																	· He
Li	X Be											X B	· C	· N	· O	· F	· Ne
Na	Mg											X Al	X Si	P	· S	· Cl	· Ar
K	Ca	X Sc	X Ti	X V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	· Br	· Kr
Rb	Sr	X Y	X Zr	X Nb	Mo		Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	· Xe
Cs	Ba	X La	X Hf	X Ta	X W	X Re	X Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	· Po	· At	· Rn
	· Ra	· Ac	X Th	· Pa	X U												

X Ce	X Pr	X Nd	· Pm	X Sm	X Eu	X Gd	X Dy	X Ho	X Er	X Tm	X Yb	X Lu

☐ Elements determinable in air-acetylene flame.

☒ Elements determinable in nitrous oxide-acetylene flame.

☐· Elements are not determinable by normal direct flame methods.

Examples are Al-O, 5.98; Ti-O, 6.9; Zr-O, 7.8 eV. Nitrous oxide as an oxidant with acetylene, hydrogen and propane produce a higher temperature (3000°K) compared with an air-acetylene flame (2450°K). The use of N_2O improves the sensitivity of the method for elements in this class. The sensitivity reported by these direct methods is high for several elements, 0.01-5 μg/ml.

iii) finally, those elements which may not be determined directly using the available equipment, or may be determined with very low sensitivity. Those elements are mainly the non-metals, and the indirect methods may be used for their determination using conventional equipment. The non-metals and organic species are disscussed in detail (see below).

1.4. Indirect Determination of Non-metallic and Organic Species by AAS

Many non-metallic elements, radicals or metallic elements with poor detection limits can be determined by indirect atomic absorption methods. Chloride, for instance, can be determined by adding an excess of silver nitrate, filtering off the silver chloride, estimating the silver content either by dissolving the precipitate in an ammonia solution or measuring the excess (unreacted) silver in solution (21).

Non-metallic species such as F, Cl, Br, I, S, P, O and N in which the main analytical lines lie in the vacuum ultra-violet ($<$200 nm), can be determined by indirect methods. Other species including metallic, non-metallic and radicals have been reported and are reviewed by Kirkbright and Johnson (22). Examples are Al, As, ClO_4^-, CN^-, SCN^-, Ge, Hg, IO_3^-, Nb, NH_3, NO_3^-, PO_4^{3-}, Se, Si, SiO_4^{2-}, Th, Ti, Tl, V. Examples of methods used for some non-metallic species are found in the table 1.2 (22).

Table (1.2) Indirect methods of atomic absorption for some non-metallic species

Species	Reagent(s) added	Principle of the Procedure	Element Measured by AAS
F^-	Mg	change in Mg absorbance in air-coal gas flame	Mg
F^-	Zr	change in Zr absorbance	Zr
Cl^-	$AgNO_3$	precipitation of AgCl	Ag
ClO_4^-	Cu(I) + neocuproin(nc)	solvent extraction of $Cu(nc)_2ClO_4$ into ethylacetate	Cu
I^-	Cd + 1,10-Phenanthroline	Tris(1,10-Phenanthroline)CdI_2 extracted into nitrobenzene	Cd
IO_3^-	Fe(II)	$Fe(II)IO_3^-$ - Fe(III) solvent extraction of Fe(II) into diethylether (9M HCl)	Fe
NH_3	Zr	enhancement of Zr absorbance	Zr
CN^-	Ag	add metallic silver and shake for one hour	Ag
SCN^-	Cu + pyridine	extraction of $Cu_2(Py)_2$ SCN $_2$ into chloroform	Cu
NO_3^-	Neocuproin + Cu(I)	extraction of $Cu(nc)_2$ NO_3 into MIBK	Cu
SO_4^{2-}	Ba	filter precipitate, and either redissolve in Na_2 EDTA or measure residual Ba	Ba
PO_4^{3-}	ammonium molybdate	solvent extraction of molybdophosphoric acid into n-butylacetate	Mo

An indirect method is usually applied either (i) when the element of interest does not possess a useful absorbing wavelength in the normal spectral region-visible and near ultra-violet region below 190 nm, or (ii) when the element to be determined forms refractory compounds (i.e. very stable oxide species) in the flame, e.g. B, Ce, Nb, Th, U or, in general gives poor sensitivity, or (iii) when the concentration of species radical or organic species are to be determined. Table 1.3 shows the available indirect methods for metal and non-metals (22).

The determination of halogens and certain non-metals in the vacuum ultra-violet region was one of the main limitations of AAS. This limitation is defined by two factors : (a) the molecular absorption of oxygen which begins to develop at less than 195 nm, and (b) the absorption of 'quartz' which is used in the optical system. The air-acetylene flame strongly absorbs any radiation between 190-230 nm i.e. in the wavelength region in which arsenic, antimony, selenium, tellurium, zinc and lead have their analytical lines. This absorption leads to background instability and a reduction in the available energy reaching the detector.

Two approaches have to be developed in order to overcome this kind of difficulty in the region below 200 nm. One approach is the development of unusual instrumentation in which many techniques and devices have been developed. Vacuum grating spectroscopic devices for instance have been used for observing the spectra. Lithium fluoride (LiF) or fluorspar (CaF_2) which transmit up to 105 and 125 nm are used for the windows and lenses. Above 150 nm, sapphire (α-Al_2O_3) or very thin layers of optical quartz can be used for making windows. The sputtering technique has been used in which the sample is made the cathode of hollow cathode tube as the mean of atomizing and no flame is involved. This

Table (1.3) Summary of available indirect methods for metals and non-metals by AAS.

Ia	IIa	IIIa	IVa	Va	VIa	VIIa	VIIIa			Ib	IIb	IIIb	IVb	Vb	VIb	VIIb	0
H																	He
Li	Be											B	C	N	O	F	Ne
Na	Mg											Al	Si	P	S	Cl	Ar
K	Ca	Sc	Ti	V	Cr	Mn	Fe	Co	Ni	Cu	Zn	Ga	Ge	As	Se	Br	Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	Ag	Cd	In	Sn	Sb	Te	I	Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	Au	Hg	Tl	Pb	Bi	Po	At	Rn
Fr	Ra	Ac															

Ce	Pr	Nd	Pm	Sm	Eu	Gd	Tb	Dy	Ho	Er	Tm	Yb	Lu

Th	Pa	U	Np	Pu	Am	Cm	Bk	Cf	Es	Fm	Md	No	Lw

Elements not determinable by AAS with conventional equipment available.

Elements determinable by indirect AAS procedures.

technique has employed for carbon, phosphorous and noble gases (He, Ne, Ar, Kr, Xe). Another technique which has been very useful for the elements having analytical lines shorter than 200 nm is the graphite cuvette.

The second approach in overcoming problems in the low wavelength region is the indirect method. This technique is more convenient since the available equipment may be used and it has been widely employed in many fields of chemical analyses.

Indirect AAS methods should be possible for organic compounds when the species being determined (A) combines in a definite stoichiometric proportion with metal (M) as in the equation.

$$M + n\,A \rightleftharpoons MAn \qquad (16)$$

hence (M) has to be determined using AAS in order to obtain (A). The effective utilization of any indirect AAS method, as a useful analytical technique, is based upon the knowledge of the stoichiometry of the chemical reaction. The difference between the indirect spectrophotometric methods and indirect AAS is that the first depends upon absorbance of light by coloured species, whereas the second based upon the absorption of radiation by free atoms of elements in the atomization cell.

1.5 Objectives of the Present Work

After a thorough search of the literature, it has been found that the atomic absorption spectrophotometry technique has played a very important role in the indirect methods of chemical analysis. The procedure

can be applied widely particularly in the determination of organic compounds encountered in environmental analysis.

It has been apparent that the topic of 'indirect methods' occupies either little or no space in books on AAS, the principles of the technique being stated only briefly. The reason for this most probably lies in the high specificity and selectivity in metal determinations which has somewhat overshadowed the estimation of organic species. Moreover much of the sporadic work that has been published or reported is in the Japanese literature.

Indirect methods have been developed in order to extend the applicability of AAS to include non-metals as well as organic species. One of the main aims to be achieved has been to obtain high sensitivity and selectivity for organic compounds and to present an alternative means of analysis for many compounds.

It is worth emphasising here, however, that the utilisation of an atomic absorption method for the determination of an organic species (i.e. one in which there is a relationship between the concentration of organic species and the atomic absorption signal); may have distinct advantages. For instance, it may offer a route to the analysis of a species not easily determined by another route. It may offer a route which utilises existing equipment, also the indirect method may be cheaper and more rapid than alternative procedures.

In reviewing indirect methods, certain observations and conclusions emerge which can be summarised as follows :

1) Numerous methods of indirect analysis have been reported and these have shown the feasibility of AAS in order to determine organic species. All the methods are based on selecting an appropriate chemical reaction

which is assumed stoichiometric.

2) There are few reviews dealing with the determination of organic species. Christian and Feldman (23) have considered a wide variety of indirect techniques, i.e. applications for the determination of organic compounds and inorganic anions and have demonstrated the practicability of obtaining a direct relationship between the atomic absorption signal and the concentration of substances such as orthophosphate, sulphate, iodide, sulphide, iodate, glucose, protein, 8-hydroxyquinoline, EDTA and APDC. However, the review covered very few organic species. Pinta (24) reported many indirect techniques for determining many metals and non-metals, but only a few organic compounds. Kirkbright and Johnson (22) reported on the general considerations and techniques required for some twenty elements or ions, but mentioned only a few organic compounds. This work was carried out before 1973 and since this time much work on indirect methods has been reported. Another useful review by the same authors (25) has covered the heteropoly amplification procedures used in AAS. The elements As, Ce, Ge, P, Nb, Si, Th, Ti, and V can be determined by this technique. These methods in general showed better sensitivity than the direct ones with a two fold increase in sensitivity. The work reported in this review included no organic determinations. Finally, the Annual Reports On Analytical Atomic Spectroscopy (ARAAS) (26) which gathers some 1600 abstracts annually from forty contributors all over the world and can therefore be considered one of the best sources of current information on atomic absorption, emission and fluorescence spectroscopy but contains very few pages covering indirect methods which are reported annually. It is clear, however, that these reviews have been orientated towards the determination of metals and non-metals rather than organic species.

No attempts have been made to include a fully comprehensive treatise on the applicability of AAS to organic species. There is now a need of such a review.

3) It seems apparent that these methods were developed either to meet a certain need in chemical analysis or on the other hand, some of these methods were described in order to demonstrate the applicability of AAS, i.e. there have been no systematic investigations to determine different organic classes or functional groups. The technique however has found practicability in certain organic classes; such as; alcohols, aldehydes, sugars, proteins, amino acids, primary and secondary amines, organic acids and the nitro-group, etc. The methods should be applicable to many other organic compounds.

4) In spite of losing selectivity for the method (in some cases) due to the nature of the initial reaction itself, the technique can still be quite valuable in some determinations.

5) Some spectrophotometric methods are modified and/or adapted to finish with AAS but all the methods involve a metal determination rather than a colour. Observation of a change in colour is not included in these methods as in spectrophotometry.

6) Of the indirect methods reported, solvent-extraction is extensively employed. The other techniques have been used to a lesser extent but in practice solvent extraction has greater applicability.

7) There is no AAS method available for the determination of saccharin or N-nitrosamines compounds.

8) The considerable sensitivity achieved by using the graphite furnace has not been exploited enough. A few cases in which a high sensitivity has been achieved, indicated the possibility of using the indirect

methods of AAS in trace organic analysis.

In the present work, attempts have been made to extend the applicability of the technique further. For this purpose it was decided to develop indirect atomic absorption methods for the determination of saccharin and N-nitrosamines. These compounds were chosen because of the interest shown in recent years about the carcinogenic properties of these compounds. It was thought that a new atomic absorption method for these compounds would perhaps lower the limits for their detection or at least provide an alternative (faster and possibly simpler) method to the existing techniques.

Thus, the main objectives of the present study can be summarised as follows :

a) to provide a comprehensive and up-to-date review on the indirect methods of AAS.

b) to examine in detail the existing spectrophotometric methods involving metal complexation of saccharin and N-nitrosamines, and the possible alternative routes for these determinations.

c) to develop improved spectrophotometric methods for the compounds of interest,

d) to extend these methods to the determination of the organic compounds by AAS,

e) to examine the use of the graphite furnace atomizer in an attempt to increase the sensitivity of the determinations, and finally,

f) to study the applicability of the methods using commercial samples.

Chapter two has been devoted completely to organic compounds in order to provide a current review on indirect AAS methods for the

determination of these species, together with the general principles of these methods. The possibility of determining saccharin by AAS via the complexation with 'Ferroin' $\left[Fe(Phen)_3\right]^{2+}$ and solvent extraction of the ion pair formed into nitrobenzene, has been discussed in chapter three. Problems associated with this determination using both the flame and flameless techniques, and the use of nitrobenzene have been demonstrated. Chapter four deals with development of a new spectrophotometric procedure for saccharin determination based on new technique for sorption and desorption process using a modified silica gel as adsorbent. This chapter introduces a new approach for the indirect AAS determination and uses silica gel matrix for this purpose. The use of other organic solvents, more suitable than nitrobenzene, is discussed. In chapter five, preliminary studies on the possible routes for developing an indirect AAS method for the determination of N-nitrosamines is discussed together with the chemistry involved. Finally, chapter six concludes with a discussion of the achievements and recommendations for the analytical procedures.

CHAPTER TWO

INDIRECT DETERMINATION OF ORGANIC COMPOUNDS BY AAS - A REVIEW OF THE CURRENT POSITION

2.1 Objectives

In chapter one, the lack of a comprehensive treatise in the literature on the determination of organic species by employing the indirect AAS methods, has been discussed. The present chapter is devoted to a such treatise.

The intention of this review is to provide a fully up-to-date guide to published applications of the indirect AAS methods. Literature from 1966 to 1980 is reviewed, not only to give a simple practical catalogue to the available procedures so far; but also to show the role of the indirect methods in analytical chemistry. It also demonstrates the applicability of the atomic absorption spectrophotometry.

A brief discussion of the general principles of the classified techniques employed prior the direct and indirect methods is included. These indirect techniques should be feasible for many other new applications.

As a conclusion in this survey; the general appreciation of the advantages and disadvantages of these methods are also discussed.

2.2 Techniques Employed Prior to the Use of Direct AAS Methods

Most elements can generally be determined directly after dissolution without prior separation. Occasionally, however it may be necessary to use one of the chemical preparation techniques. The purpose of these techniques is either to preconcentrate the element of interest to the limit of the determination, or to remove it from the bulk of the matrix for convenient atomization, or to remove the element

of interest from interfering materials. Solvent extraction, ion exchange and precipitation are the most powerful techniques employed for this purpose. They extend the applicability of AAS.

Solvent extraction is the most useful chemical method of sample preparation for AAS. The principle of the technique is to convert the ionic form of the metal in aqueous solution into a neutral species soluble in an organic medium. This can simply be done, either by utilizing the greater solubility of certain inorganic salts in organic solvents or by chelating the element of interest and extract the chelate into the organic solvent. Chelate extraction and ion-association systems are known. In the former technique, the metal ion coordinates with an organic ligand (e.g., ammonium pyrrolidine dithiocarbamate, 8-hydroxy-quinoline, cupferron) to form covalent compounds which are soluble in organic solvents. The second system can be achieved by either reacting the metal ion with an organic ligand to form an organic cation, which is often "neutralized" by electrostatic attraction to an anion or by combination of anions and organic solvents to dehydrate the metal salt by displacing the water molecules. One advantage of solvent extraction is that sensitivity is enhanced in the flame by a factor of 300 to 500% when an organic solvent replaces an aqueous solution. Esters and ketones have found to be the most satisfactory organic solvents to be used in flame AAS.

Ion exchange refers to the exchange of ions (cations) between a solution and an insoluble solid. The solid is often synthetic organic resin. Two kinds of procedures are used in ion-exchange; column and batch operations. In the former, a burette or column is packed with a certain resin and the sample is passed through. With the batch operation

the resin is added to the sample and shaken in suitable container. In both techniques, the sample solution is in contact with the resin and equilibrium is established between the sample ions and resin. The element of interest is adsorbed onto the resin and then eluted by strong acid. Batch operation is quicker than the column method.

Precipitation methods also offer a powerful technique for separation and concentration of an ion. Some organic reagents have been employed as precipitants for metals e.g. cupferron, 8-hydroxyquinoline and dimethylglyoxime. The precipitates formed can be redissolved into an aqueous solution or into an organic solvent before direct aspiration into the flame.

2.3 The General Principles of Indirect AAS:- Methods and Applications

Ever since the introduction of atomic absorption spectrophotometry as an analytical technique for metals, many attempts have been made to extend its applicability to the determination of non-metals and organic compounds. In general, there are seven different approaches that can be adapted for the indirect determination of non-metallic or organic species by AAS. These techniques are discussed under the following headings :

(1) Chemical interferences in the flame.

(2) Precipitation procedures.

(3) Ion-association or chelate complexes of metal using solvent extraction.

(4) Chemical amplification procedures.

(5) Reduction or oxidation of a metal.

(6) Direct measurements procedures.

(7) Interfacing techniques.

The principle of each technique and examples are discussed below in detail.

2.3.1 Chemical Interferences in the Flame

(i) Principle

When an element is 100% atomized, it should give a maximum possible absorbance. The presence of certain species produce an 'enhancement' or 'depression' on the absorbance for a given element. These 'enhancing' and 'depressive' effects are known interferences in AAS.

The chemical interference effect in AAS, however, is one phenomena which is utilised in indirect determinations. In this method a reaction takes place in the flame during the atomization process, between a certain element and the species of interest. This reaction causes a change in absorption signal of this element. The result is either a decrease in the signal (suppression) or increase (enhancement). This amount of interference effect may be determined giving a quantitative measure for the concentration of the species to be determined.

The depression of the absorbance signal is due to either, (a) the formation of stable species (refractory compounds) which may be a compound or radical combining with an element resulting in difficulties in dissociation in the flame, or (b) ionization, as for example when a calcium solution is aspirated to nitrous oxide-acetylene flame, calcium ions are formed and will not absorb the charcteristic radiation of the ground-state atom causing a depression of absorption.

An indirect determination exploiting the depressive effect is therefore a 'direct chemical interference' in the flame, whilst the enhancement effect is actually elimination of the chemical interference, i.e. a constant concentration of a substance is added in order to complex with metal and this removes the interference effect.

Materials, such as oxyacids (Aluminates, Phosphates, Sulphates, Silicates) and some others have appreciable depressive effect on both emission and absorption signals of particular elements such as calcium and strontium in air-acetylene or air-propane flames. The process is utilized to determine these oxyacids, by simply deriving the relationship between the concentration of the material and the absorption of metal affected by the depressive effect of the addition.

It is not only anions which give this, but also some organic compounds such as glucose and proteins which cause a similar decrease in absorption of calcium.

Some other species such as fluorides have the same effect on the response of magnesium absorption, but they have an increase effect on zirconium and titanium in nitrous oxide-acetylene flame. This effect is exploited for fluoride determination. The response of zirconium absorption signal is increased by the effect of nitrogeneous materials such as, ammonia, amines, aminoacids and other organo-nitrogen compound. Trace metals also may be determined by the increase of iron absorbance, such as aluminium and titanium.

(ii) Applications

Chemical interferences have been investigated by Christian and Feldman (23) in order to determine the feasibility of obtaining a direct

relationship between the atomic absorption signal and the concentration of the species of interest. Some organic compounds have been determined utilising the direct chemical interference in the flame or the removal of this interference.

Proteins (23), such as glucose oxidase and ribonuclease, at the 2.5×10^{-4} g % level caused a decrease in calcium percent absorption (in concentrations 4.6×10^{-4} M Ca). This decrease was proportional to the protein concentration and was different from one protein to another.

Glucose (23) showed some interesting effects in which a very small concentrations (less than 10^{-6}M) caused a noticeable decrease in absorption of calcium (of concentrations of 6×10^{-4}M). At glucose concentrations above 10^{-6}M, the absorption was gradually increased with concentration increase and then levelled off at concentrations of about 10^{-5}M. It was also found that glucose in a concentration range (10^{-6}-5×10^{-5}M) gives a linear enhancement in the calcium absorbance of a solution containing 3.75×10^{-4}M calcium and 4×10^{-4}M sulphate ion. The addition of glucose in this technique would eliminate the sulphate interference in the flame.

Nitrogen-containing compounds (30) have been found to enhance the absorption of zirconium in the nitrous oxide-acetylene flame. The magnitude of the enhancement has been found to be proportional to the concentration of the nitrogen-containing compound present in the solution. This effect has been utilized in order to determine ammonia , aliphatic amines , aromatic amines , amino acids and heterocyclic compounds. The authors attributed the enhancement to the formation of relatively volatile and easily atomized compounds containing zirconium-nitrogen bonds. The concentration of ammonia in this determination was over the range 1×10^{-4}

to 5×10^{-3}M.

2.3.2 Precipitation Procedures

(i) Principle

Precipitation of an equivalent amounts of metal can be used to determine many anions and other organic compounds. In these procedures, the element or the species of interest is precipitated using another element measurable by AAS. The absorbance of the element may be measured either in the precipitate after dissolving in suitable solvent, or in the filtrate (i.e. unreacted element). A direct relationship may be obtained between absorption by a metal and the amount of species to be determined.

(ii) Applications

The determination of sugar in plant materials, using AAS reported by Potter et.al (31), has the advantage of simplicity besides a good agreement with the official method. The method is a combination of two processes; the reduction of copper(II) and the precipitation. The reducing effect of sugar on copper takes place in alkaline solution forming insoluble copper(I) oxide. The copper(I) oxide was centrifuged from solution and the unreduced copper(II) in solution was measured by AAS using a standard calibration graph. Reducing agents may be determined using this principle of precipitation for example dextrose in the concentration range 0.1-5μ g/ml.

The practicability of determining several organic compounds by precipitation as their insoluble silver compounds and measuring the amount of excess of silver(I) by AAS, has been demonstrated by Gupta and

Boltz (32). Compounds such as iodoform , theobromine sodium salicylate , mercaptobenzothiazole and xanthate have been determined by aspirating the supernatant solution after addition of silver nitrate and centrifuging. The excess of silver was determined by AAS. At high levels of iodoform (50 mg) an excellent recovery was achieved, but at low levels(5-and 1-mg) resulted in lower recoveries. Also, excellent recoveries at low levels of mercaptobenzothiazole (0.1-1 mg) were obtained, while slightly higher recoveries were found for the determination of potassium ethylxanthate due to the decomposition of the precipitate in the presence of excess silver nitrate. However, the authors suggested that an indirect AAS method may be convenient due to its sensitivity and speed.

In clinical research the indirect AAS methods have found useful applications. For instance, a sensitive, reproducible and specific method has been proposed for determination of the total urinary amino acids (33). The method was useful in the investigation of total hypo- or hyper-excretion of urinary amino acids and is based on the formation of copper complex by addition of copper chloride solution with borate-phosphate buffer solution. The liquid after centrifuging is assayed for copper content using air-acetylene flame at 324.7 nm copper line. Gawargious et.al (34), have described another rapid method for determining ∝-amino acids after conversion to copper(II) chelates. The method was successfully applied to the determination of some ∝-amino acids with average error 0.18%.

The reaction of secondary amines with carbon disulphide to give dialkyldithiocarbamic acids (DTCH), has been utilised in order to develop an indirect AAS method for the determination of micromolar quantities of aliphatic secondary amines (35). The method involves the formation

of nickel-complex $Ni(DTC)_2$ resulting from the reaction between the carbon disulphide and the secondary amine in the presence of ammoniacal nickel reagent. The precipitate was separated from the reaction medium, dissolved in benzene-acetone (1:1), evaporated, digested with $HCl-HNO_3$ and then diluted. The resulting solution was analyzed for nickel content using flame AAS. The method was applicable to nine secondary amines. The linear part of the calibration curve for nickel was reported to be 2-25 ppm Ni which corresponding to 0.68-8.5μmol of secondary amine. The practical detection limit was about 0.30μmol/ml of secondary amine solution. At this level, the relative standard deviation obtainable was found to be from $\pm$ 7.3% to $\pm$ 12%. The reaction in this method takes place over 1.5-2 hours. The method has disadvantages-long time for the reaction to take place and high standard deviation values.

An indirect AAS method for the determination of total strong heavy metal chelating agents in water and waste water has been reported (36). The method is based on the solubilization of copper by the chelating agents at alkaline medium followed by filtration. The copper content in the filtrate is measured.

Disodium edetate dihydrate (DED) in small amounts may be added in the production of pharmaceuticals in order to remove alkaline earth impurities and also to improve the efficiency of the production processes. An atomic absorption spectrophotometry method for determining DED in antibiotic streptomycin has been described (37). The method involves the formation of a nickel-DED complex. The reacting amount of nickel is directly proportional to the amount of DED present in the sample. Precipitation of excess of nickel with dimethylglyoxime was carried out and the complexed nickel with DED was released by pH adjustment and determined

by AAS. The reproducibility was found to be $\pm$ 1.3 μg/g and the detection limit was 4 μg/g. The results in this method indicated that there was no interference from phosphate species.

Atomic absorption spectrophotometry using indirect techniques has been applied in toxicity studies for the determination of the chelating agent nitrotriacetic acid NTA (38). This determination was required in order to evaluate the toxicity and biological effects of the compound in aquatic systems. The method involves the quantitative solublisation of lead carbonate by NTA to form a soluble lead-NTA chelate equivalent to the amount of NTA present initially in the sample. The amount of lead-NTA chelate is measured using AAS.

Mitsui and Fujimura (39) have described a method for the determination of the primary amines at concentration $\geq$1 mg. The method is based on a complexation of amines with a reagent solution containing (triethanolamine + 5-nitrosalicyladehyde + acetaldehyde + copper sulphate). The copper content was determined, either in the precipitated copper complex after dissolving it in nitric acid or in the excess of copper in the filtrate. The method suffers from some interferences; zinc, magnesium and calcium while the other amines (secondary-and tertiary-) do not interfere in the determination if they are present in 20-30 fold excess.

The determination of micromolar quantities of aldehyde has been reported by Oles and Siggia (40). In their method, silver-ammonia complex (Tollen's reagent) was used to oxidize aldehydes to their corresponding carboxylic acids. The reduced silver was filtered off, dissolved in nitric acid and the silver content either in the resultant solution, or in the filtrate was determined. The method was found to be

advantageous for determining total aldehyde content in many systems containing a number of aldehydes, e.g., distilled liquors, flavouring, or perfumes. The low working range for the aldehyde concentration determinable (0.1-1.0 μmol for 10 ml or 0.5-5.0 μmol for 50 ml) reflected the practicability of the indirect AAS. This concentration range of aldehyde corresponded to 2-20 μg/ml silver under the working conditions for the AAS instrument. The relative standard deviations were found to be 1.2-5.8% in the 1-4 μmol/ml range. The very same technique has been used by Mitsui and Kojima (41) for determination of several aldehydes in which 0.002-0.231 g/l detection limit range has been obtained. Moreover, some compounds have been investigated in order to check their interferences.

Oles and Siggia (42) have developed an AAS method for the determination of micromolar quantities of 1,2-diols compounds in organic mixtures.

These compounds were oxidised with periodic acid, the iodate formed separated by precipitation as silver iodate. The precipitate was then dissolved in ammonium hydroxide and the silver content determined in the solution. The precision of the method was found to be $\pm$ 0.9-5.0% in the range of 0.3-4.0 μmol per ml of 1,2-diol. The practical limit of detection was 0.2 μmol per ml of 1,2-diol. This procedure was found to be particulary useful for determination of 1,2-diol impurities in compounds containing non-adjacent hydroxyl groups. The authors claimed that this indirect method offered some advantages such as speed of analysis, sensitivity and detection limit comparable to the most sensitive method currently available.

A convenient and selective procedure for estimation of

phenylacetylene using the indirect AAS approach has been described by Smith and Bailey (43). They made use of the reaction of phenylacetylene solution with an ammoniacal silver nitrate reagent at room temperature to form silver acetylides. The precipitate was washed, separated and dissolved in piperidine, and the amount of silver determined, either in the precipitate or in the supernatant solution. In the former technique, the calibration curve was found to be linear over the range 1.0-4.0 ppm (silver) and gave a mean percent recovery of 100.4% with a relative standard deviation of 6.5%. In the second technique, the calibration curve was found to be linear over the range 2.5-7.5 ppm (silver) and gave a mean recovery of 100.3% with a relative standard deviation of 2.9%. Although the first technique was as twice sensitive as the second technique, the precision of the second method was proved to be better and the method was quicker. Because silver phenylacetylide is insoluble in most solvents, piperidine was therefore used to solubilize the precipitate and diluted with DMF. These solvents are unusual for AAS use. However, the second technique could be used.

In the recent years, there has been an increasing demand for a method of determination of oxalic acid in urine samples. This is due to the formation of calcium oxalate stones in the patient's urinary system and routine clinical tests are required for urine samples. Two routine micromethods for determination of oxalic acid in urine by AAS have been developed in order to meet this need of a rapid method of analysis (44, 45). The methods are based on the precipitation of oxalic acid with excess of calcium ions at pH 5. The calcium in the precipitate is determined indirectly by AAS, subtracting the excess of calcium measured in the liquid from the total calcium present and added to the urine

in a similar sample at pH 2. The oxalic acid may be calculated on this basis. The percentage recovery for the normal range specimens was 95% of oxalate for both methods. Although the method (45) gave better precision than the other method (44), it seems that it is time-consuming method in which the completion of the precipitation takes from 12 to 72 hours. Precipitation times was investigated and the percentage of oxalic acid recovered found to be 90.4%, 94.5%, 95.2% and 95.1% at different precipitation time of 12, 24, 48 and 72 hours respectively. While in the other method the precipitation time was one hour giving 95% recovery.

Indirect AAS techniques have found a wide range of applications in drug analysis for the determination of certain organic species. Some examples are mentioned here, but many other examples will be mentioned under other subheadings. Methamphetamine hydrochloride (46) down to 16μg ml^{-1} has been determined in drugs via the precipitation as its bismuth complex. The precipitation takes place by the addition of hydrochloric acid (7%) and potassium iodide solution (7%) saturated with bismuth(III) chloride to the test solution. The unconsumed bismuth in the filtrate was determined by AAS using the line at 223.1 nm. The tolerated amounts of some metals and organic species have been reported and the interference could be eliminated by prior extraction of methamphetamine hydrochloride into chloroform.

Barbituric acid derivatives (at levels 0.51-16.21 mg) have been estimated in pharmaceutical products (47), by precipitation as copper-pyridine barbiturate complexes. The copper content, either in the precipitate (dissolved in concentrated nitric acid) or in the filtrate was determined. The method seems to be simple and rapid, however some interferences were reported including metals and some organic species.

This problem was overcome by precipitating these species with the addition of sodium carbonate solution.

Indirect AAS methods for determination of biuret in mixed fertilizers and urea has been investigated (48-50). It has been claimed in the previous reported method (48) that the only disadvantage is that the determination can be carried out after keeping the copper hydroxide (for complexing) in suspension for about five hours. Woodis et.al (49) have developed an indirect method based on the treatment of an ethanolic solution of biuret with copper(II) and alkali to form a biuret-copper complex. The complex remains in solution and the excess of copper is precipitated. After filtration, the complexed copper in solution is determined using the AAS technique. The copper-biuret complex forms immediately after the addition of alkali containing copper and biuret. The method has been developed in order to avoid the colour formation methods which suffer from colour interference as a source of problem. Corominas (50) has concluded from his study on biuret determination; that the AAS method of Woodis et.al was found to be the more reliable for mixed fertilizers than the AOAC method 2.072 which involves spectrophotometry. The AAS method was found to be accurate and applicable to mixed fertilizers and urea samples containing biuret in the 0-10 mg concentration range.

Sugars, such as glycerol, lactose, saccharose, glucose, tartaric acid (51) have been determined in ethanol samples in which they react with periodic acid to yield iodate which was precipitated by silver nitrate. The precipitate was washed with dilute nitric acid and water, dissolved in ammonia for subsequent silver determination using the flame technique and the silver 328.1 nm line. The limits of determination were

from 0.2 mg to 1.93 mg. Acetaldehyde in an ethanol sample was treated with Tollen's reagent to give silver. The precipitated silver was filtered, washed and dissolved in nitric acid and water and analyzed for silver content. The limit of determination was reported to be from 2 μg (51).

Tollen's reagent (ammoniacal silver nitrate) has been widely employed in indirect AAS methods. Another application of this reagent has been reported for determination of some nitro-compounds (52). The nitro-group was reduced to the substituted hydroxylamine with zinc powder, oxidized by Tollen's reagent and filtered. The silver content was then measured by AAS after dissolving the precipitate in nitric acid. The method suffers from serious interferences from resorcinol (0.25-fold), benzoin, P-aminophenol, or ninhydrin. The effect of interferences from some metal ions could be eliminated by prior extraction step of nitro-compounds with benzene.

The applicability of the atomic absorption spectrophotometry technique has been demonstrated on the determination of tannins in equeous tea extracts (53). The method involves precipitation of tannins with copper acetate followed by AAS determination of copper in either the precipitate or filtrate after digestion with a mixture of nitric and sulphuric acids. However, the authors have reported that systematic differences in the results were found a comparison with another techniques. They claimed that these differences can be overcome.

Hassan et.al (54) have developed a simple, rapid and accurate method for the determination of vitamin B1 in pharmaceutical preparations. The method is based on the desulphurization reaction with potassium plumbite by precipitation of lead sulphide. The unreacted lead(II) ions

are measured at 217 nm using AAS technique. The method offers several advantages in terms of simpilicity, rapidity (15 minutes for the analysis), sensitivity (linear curve 1-10 μg Pb/ml) and accuracy. Recoveries of pure vitamin B1 was found to be 99.1%, with a standard deviation of 0.8%. Moreover, several common excipients and diluents used in the preparation of capsules, tablets and suspensions were examined. Magnesium stearate, talc, sodium citrate, carboxymethyl cellulose, tween 80, polyvinyl pyrrolidone, glucose and lactose in large amounts showed no interference. There was no effect also from vitamins B2, B6 and B12.

An indirect AAS method for the determination of micromolar quantities of thiols has been described (55). An alcoholic silver nitrate reagent was used to precipitate the silver salts of thiols. The silver content in the precipitate after dissolving in nitric acid, was estimated by AAS. Thiols in the 0.8-20 μmol/ml range may be determined using this method. Interference was caused by hydrogen sulphide.

Aliphatic esters in 0.035-3.482 mg/ml range concentration have been estimated using an indirect AAS method (56). Interferences have been reported for copper and aluminium ions but many other species such as potassium, magnesium, calcium, ethylether, acetone, acetic acid, aniline, nitrobenzene, propionaldehyde and phenol did not interfere. Recoveries were reported to be between 92.4-105.0%.

The precipitation technique has been applied to the determination of non-ionic detergents in water and sewage samples by AAS (57). In this method non-ionic detergents in 0.1-1.2 mg range may be determined. The detergent in a five-litre sample was extracted into ethylacetate by a foaming technique, evaporated and the dry residue was mixed with molybdophosphoric acid solution and barium chloride. The unconsumed, Mo

(molybdophosphoric acid) after centrifugation or filtration was determined by AAS. The recovery ranges were from 97.3 to 102.1%. The method may be applied to samples containing proteins or calcium, magnesium or manganese salts. Many other methods of detergent determination are given under other subheadings in this review.

Mitsui and Fujimura (58) have developed an indirect AAS method for determination of biacetyl in sample solutions containing 0.05-2 mg of this species. The sample solution was treated with hydroxylammonium chloride-sodium acetate solution and heated on a water bath at 75°C for 10 minutes. Nickel solution (6.3 mg ml^{-1}) was added after cooling. The nickel content in the precipitate was determined by AAS. The method is free from interference which may be caused by many species including cations and organic compounds.

A very simple method has been reported for the determination of chlorprothixene (59) in drugs. The method involves a precipitation process by adding a freshely prepared solution of ammonium reineckate in excess. The precipitate formed was filtered off and the chromium in the filtrate was determined by AAS. The calibration curve was linear in range 8-10 $\mu g\ ml^{-1}$ chromium concentration and the mean recovery was 98.6 to 100.9%. No interferences were reported.

2.3.3 Ion-Association or Chelate Complexes of Metals Using Solvent Extraction

(i) Principle

The anionic or organic species of interest may form an uncharged complex with a metal ion in the aqueous solution. The metal

complex is extracted into an organic solvent. The absorbance of the metal content in the organic solvent is measured after aspiration of extracted species in the organic phase into the flame, or alternatively the excess of unreacted metal remaining in the aqueous phase may be determined. A linear relationship is found between the metal content (in terms of absorbance) and the concentration of the added anionic or organic species forming the unchanged complex or the ion-pair.

In some instances the species of interest acts as a masking agent for the solvent extraction of a metal-chelate. The decrease in absorption by the extracted metal is proportional to the species of interest present in the aqueous phase.

In this indirect technique, an excess of metal ion is added rather than an excess of the chelating agent.

The combination of solvent extraction and atomic absorption offers the most powerful means to extend the applicability of AAS in order to cover the area of major and micro analysis of anionic and organic species.

Many organic solvents including ketones, esters, alcohols, ethers, aliphatic and aromatic hydrocarbons and nitro-compounds have been employed for flame AAS using the solvent extraction technique. This technique also has been extended to AAS analysis utilizing electrothermal atomization. The electrothermal atomizers possess an advantage over flame atomizers, in that when solvent extraction is employed, the range of solvents which may be used is less restricted. The problem of toxic combustion products and other undesirable combustion characteristics is totally eliminated when electrothermal atomizers are employed. This is because very small aliquots of sample solution is

injected into the atomizer. In general, the use of organic solvents in AAS method improves the sensitivity.

Most of the chelating reagents of interest are of the type HL, in which H can be replaced by an equivalent of metal, for instance; -OH, -COOH, $-NH_2$, =NOH and -SH. 1,10-phenanthroline has proved to play a significant role in the indirect AAS methods employing the solvent extraction technique. 1,10-phenanthroline, for instance, with iron(II) ion (and other divalent metals) gives a stable five-membered ring with the coordinated metal atom. The extraction of the iron(II)-complex requires neutralization of the charge with a bulky anion such as chlorate or with an organophilic anion.

The above mentioned facts make the indirect determination of some of the anionic or organic species which have the characteristics of those chelating agents, possible.

A wide range of applications, particularly, in drug preparation analysis has been reported which are based mainly on either; (i) ion-association extraction system or (ii) chelate extraction systems. Neutral complexes formed using the second system are readily extractable into suitable solvents and this makes the determination of numerous organic species feasible.

(ii) Applications

It has been found that neocuproine (2,9-dimethyl-1,10-phenanthroline) (NC) dissolved in chloroform was a selective extractant for phthalic acid in aqueous solutions containing amounts of copper(I). This principle has been applied to the atomic absorption for determination of phthalic acid (60, 61) via its extraction as the ion-pair ;

$\{[Cu(NC)_2];[C_6H_4(CO_2)_2]\}$ into MIBK. The copper content measured by AAS is proportional to the concentration of phthalic acid present in the aqueous phase. The linearity of the calibration curve was over the concentration range 0-4 X 10^{-5}M of phthalic acid.

Similarly, Yamamoto et.al (62) have reported a new method for the determination of pentachlorophenol (PCP) by AAS. The anions of PCP may be extracted into nitrobenzene as $\{[Fe\ Phen_3];[C_6Cl_5O]_2\}$ from an aqueous phase containing an excess of tris-(1,10-phenanthroline)iron(II) cations. It was possible to determine a small amount of PCP in the concentration range (0-3 X 10^{-4}M) in the aqueous solution through the aspiration of nitrobenzene phase into an air-acetylene flame using the iron 248.3 nm line.

Solvent extraction in flame AAS has been applied to organic species that are capable of forming metal chelate complexes which are extractable into organic solvents. Christian and Feldman (23) have demonstrated the practicability of AAS for the determination of various organic species of this kind. Ammonium pyrrolidine dithiocarbamate (APDC) has been determined through its extraction with an equivalent amount of copper or cobalt into MIBK at pH 3. The final concentration of APDC in the organic phase ranged up to 10^{-5}M. Similarly, 8-hydroxyquinoline (oxine) has been determined by extracting its copper complex into MIBK or ethylacetate from an ammonium acetate buffer at pH 6.5. The concentration of oxine in the organic phase was in the range 0-2 X 10^{-5}M (23).

Masking of the solvent extraction of a metal chelate was another way of determining some organic compounds. Ethylenediaminetetraacetic acid (EDTA) for instance, has been determined (23) via the formation of

charged complex with copper and masking the extraction of the copper-oxinate complex into MIBK at pH 6.5. The result of this was, a linear decrease in absorption by the extracted metal which was found to be proportional to the EDTA concentration present in the aqueous phase ($0-4 \times 10^{-5}$M). The above mentioned methods showed a high sensitivity and a rapid way of analysis. Small volumes of test substances were used.

β-Hydroxynaphthoic acid has been determined indirectly via the solvent extraction of tris-(1,10-phenanthroline)nickel(II)-β-hydroxy-naphthoate species into nitrobenzene, followed by measurement of the nickel concentration in the organic phase (63). An air-acetylene flame has been used with the Ni 232.0 nm line. The nickel content was a function of the amount of β-hydroxynaphthoic acid initially present in aqueous phase. The calibration plot gave a linear relationship in the range from 8×10^{-5} to 4×10^{-4}M of β-hydroxynaphthoic acid.

An indirect AAS method has been developed for the determination of nitro-group (64). The method involves the oxidation of nitro-group to nitrate using cerium sulphate or potassium permanganate. The nitrate reacts with copper-neocuproine complex to form an ion-pair which is extracted into MIBK and the copper content is determined by AAS.

Butylscopolamine bromide and butylscopolamine tannate which are parasymathetic nerve blocking agents have been determined in blood, urine and feces by forming the ion-pair complexes with $Co(SCN)_4^{2-}$ in an acidic medium in the presence of tartaric acid (65). These complexes were extracted into chloroform, and the cobalt in the extracted species was determined in a medium of isobutyl methyl ketone or ethyl acetate. The calibration graph was linear over the range 3 to 15 μg of butyl-scopolamine tannate in isobutyl methyl ketone.

Two indirect AAS methods have been developed by Kidani et.al (66, 67) for the determination of anthranilic acid. The first method is based on the formation of the iron(III) complex which is extracted from acetate buffer solution pH 5.9 into MIBK. Alternatively, it may be determined by extraction as cobalt(II)-anthranilic acid complex into MIBK from pH 6.0-7.5 acetate buffer in the presence of bathophenanthroline (4,7-diphenyl-1,10-phenanthroline). The cobalt in the organic phase is measured using an air-acetylene flame. A linear calibration curve was obtained in the concentration range 3-22 μg/ml of anthranilic acid with 99.5% recovery. The method is rapid and sensitive.

The analysis of dissolved free fatty acids (FFA) has been of great interest and importance for the oceanographers for many years. Treguer et.al (68) have contributed to this field by proposing a new method for determination of total dissolved free fatty acids in sea water. The (FFA) was extracted with chloroform, evaporated to dryness and dissolved in chloroform-heptane solution. A copper complex was formed when a mixture of (triethanolamine, acetic acid and copper sulphate) was added. The mixture was centrifuged and an aliquot of the organic phase was evaporated to dryness before ammonium pyrollidine dithiocarbamate in MIBK was added. The complexed copper was determined by AAS. The determination of total FFA in sea water could be carried out with good selectivity and 10% precision. Rapidity of the method and the fact that only one litre of water is required for this determination are the main advantages. The method is also applicable to the determination of long chain FFA (longer than C_{10}) in concentrations of 10-40 μg/l. Another method for fatty acids determination in serum, in concentration range 70-1300 μ moles/l, has been reported (69).

Some drugs, such as cyanocobalamin, copper chlorophyllin sodium, and calcium pantothenate, contain metals in their matrix which can be subjected to AAS directly. The stoichiometric ratio between the metal content and the molecules of the matrix is the basis for an indirect AAS determinations. Many other drugs possess groups capable of forming chelate compounds with certain metals. This principle was employed for the determination of several drugs of this kind, and a series of papers have been published, particularly, in the Japanese literature.

Chinoform (5-chloro-7-iodo-8-quinolinol) in drug preparation posseses a group capable of forming an oxine-type compound and this form the basis of the determination by extracting its 2:1 zinc chelate into MIBK (70). The absorbance was measured at Zn 213.8 nm line. A linear calibration curve was obtained for 6-30 μg/ml of chinoform, with standard deviation 0.495.

Kidani et.al (71) have described the formation of 2:1 isonicotinylhydrazine-copper chelate which was extracted into MIBK as the basis of an indirect AAS determination for isonicotinylhydrazine in pharmaceutical products. The relationship between the absorbance of copper content in the complex and the concentration of isonicotinyl-hydrazine initially present in the aqueous phase was linear in the range 0.2-1.7 X 10^{-4} M with relative standard deviation 0.44%.

Minamikawa et.al (72) have noticed that flufenamic acid, a non-steroid antiphlogistic forms a chelate compound with copper in the presence of 2-(2-hydroxyethyl) pyridine and they developed an indirect AAS method for the determination of flufenamic acid in biological samples and preparations. Their method involves extracting the chelate compound with propylacetate followed by determination of copper content. A

higher sensitivity than the thin-layer chromatographic method has been claimed in this method and it could be applied to samples without any separation or preconcentration procedures and was not affected by water as in the fluorometric method.

Chloropheniramine maleate forms a 1:1:1 ternary complex when treated with copper-zincon chelate in water at pH 4.3. This was used as the basis of a new method for the determination of this organic species in drug preparations (73). The ternary complex was extracted into chloroform and the copper content determined in the organic phase by using a mixture of chloroform and methanol. A linear relationship was found for the range 1.9-27.4 μg/ml of chlorpheniramine maleate present initially in the aqueous phase and the relative standard deviation was 2.18%. The method offers simplicity and good sensitivity, however, the disadvantage of this method that some species such as methylephedrine and diphenyl hydramine, interfere with the determination.

Under 'precipitation procedures' subheading, mention has been made of the determination of methylamphetamine hydrochloride(I) in the presence of ephedrine hydrochloride (46) via its precipitation as bismuth complex. Down to 16 μg ml^{-1} of those species may be determined in presence of ephedrine hydrochloride(II). The tolerated amount of (II) relative to (I) was reported to be 7.3. However, a more sensitive method has been described for determination of three aliphatic secondary amines (74). Diethamine hydrochloride, ephedrine hydrochloride and methamphetamine hydrochloride were estimated by reacting the amines with carbon disulphide in presence of copper(II) and ammonium hydroxide solution. The copper complex was extracted into MIBK for determination of copper content. A linear calibration curve for 1.1-6.6 μg/ml

diethylamine hydrochloride, 1.2-8.0 μg/ml ephedrine hydrochloride, and 1.9-8.0 μg/ml methamphetamine hydrochloride was reported. The recovery of aliphatic secondary amines ranged 93.3-103.2%. The method is quicker and more sensitive than the precipitation method with bismuth.

Mitsui and Fujimura(75)have published a method for the determination of number of phenols, such as phenol, o-, m- and p-cresols, o- m- and p-hydroxybenzoic acids, p-ethylphenol, o-isopropylphenol, o-sec-butylphenol, 3,4-xylenol, 3,5-xylenol, o-aminophenol, o- and p-chlorophenols, p-hydroxy phenylacetic acid, propyl p-hydroxybenzoate, p-nitrophenol, α- and β-naphthols and α-nitroso-β-naphthol. The method is based on the formation of cobalt-complex between the phenol and sodium cobaltinitrite in acetic acid at 100°C. The complex is extracted into MIBK and the organic phase analysed for cobalt content. Recoveries of 94.5-104.5% were obtained. The interference of copper(II), iron(III) and the presence of aniline in the determination is a disadvantage.

A simple indirect method was proposed by Kidani et.al (76) for the determination of catechol by the extraction of its 2:1 catechol-copper(II) chelate into chloroform in the presence of trioctylmethylammonium chloride. The extract was diluted with methanol and the copper content was determined by AAS. The concentration of copper in the organic phase was linearly related to the catechol concentration present in the aqueous phase in the range 11.0-176-2 μg/ml. There is interference from various cations and anions present.

The determination of porphyrin (77) by AAS method has been described. Coproporphyrin, uroporphyrin and erythroytic protoporphyrin were separated as copper chelate complexes. The method is more simple and suitable for routine use compared with the spectrophotometric methods.

A highly selective procedure for the determination of perrhenate has been developed by Senise and Silva (78). Perrhenate was extracted into MIBK from aqueous solution containing copper(II), azide and excess of 2,2'-bipyridine. The copper in the extracted species $CuN_3(bipy)_2\ ReO_4$ was determined by AAS permitting the determination of perrhenate in the range 3-16 μg/ml in the final dilution. The method is stated to be applicable in the presence of a large concentration of molybdate and a considerable number of foreign substances. The method of extraction was applied to a spectrophotometric finish, and the sensitivity was enhanced by using an AAS finish.

A rapid and simple method has been reported (79) for determination of p-aminobenzoic acid in pharmaceutical products using the indirect AAS technique. The reaction of p-aminobenzoic acid with copper(II) to form a 2:1 complex of bathophenanthroline is the basis of this determination. The copper in the ternary complex extracted by MIBK is finally determined and calibration curve was found to be of 13.7-37.0 μg/ml of the organic species and the recovery stated to be 100.6%.

Low concentrations of benzylpenicillin , in the range of 18.6 to 111.6 μ g/ml have been determined using an indirect approach in which an ion-pair was formed when benzylpencillin was added to tris-(1,10-phenanthroline) cadmium chelate in nitrobenzene (80). The absorbance of cadmium concentration versus the concentration of benzylpenicillin was in linear relationship over the range mentioned above. The relative standard deviation was 2.14. The procedure is simple and there is no interference from compounds such as starch, lactose, dextrin and sodium saccharin.

The same authors (81) have shown that folic acid may be

oxidised, using $KMnO_4$ to give 2-amino-4-hydroxypteridine-6-carboxylic acid which reacts with nickel(II) to form a metal complex. The nickel complex may be extracted with MIBK in the presence of bathophenanthroline and the nickel content in the organic phase may be determined by AAS if the extract is nebulized into an air-acetylene flame, and thus provides a method for the determination of folic acid. The method has been applied to the pharmaceutical preparations containing 1-20 μg/ml folic acid in the extract. Besides the high sensitivity obtainable in this determination, the method showed no interference from organic species. such as nicotinamide, pyridoxal phosphate, thiamine, cyanocobalamine and others.

Some organic bases have been determined by different techniques, such as quaternary ammonium compounds (82), azepine (83), phenothiazines (84) and noscapine (85). Alary et.al have investigated the applicability of AAS to the first three organic bases. Quaternary ammonium compounds in the range of 1 to 4 μ mol, were added to sodium dioctylsulphosuccinate(I) in presence of sodium chloride. After shaking the mixtures, a solution of copper-1,10-phenanthroline was added to form a complex with excess of (I). The complex was extracted with MIBK and the copper was determined by AAS in an air-acetylene flame at 324.8 copper line. The method is convenient for the assay of pharmaceuticals containing a long hydrocarbon chains or those that are heavy heterocyclic compounds. It is not recommended to employ this method to pharmaceutical products containing ester, amide or hydroxy groups. However, the method offers a good sensitivity (82).

Similarly, a number of bases in the diazepine series may be determined on the same basis mentioned above. At low pH (usually 3), the base forms a 1:1 complex with sodium bis-(2-ethylhexyl) sulphosucc-

inate(I). The excess of (I) is reacted with copper-1,10-phenanthroline; the complex is extracted into MIBK and the copper is determined by AAS. One of azepine series; opipramol, forms 1:2 complex with (I) (83).

A small amount of phenothiazine (1-4 μmol) may be estimated by its reaction with dioctylsulphosuccinate(DOSS)at pH 3. The excess of DOSS was complexed with copper-1,10-phenanthroline, extracted with MIBK and the copper was determined by AAS. If the complex formed between DOSS and phenothiazine is not sufficiently stable an extraction step with chloroform is included(84).

A complex was formed between noscapine and Reinecke's salt at pH 11.7 in the presence of tartaric acid. This complex was extracted into chloroform and the chromium determined in the organic phase (85).

The flameless AAS was the method of choice in the determination the metabolites of tetramethylthiuramdisulphide (TMTD) [thiram](86), and tetraethylthiuramdisulphide (TETD) [disulfiram](87) in over dosage in urine. The method is based on treating the urine sample with a buffer at pH 4 and an excess of copper(II). The resulting copper complex was extracted into carbon tetrachloride for copper content measurements using the 324.5 nm copper line and a carbon furnace. The main metabolites of thiram and disulfiram are sodium dimethyldithiocarbamate and sodium diethyldithiocarbamate respectively. The method is rapid and sensitive. The calibration curves are linear over the range of 1-10 mg/l and the lower limit of determination is 0.5 mg/l.

Surfactants are usually classified, according to the charge carried by their head group, into anionic surfactants (e.g. $CH_3(CH_2)_{11}OSO_3^-\ Na^+$), non-ionic surfactants (e.g. $CH_3(CH_2)_{11}(OCH_2CH_2)_8OH$) and cationic surfactants (e.g. $CH_3(CH_2)_{17}\ _2\overset{+}{N}(CH_3)_2\ Cl^-$). These are materials of great industrial importance and their major uses are in

domenstic fabric washing and dish-washing products. Some methods have been reported for the determination of this class of material in sea water and fresh water using the indirect AAS technique.

Le Bihan and Courtot-Coupez (88-92) have described several methods for the determination of anionic and cationic detergents in water and fresh water. In one of these methods (89), it was possible to determine anionic detergents in water in very low concentration range 3n g/ml to 2.5 μg/ml (as the sodium salt). In this method, an ion-association compound was formed when 1,10-phenanthroline-copper was added to the anionic species, followed by an extraction step with MIBK. The organic phase was nebulized into an air-acetylene flame in order to determine the copper content.

Traces of cationic surfactants, at ppb level (5-200 ng/ml) have been determined (90) via extraction as cationic-cobalt-thiocyanate surfactant complexes into benzene and determination of the cobalt in the organic phase using the graphite furnace and the cobalt 240.7 nm line.

A modified technique for determination of anionic and non-ionic detergents using flameless AAS has been described by the same authors (91). In this technique, smaller water samples and simpler extraction processes have been used permitting the determination of surfactants at concentration of 10 μg/l.

The carbon-rod atomiser has been employed for determination of non-ionic surfactants down to 50 μg/l (of Triton X-100) in the presence of $<$ 1 g/l of polyoxyethylene glycol (92). The coefficient of variation was found to be 15% and the method suffers from dithiocarbamates and humic acids interference.

The determination of anionic detergents at ppb levels (below 50 μg/l) using the graphite furnace has been reported (93). The method involves solvent extraction of detergent anions into chloroform as an ion-association compound with the bis(ethylenediamine)copper(II) cation. The determination was carried out by injecting 50-μl aliquots of the chloroform extract into the carbon tube of AAS, in order to measure the copper content. The limit of detection was 2 μg/l for linear alkyl sulphonic acids. The simplicity, high selectivity and single extraction step were the advantages claimed by the authors. The method was applicable to fresh, estuarine and marine waters with modification.

Water samples containing 0.05-2 mg/l of non-ionic surfactants may be determined indirectly using different approach (94). A reagent of potassium tetrathiocyanatozincate was added to the sample solution (pH 6-8) and shaken with 1,2-dichlorobenzene. The extracted species were stripped back into dilute hydrochloric acid. The zinc content was determined by aspirating the aqueous phase into an oxidising air-acetylene flame, using the 213.9 nm zinc line and a solution of Triton X-100 as standards. Tolerant amounts for other species are reported.

Better limits of detection, (0.3 μg/l) have been achieved in the determination of anionic detergents in natural waters at the ppb level (95). A rapid and sensitive method has been described on the basis of the above mentioned method (93). A smaller volume size of the chloroform extract containing the copper complex is injected into the graphite furnace i.e. 10 μl. The calibration graph was found to be linear over the range 0-50 μg/l. The method was applicable to fresh and saline waters.

In the methods reported above for the determination of anionic

and cationic detergents a very high sensitivities were achieved and this is one of the major objectives in employing an indirect AAS method. The methods also have found useful applications in the analysis of fresh, estuarine and marine waters.

Kidani et.al have described indirect AAS methods for the determination of some amino acids ; glycine, valine, phenylanine, tyrosine and tryptophanan (96). The same authors have reported the determination of some other species such as L-methionine and L-histidine (97). The determinations were carried out by conversion of the acid to the copper(II) chelate by reaction of acid with salicyladehyde in alkaline solution to give their Schiff's bases which complexed with copper(II) and subsequent extraction into MIBK in the presence of bathophenanthroline. The copper content was determined by AAS and linear calibration curves were obtained in the range 1.5-15.0 μg/ml for glycine, 3.0-30.0 μg/ml for L-methionine and L-histidine. The last two amino acids were determined simultaneously in a mixture by the atomic absorption spectra of the copper(II) complexes. The standard deviation and the coefficient of the variation were 0.71 and 1.45%, respectively. The recovery was stated to be 99.8%.

The determination of thirteen different alcohols (98) may be carried out indirectly by AAS using a simple technique. A chromium complex was formed when a benzene solution of alcohol was added to a reagent solution of (CrI_3 and pyridine in acetic acid). The benzene phase was separated, dried, filtered and mixed with methanol followed by chromium determination in the extract. The method was practicable for thirteen different alcohols, but not for iso-propyl alcohol.

Long-chain primary amines are usually added to the boiler

water in order to protect against corrosion. A sensitive AAS method for monitoring octadecylamine in cooling circuits of power stations has been developed capable of determining 0.1 to 1.0μ g/ml of the amine (99). A mixture of 10% sulphuric acid, 5% aqueous potassium chromate, sodium sulphate and 1,4-dioxan was added to the sample containing the amine and was shaken for 1 minute. The amine-chromate complex was extracted into nitrobenzene and the chromium content was determined using air-acetylene flame. The detection limit was stated to be 0.02μ g/ml octadecylamine. The interfering materials are normally present in negligible concentrations in boiler waters.

Ethambutol (up to 400μ g/ml) has been estimated by formation of its copper complex in alkaline medium (pH 8-11.5), extracted into ethylmethylketone and the copper content was determined in the extract (100).

A rapid detection method for organic pollutants in water has been reported (101) utilizing vapour-phase uv absorption spectrometry. The method is based on the extraction of the water sample with hexane or chloroform. A small amount of the extract (0.1 to 10μ l) is injected into a graphite tube and the absorption of the vapour is detected at 190 nm. At this wavelength an optimum sensitivity is obtained. The method is rapid and sensitive and very useful for both monitoring and comparitive evaluation of gross organic pollution.

A suitable method for determination of ethinyloestradiol in tablets (102) has been reported. It was converted by nitrous acid into the O-nitroso-derivative. The cobalt chelate of the nitroso-derivative was formed by the action of $Na_3Co(NO_2)_6$ in acetic acid and extracted into MIBK for cobalt determination at 240.7 nm. The graph was linear over

0.5-1.0 mg of ethinyloestradiol.

Recently, the feasibility of determining malathion indirectly by AAS has been investigated (103). In this method, malathion was hydrolysed with 6M NaOH, the resulting dimethyldithiophosphate (DMDTP) complexed with bismuth and extracted into MIBK. The organic phase was then aspirated into the flame. For this purpose a standard curve has been constructed by extracting bismuth-DMDTP complex in MIBK.

2.3.4 Chemical Amplification Procedures

(i) Principle

Amplification reactions are widely used for determining traces of some metals and non-metals. In his exhaustive review (104), Belcher has defined the amplification (or multiplication) reactions as "reactions in which the normal equivalence is altered in some way so that a more favourable measurement can be made".

In indirect flame spectrometric methods, it is possible to select a chemical reaction to provide an extractable species in which more than one atom of the element to be measured is extracted with each atom, molecule or ion of the initial species of interest. Phosphate as an example, may be determined utilizing this principle, in which phosphomolybdic acid $\{ H_3PO_4(MoO_3)_{12} \}$ is formed and extracted into an organic phase. In this reaction, 12-molybdenum atoms are associated with every atom of phosphorous, the molybdenum content of the organic phase may be determined by AAS.

Chemical amplification procedures through heteropoly acid formation are very well known in several branches of analytical chemistry,

This offer the possibilty of trace determinations with a high precision. The heteropoly molybdic acids are formed when an excess of molybdate reacts with oxo anions such as phosphate, silicate, arsenate, germanate, titanate, vanadate, niobate and other ions. A typical heteropoly anion of the series of the 12-molybdoheteropoly acids has the formula $\left[PMo_{12}O_{40}\right]^{3-}$ in which phosphorous is the central atom and the 12 molybdenum atoms are coordinate through oxygen atoms. The yellow heteropoly acids formed can be reduced to intensely coloured blue forms. Both may form the basis for spectrophotometric methods in trace analysis. These compounds are stoichiometric under certain conditions and are extracted with oxygen-containing solvents.

Heteropoly acids have been also used extensively as precipitants for organic bases such as alkaloids and other nitrogen heterocyclic compounds, in which gravimetry and colorimetry techniques have previously been employed. Recently, these methods utilizing heteropoly acids, have been applied successfully to atomic absorption spectrophotometry. They are only used for the determination of elements which can not be satisfactorily determined by direct AAS method i.e. those elements which give poor sensitivity due to either they do not possess a useful absorbing wavelength in the spectral region normally available (the visible and near u.v. region) or, they form refractory compounds in the flame as it has been discussed before.

Some methods employing the heteropoly acids as precipitants have been discussed already under the other subheadings; precipitation and solvent extraction processes. In this section, some other examples are given.

(ii) Application

Indirect determinations using chemical amplification procedures through the formation of a binary or ternary heteropoly acid systems have been applied to various metal and non-metal species. A few organic species may also be determined by this procedure.

Organic bases such as alkaloids have been determined utilising their reaction with heteropoly acids in which 3:1 complexes were formed (105). Two high molecular weight alkaloids; strychine and brucine, react with molybdophosphoric acid (MPA) to give a precipitate. The excess of MPA was masked with citric acid allowing extraction of the remaining organo-MPA complex into MIBK. The molybdate equivalent to the organic base was stripped by a basic buffer and measured by AAS using the molybdenum line at 313 nm. Alternatively, the organic phase was directly aspirated to the flame for determination of the molybdenum content. A lower molecular weight organic base; quinoline, was precipitated, centrifuged and dissolved in an alkaline buffer. The molybdenum equivalent to the quinoline was then determined by flame AAS. This method may be applied for 0-40 ppm of strychine; 0-45 ppm of brucine and 2-16 ppm of quinoline. The sensitivities (for 1% absorption) obtained for strychine and brucine were, 0.65 ppm and 0.75 ppm respectively.

2.3.5 Reduction or Oxidation of a Metal

(i) Principle

This is another approach used for indirect determinations in which a metal ion is oxidized or reduced followed by solvent extraction of the oxidized or reduced form. The equivalent amount of metal reduced or oxidized in either the organic or aqueous phase may be determined.

Alternatively, the excess of unreacted metal can be determined. Making use of this property the reducing and oxidizing agents can be determined using AAS. This indirect technique has been very useful in the determination of anions such as iodide and iodate(22). The oxidised or reduced forms are also associated with precipitation procedures.

(ii) Application

The classification of some organic species under the 'reduction-oxidation' or precipitation procedures is difficult. This is because the two processes are involved in the determination. It has been already mentioned under the subheading 'precipitation procedures', that the reducing effect of sugar on copper takes place in alkaline solution forming insoluble copper(I) oxide and this effect may utilised for sugar determination by AAS (31). Some other applications have been also mentioned; aldehyde (40), acetaldehyde (51), and nitro-compounds (52, 64).

An indirect AAS method for the determination of chloramphenicol in several pharmaceutical preparations has been proposed (106). The method is based on a combination of an easy reduction procedure for chloramphenicol and its esters without prior extraction by use of cadmium metal, followed by rapid instrumental measurement of the released cadmium ions and amine by AAS. The method is simple and accurate.

2.3.6 Direct Measurements Procedures

(i) Principle

Some organic species contain metals in their matrix which can

be subjected to AAS directly. The stoichiometric ratio between the metal content and the molecules of the matrix is the basis for this indirect AAS determinations. This principle has found to be useful in drug analysis hence many drugs contain a metal in their matrices.

(ii) Application

Vitamin B12 (cyanocobalamin) contains one atom of cobalt per each molecule of vitamin. Such compound may easily be determined by AAS directly after an appropriate pre-treatment. The determination of vitamin B12 in some pharmaceutical preparations has been reported (107-111). Most of the other methods which employ, spectrophotometric, fluorometric, titrimetric, gravimetric, electrometric and chromatographic techniques, require time-consuming extraction of chromogen and careful treatments in order to obtain the optimum reaction conditions. They also suffer from some interferences due to the presence of some other species. The proposed procedures using an indirect AAS method eliminate the usual initial separation or chemical conversion. The tablets were dissolved in hot water, the solution was filtered and the cobalt content in the filtrate was determined by AAS using acetylene-oxygen flame (107). The method was applicable to samples containing 25-100 μg of cyanocobalamin per tablet. Species such as aluminium, strontium, zinc, copper, iodide, nitrate, sulphate and citrate caused some interference when present in $>$ 200-fold excess of cobalt.

Direct determination of cobalt content in cyanocobalamin, hydroxocobalamin, cobalt acetate and cobalt glycinate in cyanocobalamin preparations (injections and tablets) have been reported (109). Samples containing 0.7-0.9 ppm cobalt may be determined.

Peck (111) has applied the graphite furnace AAS to analysis of vitamin B12 in a variety of matrices (liquid formulations or tablets). The method is based on the dissolution of samples in distilled water and the resulting solution was acidified with hydrochloric acid. The calibration graph used for this determination was in the range 0 to 30 ng/ml of cobalt in 5% HCl. Higher sensitivity has been achieved by using the carbon furnace technique instead of the flame AAS; samples containing 15-20 ng/ml of cobalt may be determined.

The determination of the soap content of a number of commercial samples of refined olive oil in range 8-25 ppm (as sodium oleate) has been reported (112). The oil is treated with absolute ethanol, the mixture dissolved in ethylmethylketone and the solution directly aspirated into the flame for sodium determination. A linear standard curve using virgin olive oil and known amounts of sodium oleate in range 3-1000 ppm may be obtained.

The indirect AAS methods have found applications in rubber technology. The graphite furnace AAS has been employed to develop a quick and accurate test for tyre cord dip pick up (113). This test is significant because the amount of adhesive dip pick up on tyre cords will affect both the performance of a tyre and processing economics. In this method, a solution of strontium nitrate was added to either predips or resorcinol-formaldehyde latex dips at predetermined levels of a few parts per million. The graphite furnace allowed direct analysis of milligram sized dipped cord samples with no preparation. The technique also, offers the advantage of total analysis time (two to three minutes). The method has replaced the current ASTM method which involves a wet gravimetric procedure using hazardous chemicals with several hours of time-

consuming procedures.

2.3.7 Interfacing Techniques

(i) Principle

This technique has been mainly used for the determination of organometallic species, such as chromium organometallic, organosilicons and tetraalkyllead compounds. The technique is based on separation or/and preconcentration of the species of interest prior the determination or detection using the AAS. Techniques such as ion-exchange column, gel filtration, high-speed liquid chromatography (HPLC) and GLC have been employed prior the AAS measurements. The development of the interfacing techniques employing GLC and AAS has extended the applicability of both, hence the AAS has been used as specific detector for GLC in some of those determinations.

(ii) Application

Dimethylpolysiloxane, has been determined in foods at μg/g levels (114). The species was extracted first with diethylether, separated on a column and then the silicon content was determined using nitrous oxide-acetylene flame and the 251.6 nm silicon line.

The recovery was stated to be 95.0-100.4% and the calibration curve was linear over the 1-20 ppm range. There were no interferences from 1000 μg of SiO_2, sucrose fatty acid esters and sorbitan fatty acid esters on the determination of 100 μg dimethylpolysiloxane.

An indirect AAS method has been developed by Suzuki et.al (115) for determination of protein thiol groups . The method is based on the interaction between the thiol group and p-hydroxymercuribenzoate, the

excess unbound reagent is removed by dialysis or gel filtration chromatography. This is followed by direct measurement for the amount of mercury bound to thiol group of the sample. The detection limit was found to be 0.1 μg Hg/ml.

Another indirect AAS method has been developed by Carlsen (116) for the estimation of thiol group in proteins using cold vapour technique for mercury determination. The method involves an addition of excess of p-hydroxymercury(II) benzoate to the solution of protein in phosphate solution (pH 7.3). Gel filtration (sephadex G-25) technique has been employed for the separation. A portion of eluted mercury-complex with nitric acid is digested and a solution of sulphuric acid-potassium permanganate is added. The mercury is determined by reduction and cold vapour AAS technique. The method seems to be less rapid than the method which has been reported by Suzuki et.al (115). However, the detection limit was stated to be 0.05 μg protein-bound mercury.

Atomic absorption has been used as detector for the determination of chromium-organometallic compounds after separation process by high-speed liquid chromatography (117).

The compounds determined were, the chromium(III) complexes of acetylacetone, 2'-hydroxyacetophenone and hexafluoro acetylacetone with limits of detection $\simeq 4 \mu g\ ml^{-1}$.

Organosilicon compounds have also been detected by atomic absorption after preconcentration on porous polymers and separation by molecular-seive and reversed-phase chromatography (118). Detection limit was in 0.5-5 μg silicon range.

In another approach for the determination of ionic detergents in water, Alary et.al (119) have described an indirect method based on

formation of stable copper-complex with copper via 1,10-phenanthroline. The complex is adsorbed on ion exchange column (Amberlite IR 120), followed by MIBK elution and subsequent determination of copper.

A rapid and reproducible method for the determination of high-molecular-weight quaternary ammonium salts has been proposed by Benttoni and Franchini (120). A stoichiometric amount of calcium(II) equivalent to the amount of quaternary ammonium salts may be eluted after passing the aqueous solution containing those salts through ion-exchange column (Dowex 50W in calcium(II) ions form). The calcium content is measured by AAS. An amount of inorganic salt (e.g. NaCl) more than 50:1 with respect to the quaternary ammonium salt may interfere with the determination. This may be eliminated by pre-extraction process before the determination. Salts such as; benzylkonium chloride, steramine and cetyltrimethylammonium p-toluenesulfonate were determined by this method with standard deviation 2% for the former salt.

Metal-containing compounds (e.g., chelates or organometallic compounds in petroleum) have been separated by HPLC before a sensitive and selective detection by AAS (121).

High-speed liquid chromatographic separation and atomic absorption detection of aminocarboxylic acid copper chelates technique has been reported (122).

In this technique, the copper chelates of EDTA, nitrilotriacetic acid (NTA), 1,2-bis-(2-aminoethoxy)-ethane-NNN'N'-tetra-acetic acid (EGTA) and 1,2-diaminocyclohexane-NNN'N'-tetra-acetic acid (CDTA) were separated on the weak anion exchanger Aminex A with ammonium phosphate as mobile phase. An AAS was interfaced to the liquid chromatography in order to detect the copper chelates.

Tetraalkyllead compounds in various brands of gasoline and organic lead compounds in air have been determined by combining gas chromatography with carbon furnace AAS (123).

Tetramethyllead and tetraethyllead have been determined using AAS as a specific detector for gas chromatography (124). Both, the flame and carbon furnace techniques have been examined for this determination. The detection limits were found to be 5.5 and 27 mg/l for tetramethyllead and tetraethyllead, respectively using the flame technique and 0.04 and 0.35 mg/l, respectively with the graphite furnace. Although the carbon furnace technique is the more sensitive, the flame was proved to be simpler and more rapid.

The determination of glucosaminoglycans extracted from mouse brains has been reported (28). Initial purification, by electrophoresis (at 10 mA) on cellulose acetate membrane strips has been carried out.

Another use of the carbon furnace AAS as a specific gas chromatographic detector has been reported for the determination of alkylmercury compounds in fish tissue (29). The detection limit was 0.3 ppm of mercury for 0.5 g samples.

Recently, in the present work, a very simple, rapid and accurate indirect AAS approach for saccharin determination has been described (see chapter four). The method is based on the quantitative formation of ion-association complex, $[(Fe\ Phen_3)^{2+}\ ;\ (saccharin)_2^-]$ when an ethanolic, methanolic or acetone solutions of saccharin is shaken with ferroin-impregnated silica gel. The absorbance of the decanted and filtered supernatant liquid is measured for the iron content by aspirating the solutions into the flame using wavelength 248.3 nm iron line. The calibration curves were found to be linear over the ranges :

1.25×10^{-4} -5.0×10^{-4}M (distilled water), 7.5×10^{-5} -5.0×10^{-4}M (60% ethanolic solution), 7.5×10^{-5} -4.0×10^{-4}M (80% methanolic solution) and 2.5×10^{-5} -5.0×10^{-4}M (70% actone solution). The enhancement effect of the solvents on the AAS signals offers advantages and the sensitivity of the method was found to be ; 4.62μM of 80% methanolic solution of saccharin, 6.06μM of 70% acetone solution of saccharin, 6.60μM of 60% ethanolic solution of saccharin and 20.46μM of aqueous saccharin solution. The method is hoped to be useful contribution to the indirect AAS methods and the new approach may be applied to other organic species.

2.4 Summary

In conclusion, the above survey of the methods reported for the indirect AAS methods demonstrates the wide applicability of the methods. It also shows the significant role of this technique which extends the practical usefulness of atomic absorption spectrophotometry. Although the reactions involve determinations which are mostly not specific, indirect methods may prove to be the solution for many problems for many particular applications.

The methods in general, offer a rapid and simple approach in preparing the species for the AAS technique and also are found to be very sensitive particularly when using the flameless technique. One of the major objectives is to achieve high sensitivity.

Chemical interferences sometimes limit the applications. This probably occurs because the 'enhancement' and the 'depressive' effects depend crucially on the temperature and chemistry involved of the flame. This limitation of applicability may not exist when using the carbon

furnace in which a controlable temperature programme is available. Also, in these interference methods the flame conditions must be stable and reproducible. The methods are applicable only for samples free of the interference by other species.

Examination of the plot obtained for the interference techniques, often show the relationships between the concentration of the test substance and the relative percent absorption due to the interference , are not quite linear. The plots are either curved or behave unexpectedly after a certain point on the curve i.e. as the concentration of the amount of organic species to be determined increases; the 'depressive' effect reverses into an 'enhancing' effect. Therefore the value of such methods is limited to certain concentration ranges.

The precipitation and solvent extraction techniques are widely used in the indirect methods. The solvent extraction procedure has proved to be a powerful technique when associated with AAS. Many organic solvents are suitable for AAS measurements and at the same time are also convenient for the extraction of many organic species. This applies to metal chelate and ion-association systems. The use of these solvents often offer enhancement in absorbance. The sensitivity is even further improved using the flameless techniques.

Several advantages have been offered using the indirect methods but the main disadvantages are : low selectivity and some interferences in some cases.

CHAPTER THREE

INDIRECT DETERMINATION OF SACCHARIN BY ATOMIC ABSORPTION SPECTROPHOTOMETRY

3.1 Introduction

3.1.1 Advent of the Artificial Sweeteners

a) Early sweeteners :

Ever since the beginning of civilization, man has had a desire for sweetness. This is shown by many thousand years old drawings in caves showing prehistoric man robbing bees'nests of its honey and the more recent man practising apiculture.

There is always a psychological relationship between man and his environment as shown by his behaviour and his experiences. He has the ability to experience the sensation of 'sweetness' right from the early childhood, and it has been suggested that sweetness has played a useful role in the development of man's selection of substances from his environment to be suitable as food.

Sweetness-as it is assumed-is one of the four basic tastes i.e. salt, sour, bitter and sweet. The primitive man may have used sweet and salt taste as an indicator when selecting his food in terms of calories and other essential nutrients. He also may have used the other two, sour and bitter as an indicator for the harmful substances. This suggests, therefore, that man has performed his ability to select the sweeteners in order to provide him the pleasure and, psychologically, to secure the safty of his food. Mankind, using his selection ability, started to eat from fruit-bearing plants which are a source of various sugars, including sucrose.

Man started to seek out substances of additional sweetness in order to improve food palatability. Evidence of this is available (243),

in a cave at Arana, near Valencia, in southern Spain, some 20,000 years old in which wall-drawing showing a slightly androgynous figures robbing a wild bees'nest of honey, while under attack by the occupants. Another historical record showing honey as the earliest sweetener available is an Egyptian tomb drawing, as early as 2,000 B.C., where apiculture is being practised.

Sugar in the form of honey (mixture of sucrose, glucose, fractose and water) was probably mankind's first sweetener, it remained the main source of sweetness until some six centuries ago. It only began to be supplanted by sucrose in Europe in the 14^{th} century A.D.

Sugar was being refined but was regarded as a rare delicacy. Historically and nutritionally sucrose (sugar-cane) is the most important commercial sweetener available. Sugar-beet is second major commercial source of sweetness which only came into wide commercial use less than two centuries ago.

b) Natural sweeteners and health :

World-wide consumption of sugar is increasing steadily. Sucrose has been the sweetening agent of choice for the majority of people. It provides calories, contains no vitamins or minerals and despite its long history and advantages, it has more recently been condemned as a food substance. There is strong evidence that sucrose contributes to the formation of dental caries. Also, studies of the diet have suggested that as more refined carbohydrates (like sucrose or glucose) are added to the diet and more complex carbohydrates (such as starches) are eliminated, there tends to be an increase in the incidence of coronary heart disease.

Diabetic subjects may only use sweetening agents that do not

raise their blood sugar or increase their calorie intake. As a consequence of this, the need for a substitute for sucrose has increased considerably. Various synthetic sweetening agents sweeter than, or, comparable in sweetness to sugar have been discovered and prepared to meet that need, such as cyclamate, saccharin and sorbitol. Saccharin, for instance, has no food value and it produces a decrease in the blood sugar level of normal persons.

Because the ' artificial sweeteners have no food value, they may be described as 'non-nutritive sweetening agents, (see also subsection c). They are used where the use of sucrose is undesirable; in the low-carbohydrate 'dietetic' foods such as beverages; canned fruits and vegetables; frozen derserts; backed goods; salad dressings, jams, jellies and marmalades, for people who must limit their intake of the carbohydrate sweetening agents. The artificial sweeteners do not have cariogenic potential of the sugar carbohydrates which cause the tooth decay problems.

c) The development of artificial sweeteners :

The commercial development of artificial sweeteners dates back from the discovery of saccharin in 1878, by Fahlberg and Remsen (244), followed by its commercial manufacture five years later.

Before details about the development of artificial sweeteners are discussed, it is worth pointing out the distinction between the two kinds of sweeteners: 'natural' and 'synthetic'. Practically, the difference is from the nutritional point of view. The natural sweeteners are converted to carbon dioxide, water and energy by the body, hence, these sweeteners have nutritional value, they furnish four food calories per gram (one food calorie = one K calorie).

Natural sweeteners are those which occur in nature and which

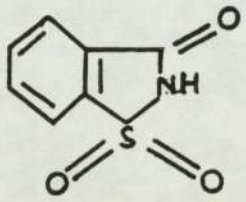
may or may not be extracted for commercial use. Examples of such sweeteners are, Sucrose ($C_{12}H_{22}O_{11}$) extracted refined from the sugar-cane or sugar-beet; Maltose ($C_{12}H_{22}O_{11} \cdot H_2O$) made by the action of the enzyme, maltase, on strach, Glucose ($C_6H_{12}O_6$) found in large amounts in grapes (old-fashioned name was grape sugar), manufactured by the hydrolysis of starch; Fructose or Laevulose ($C_6H_{12}O_6$) which occur in a large number of fruits and in honey (there is a synthetic methods today for preparing it); Lactose ($C_6H_{22}O_{11}$) occurs in the milk of all mammals and is prepared pure from cow's milk.

The sweeteners mentioned above are the common commercial sweeteners in use today, and each of them possesses a different degree of sweetness.

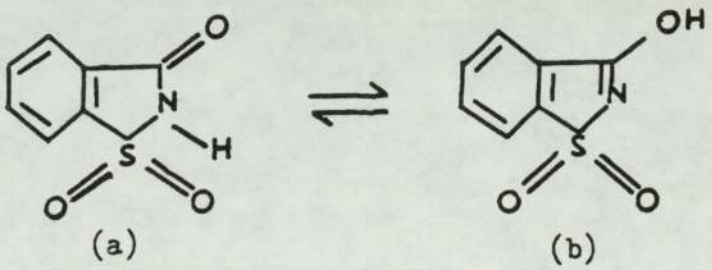
The modern understanding of chemical structures has enable some of the natural sweeteners to be synthetically prepared, such as, fructose and lactose, and that is why the distinction between 'natural' and 'synthetic' sweeteners, as a term, is often difficult to distinguish. Terms like, 'artificial' and 'non-nutritive' sweeteners are accepted terms by the food technologists. Sweetening power of these,ranges from very slight to 4000 or more unit compared with sucrose which is taken as unity.

As it has been pointed out earlier in this subsection, saccharin was synthesised in 1879 by Fahlberg and Remsen and has been in use commercially since 1900. It is commonly employed, either in the form of 'insoluble saccharin' often used in pharmaceutical tablets, or 'soluble saccharin' as soluble sodium or calcium salts which is more palatable and comparatively free from the unpleasant metallic after-taste of saccharin. Its sweetening power is considered to be about 550 times of

sucrose, depending on the strength of solution (the greatest sweetness is obtained in dilute solution).



A structural unit $O{=}\overset{|}{C}{-}NH{-}\overset{|}{S}O_2$ (a) or perhaps $HO{-}\overset{|}{C}{=}N{-}\overset{|}{S}O_2$ (b) incorporated in an appropriate ring system is held responsible for the sweet taste of saccharin.



(a) (b)

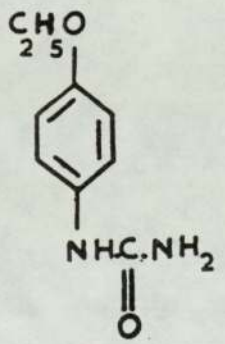
Saccharin has found extensive use for carbohydrate sweeteners in times of scarcity, and in sugar-free diets. Its production reached 100,000 lb per year in Germany before the world wars, and increased sharply during the wars, reaching a peak (about 500,000 lb per year) in U.S.A.

In Britain and Europe, the production has remained static during the first half of this century.

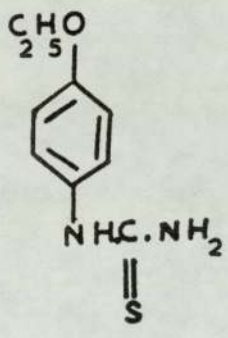
Control of nutritional intake has been focussed on carbohydrates in the 1950s by the world health authorities. It was found then, that saccharin is an alternative means for reducing carbohydrate-and hence calorie-intake.

Pharmacological, toxicological and clinical investigations have shown some evidence for the carcinogenicity of saccharin (see subsection 3.1.2).

About the same time, when saccharin was discovered, Berlinerblau discovered the intense sweetness of P-ethoxyphenylurea and within ten years, this sweetener was being marketed as 'Dulcin'. It is a very sweet



P-Ethoxyphenylurea



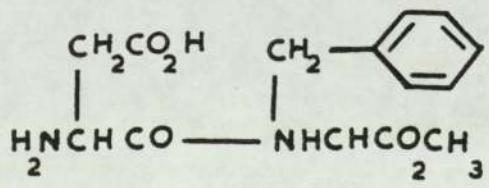
P-Ethoxyphenylthiourea

compound, it has been used in some countries as a substitute for sucrose, being about 250 times as sweet. However, it should not be used as a food additive because of its tumorigenic potentialities (245). Para-ethoxyphenylthiourea (shown in the figure above) is an extremely bitter compound. This demonstrates that a slight change in the structure may alter the taste sensation, and it is not easy task to correlate sweetness and structure.

Another sweetening agent which has been in great demand is cyclamic acid (N-cyclohexylsulphamic acid) discovered in 1937 by Audrieth and Sveda (246), and subsequently synthesised two years later.

The flavour of cyclamate is free from after-taste and is pleasant. Its sweetness power is one-tenth the sweetness of saccharin. An admixture of cyclamate/saccharin has been used in soft drinks. These discoveries increased the intake of artificial sweeteners tenfold by 1968, and led to a concern over the potential hazard of those sweeteners. Many toxicity studies have been carried out to establish the safety of cyclamates after being 'suspected' to be the cause of cancerous tumours in laboratory animals. The conclusion of those studies was: the use of cyclamic acid and its sodium salts as an artificial sweetener in food or soft drink is no longer permitted in Great Britain, U.S. and Europe(245). Consequently, the banning of cyclamate and the restrictions applied to saccharin have resulted in the search for new, safe and high-intensity sweeteners.

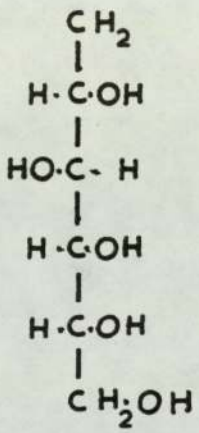
The dipeptide sweetener; aspartyl-phenylalaninemethylester, (shown below) gives a sweetness of 100-200 times sucrose depending on concentration and it is found to be free from the unpleasant after-taste.



Aspartyl-phenylalaninemethylester

Polyhydric alcohol, such as D-Glucitol (D-Sorbitol), which is

an isomer of mannitol, another sweetener, occurs in small quantities in certain fruits such as apples, cherries, pears and plums and has been synthesized. Its sweetness is half of sugar, but it suffers the disadvantage of providing nearly as many calories in the diet as sucrose and, thus it cannot be used in slimming diets.



D-Glucitol

Other examples of polyhydric alcohols sweeteners, are D-xylitol and glycerin. Compounds such as nitro-aminoalkoxybenzenes having the general name P-4000 are as 4000 times sweet as the sucrose. However, these compounds are banned because they were found to produce kidney troubles (247).

3.1.2 Carcinogenicity and Legislative Aspects of the Artificial Sweeteners as Food Additives

Recent investigations into the chronic toxicity of saccharin

and cyclamates indicated that these materials may induce bladder tumors (248). In this report by Melvin Dwaine Reuber, he reviewed the published and unpublished carcinogenicity studies on saccharin. Animal laboratory and human studies were carried out and the results were reported as tumors involving some organs, such as, the urinary bladder, reproductive system, the hematopoietic system, the lungs and the vascular system.

The earliest carcinogenic studies started in 1948-1949 did not show that saccharin was not carcinogenic for the urinary bladder and the results were not complete.

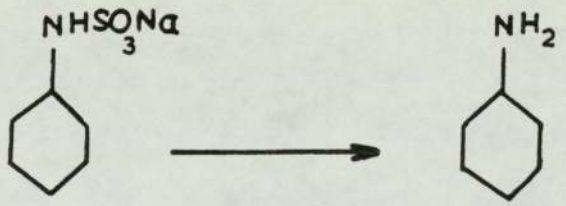
The chemical impurities present in tested saccharin was one of the suspected factors as a possible reason for the induction of bladder tumors. Analytical techniques have proved very useful in detecting, identifying and determining those impurities.

Up to July, 1974, it had not been conclusively proven from the available data whether saccharin is tumorigenic or whether a combination of other factors are responsible for the observed carcinogenic activity. However, some of the studies have shown saccharin to be safe for humans. These studies showed that saccharin is rapidly absorbed, distributed throughout the body, and excreted unchanged, except for 1% or less. However, there is no difinite answer as to whether or not saccharin is mutagenic as in the case of cyclamate.

The use of cyclamic acid and its salts in foodstuffs has increased when the early investigations on animals and man showed that cyclamates are harmless materials, and the 'Public Health Authorities' in both U.S.A and U.K approved their use. By 1965, 5000 tons annually were eaten and drunk.

The recent toxicological investigations (249) showed that

cyclamate ion was only partially absorbed and that a proportion of this was excreted in the urine unchanged. In 1967, the detection of cyclohexylamine in the urine of a human after the metabolism of ingested sodium cyclamate was reported (250). This metabolism of cyclamate was studied in animals and man. It has been shown that about 25% of humans can metabolise cyclamates to cyclohexylamine.



Socium Cyclamates Cyclohexylamine

Gas-liquid chromatographic techniques have been used for cyclamate/cyclohexylamine analysis in order to discover the relationship between the dose of cyclamate and the produced cyclohexylamine, which is hazardous material to man's blood pressure and has some toxic effect.

Cyclamate has been reported to induce bladder cancer. Large doses of cyclamates cause softness of the stools and excessive intake may produce diarrhoea.

In 1953, two orders were issued. The first one was the 'Food Standards-saccharin tablets-Order' , in which 'saccharin order of 1949' was revised for saccharin tablets and in which it is stated that "saccharin tablet or other sweetening tablets containing saccharin shall

contain not less than 0.18 grains (10.8 mg) and not more than 0.22 grains (13.2 mg) of saccharin or the equivalent weight of soluble saccharin". The second order was the 'Artificial sweeteners in food' order, in which the use of artificial sweeteners other than saccharin was prohibited for human consumption.

Since 1954, the Food and Drugs Act has controlled the use of food additives and contaminants including the use of saccharin. In 1954, soft drinks regulations permitted use of saccharin and artificial sweeteners for soft drinks.

Under the artificial sweeteners in Food Regulations 1967, saccharin, saccharin calcium, saccharin sodium, cyclamic acid, calcium cyclamate and sodium cyclamates became permitted artificial sweeteners in England and Wales for use in food intended for sale for human consumption.

After the banning of cyclamic acid and its salts as permitted food additives under the "Artificial Sweeteners In Food Regulations 1969", some form of control in the interest of public health and for the protection of the consumer, seems desirable. The present regulations came into force on 1970, are the same as the 1967 regulations except for the elimination of cyclamic acid and its salts from the list of permitted artificial sweeteners.

The concept of the acceptable daily intake (ADI)is of great importance for the regulating authorities to exercise appropriate control on food additives. ADI is defined as a 'biological measure of the maximum amount of a substance that could be consumed throughout life without demonstrable ill effects'. Saccharin ADI for a man is estimated to be up to 5 mg per Kg body-weight by the "Joint FAO/WHO Expert

Committee on Food Additives", in 1968 (245). In view of doubts about the safty of cyclamates, no ADI for man could be reliably recommended by the same committee in 1971.

From the discovery of new hazards in the environment arises the need for developing new methods of analysis. Sensitive analytical techniques are required to implement the control of the food additives. The methods available for saccharin determination are discussed in the next subsection.

3.1.3 Review of the Analytical Methods Available for the Estimation of Saccharin

From varying standpoints it is necessary to know the composition of foods. Essential elements must be present to aid growth, and to maintain body tissue. Calories are needed for the fuel supply. The presence of compounds likely to be undesirable from the standpoint of health must also be known.

Saccharin was once suspected of being potentially carcinogenic. The need for more sensitive and convenient methods is increasing both for routine and research work. Saccharin has been in use in the United Kingdom and other countries for many years during which, several methods have been reported in the literature for its determination in various foods, pharmaceutical products, baby foods and biological samples.

There are many well established basic analytical techniques used in the determination of saccharin. In general, each analysis consists of three main steps :

(A) Extraction, isolation and identification.

Table (3.1) Applications and detection limits of methods available for the determination of saccharin

Technique	Matrix	Detection limit or sensitivity *	Reference
Paper Chromatography	biscuits & chocolate	40 μg (D.L)	125
	---	3 μg (D.L)	126
	soft drinks	>56 μg (D.L)	127
Thin-Layer Chromatography	soy sauce	5 μg (D.L)	128
	--	1 μg (D.L)	129
	soft drinks & soy sauce	0.001-0.02 % (D.L)	130
	--	2 μg (S)	131
	beverages	0.004% (D.L)	132
	--	0.2 μg (D.L)	133
	food products	0.05-2 μg (S)	134
	foods & pharamceutical preparations	1-5 μg (D.L)	135
Gas-Liquid Chromatography	soft drinks	16 μg/g (D.L)	136
	wine	0.05 mg/L (D.L)	137
	biological materials(urine, faeces, blood, animal tissue	0.1 μg/ml (D.L)	138
	----	0.02-0.2 μg/ml (D.L)	139
	biological fluids (urine, plasma)	1-6 μg (C) 20 ng (D.L)	140
	---	5 μg/L(D.L)	141
	foods(soy sauce, vinegar, orange juice, tomato ketchup	0.05-0.5 mg/ml (C)	142

Table-continued next page

High-Speed Liquid Chromatography	foods	0.1 μg (D.L) 0.25-1.5 mg/ml (C)	143
Reverse-Phase High Pressure Liquid Chromatography	beverages (fruit juices, coffee)	14 ng (D.L)	144
Infrared Spectrometry	drugs	50 μg (D.L)	145
Visible Spectrophotometry	--	1-32 μM (C)	121
	soft drinks	20-400 μg/ml (C)	146
UV-Spectrophotometry	foods	1-10 mg (C)	147
Colorimetry	drugs	10 μM (D.L)	148
	--	0.1 mg (D.L)	149
	foods (fruit juice, jelly and dietetic food)	5 mg (D.L)	150
Ion-Selective Electrode	---	10 μM-0.1M (C)	151
Liquid Membrane Electrodes	---	0.1 mM (D.L)	152
Alterating-Current Oscillopolarography	saccharin tablets	8-45 μg/g (C)	153
Differential Pulse Polarography	soft drinks, tonic waters, fruit juices, sweeteners	0.5 μg/ml (D.L)	154
Polarography	beverages & solid foods	0.05-2.5 mg (C)	155
	---	0.1-100 μg (C)	156
Molecular Emission Cavity (MECA)	soft drinks	< 90 μg/ml (C)	157

* D.L. = Detection Limit S = Sensitivity
C = Concentration range for linear graph.

(B) Clean-up procedures.

(C) Final stage of analysis.

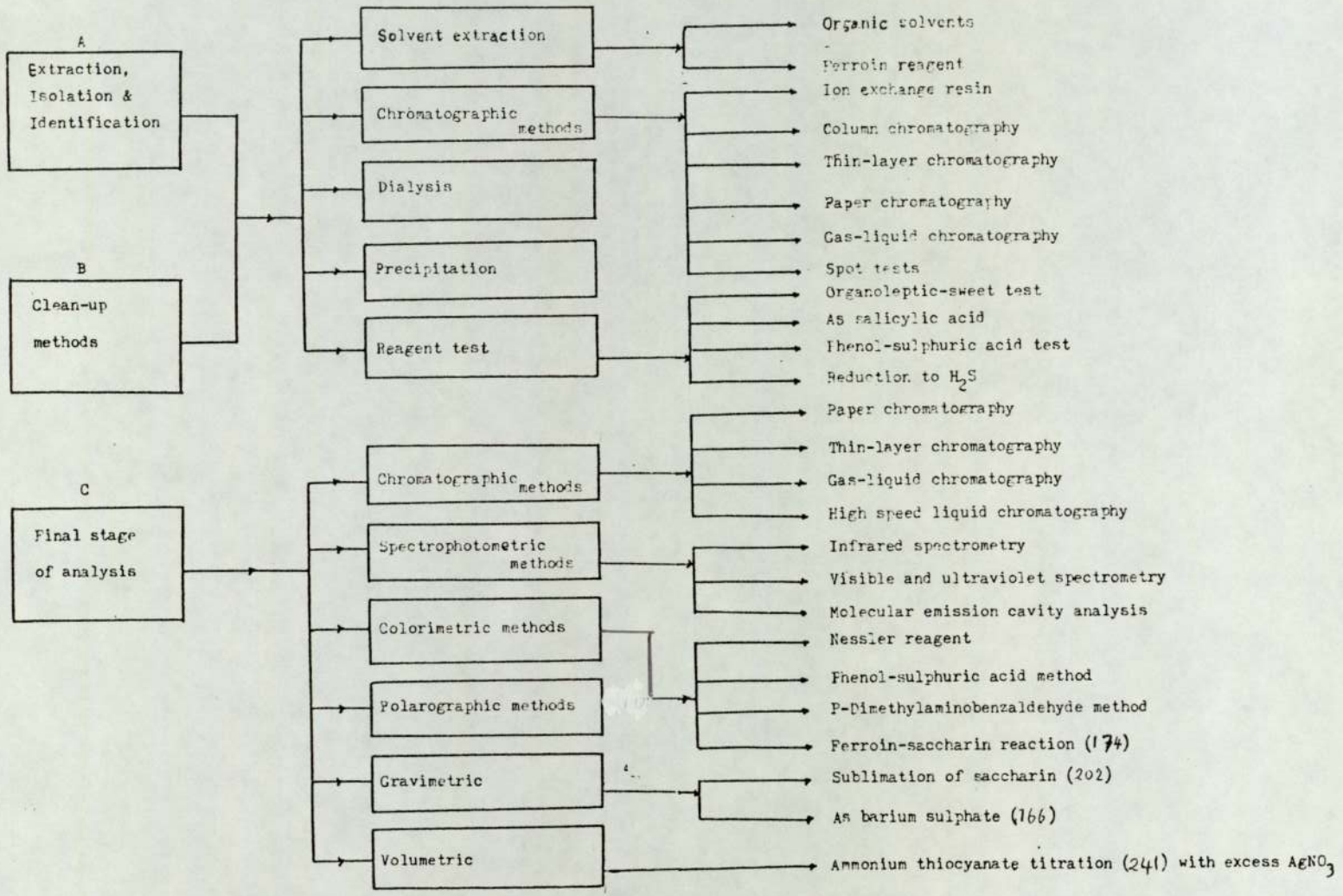
The literature contains numerous reports on the determination of saccharin in food, beverages, and pharmaceutical products. Various techniques have been used, as shown in the diagram in Fig. (3.1). A large number of these techniques continue to play a useful role in commercial or government control centres. However, the development in recent years of modern chromatographic and spectrometric methods has seen the displacement, or the beginning of the displacement, of some of the older techniques. Food analysis, is a large subject which is still developing.

The chromatographic and spectrophotometric methods are the most widely used techniques in saccharin estimation. Table (3.1) shows the detection limits and sensitivity of some of the methods available for this estimation and the variety of samples for which the technique is applied.

The most recent technique successfuly applied to saccharin determination is 'Molecular Emission Cavity Analysis', MECA (157), in which a 5-μl portion of saccharin extract is injected into the cavity in a hydrogen-nitrogen-air flame and the sulphur (S_2) emission at 384 nm is measured (peak height). The technique has been applied to saccharin determination in soft drinks achieving good results.

However, no attempt has been made using Atomic Absorption Spectrophotometry for the final stages of analysis. In the present work the applicability of both flame and flameless AAS techniques as a possible means for indirect determination for saccharin is examined.

Fig. (3.1) Analytical Methods Available for Saccharin Determination

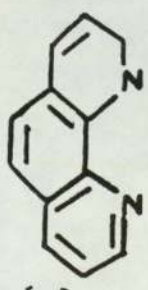


3.2 Spectrophotometric Determination of Saccharin by Solvent Extraction with Tris-(1,10-Phenanthroline)$_3$-Iron(II) Chelate Cation

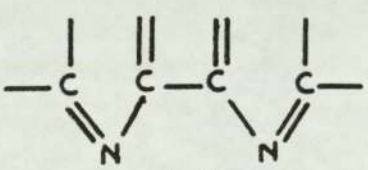
3.2.1 Introduction

1,10-phenanthroline (O-phenanthroline)[I] and related compounds are organic bases with very similar chemical properties. Phenanthroline reacts specifically with iron(II) over a wide range of pH 2-9 to give an orange-red cationic complex having the formula $\left[Fe(Phen)_3\right]^{2+}$. This forms the basis of a well-known determination method of iron in aqueous solution (158). The 1,10-phenanthroline and related compounds are widely used for the determination of low levels of iron and copper. They also serve as colorimetric reagents for determination of some other metals and other species by direct and indirect ways.

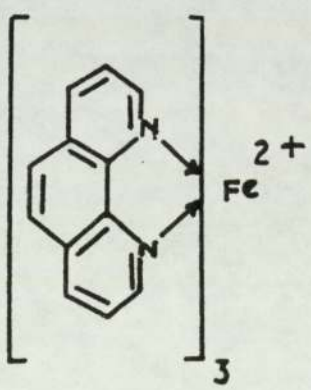
1,10-phenanthroline has the specific reactive group [II]. It forms a 3:1 charged chelate complex with iron(II), the stable 5-membered chelating rings being formed known as 'ferroin' [III], it has the formula below,



[I]



[II]



[III]

where three molecules of the reagent are coordinatively bonded through the lone pairs of electrons on nitrogen atoms with one of iron(II) ion to give a complex of coordination number six. The orange-red iron(II) chelate $[Fe(Phen)_3]^{2+}$ in aqueous solution absorbs in the visible range at 510 nm. Absorbance follows Beer's law over the range 0-8 ppm iron, with a high molar absorpitivity (1.1×10^4). The 'ferroin' ion is not extractable by the usual organic solvents. However, it can be extracted in presence of many anions in organic solvents. This extraction can be carried out after the neutralisation of the charge of 'ferroin' ion with a bulky anion such as perchlorate ClO_4^-, or better an simple extraction with an organophilic anion. 'Ferroin' has been used as a convenient system in the indirect determination of various anions by solvent extraction. Yamamoto et.al have determined iodide (159); thiocyanate (160); perchlorate (161); pentachlorophenolate (162); sodium cyclamate (163); salicylic acid (164) and saccharin (174) by using 'ferroin' ion and 2,2'-dipyridyl-iron(II) and nitrobenzene as organic extractant solvent. Hexaflurophosphate PF_6^- is extracted with 'ferroin' at pH 2.2-10.6 into butyronitrile and the absorbance measured at 505 nm for the determination of phosphorous (165).

$$[Fe(Phen)_3]^{2+} + 2ClO_4^- = [Fe(Phen)_3][ClO_4]_2$$

(ferroin) (counter-ion) (ion-pair)

$$[Fe(Phen)_3]^{2+} + 2\ \text{Saccharin} = [Fe(Phen)_3][\text{saccharin}]_2$$

In the present work, the spectrophotometric determination of anion by solvent extraction with organic solvent containing chelate cations has been chosen as a basis for the indirect determination of saccharin by Atomic Absorption Spectrophotometry AAS (174). In this method, saccharin forms a chelate with tris-(1,10-phenanthroline)-iron(II) ion, which selectively extracted into nitrobenzene as an ion-association system. This method has been thoroughly investigated in order to determine the optimum conditions. The factors affecting the solvent extraction system and the subsequent application for the determination of saccharin in soft drink samples have been investigated. The colorimetric method has been adopted to give an 'atomic absorption finish' using both flame and flameless techniques. In this indirect method both the organic phase and aqueous phase using 'stripping' technique, have been used.

3.2.2 Experimental

Apparatus :

Spectrophotometric measurements were carried out by using a SP 6-400 ultraviolet and visible spectrophotometer manufactured by 'Pye-Unicam'; or SP-800 ultraviolet spectrophotometer. An EIL model 23A pH-meter was used for measuring the pH of the solutions. 1.0-cm quartz cells were used for measuring all the absorbances

Reagents :

Iron(II) ammonium sulphate solution (5×10^{-3} M)

A solution of 5×10^{-3} M was prepared from A.R. grade by dissolving 0.98 g of the salt in 500 ml of distilled water.

1,10-phenanthroline solution (16×10^{-3} M)

A stock solution of this was prepared by dissolving 1.585 g of the A.R. grade in 500 ml of distilled water.

Standard saccharin solution (10^{-2} M)

A stock solution of saccharin was prepared by dissolving 0.1832 g of A.R. grade in 100 ml of distilled water. Dilutions were made to some solutions in range 0-100 μM.

Phosphate buffer solution (1/15 M)

Two phosphate solutions have been prepared; one is 1/15 M monopotassium phosphate by dissolving 9.08 g of A.R. grade KH_2PO_4 in a litre of distilled water, the other is 1/15 M disodium phosphate by dissolving 11.88 g of A.R. grade Na_2HPO_4 , $2H_2O$ in a litre of distilled water. Different amounts of both stock solutions were mixed together to yield the desired pH value for the extracted species. pH was measured for each mixture.

Organic solvent :

Nitrobenzene was purified using vacuum distillation. A further purification step was found necessary for AAS work as described under other section (see 3.3.1).

Procedure used :

1.0 ml of saccharin solution in the concentration ranging 0-300 μ M was transferred into a 50 ml separating funnel. 1.0 ml of iron(II) ammonium sulphate solution (5×10^{-3}M) was added, followed by 1.0 ml of 1,10-phenanthroline solution (16×10^{-3}M), and 5.0 ml of phosphate buffer solution (equivolumes of 1/15 M Na_2HPO_4, $2H_2O$ and 1/15 M KH_2PO_4 solutions) in order to bring the pH of the mixture to 6-7. Two milliletres distilled water were added in order to bring the volume of

the aqueous phase to 10 ml ; then 5.0 ml nitrobenzene was added, and the mixture shaken for about three minutes. After allowing the two phases to separate for 30 minutes, the organic phase was filtered through a small piece of cotton wool in the stem of the separating funnel to remove water. The maximum absorbance of the dried organic phase at 516 nm was measured by the spectrophotometer using reagent blank or nitrobenzene as a reference.

3.2.3 Results & Discussion

(1) Absorption Spectra

The absorbance maximum of the extract, tris-(1,10-phenanthroline) iron(II)-saccharin in nitrobenzene was confirmed as being at wavelength 516 nm. The absorbance spectra of an extract and a reagent blank using nitrobenzene as reference are shown by the graph in Fig. (3.2).

(2) The Effect of Variation of pH

In order to study the effect of pH on the solvent extraction system, a series of solutions adjusted to various pH values were prepared and extracted as mentioned in the procedure described. The graphs in Fig. (3.3) and Fig. (3.4) show how absorbance of the extracted species in the organic phase varies with pH using (0.1 M KOH/0.1 M HCl) and phosphate buffer solutions respectively. Both plots show that the degree of extraction of saccharin with $\left[Fe(phen)_3 \right]^{2+}$ is maximum from pH 4-8. The similarity of the absorbances with and without phosphate indicates

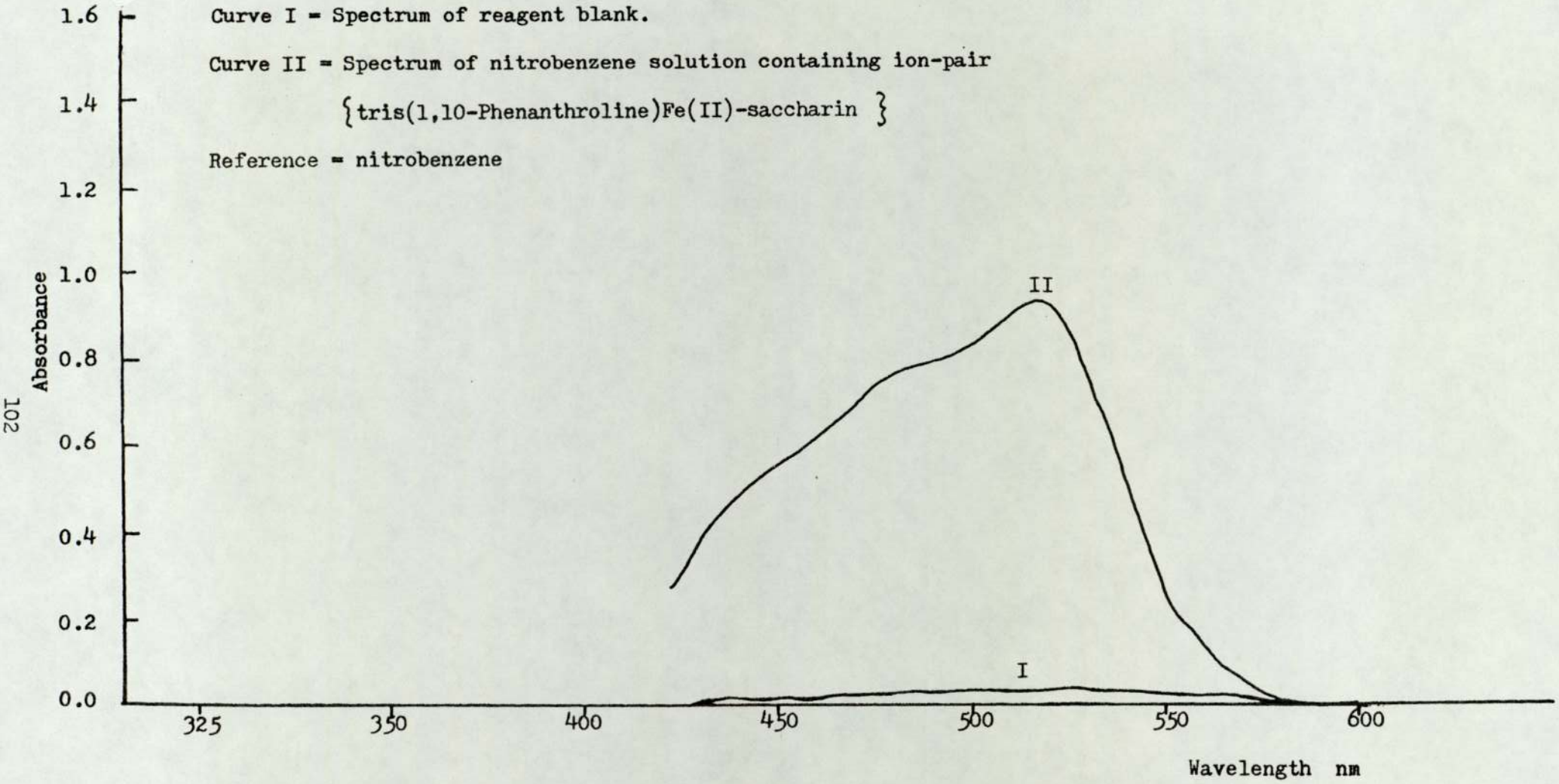


Fig. (3.2) Absorption spectra

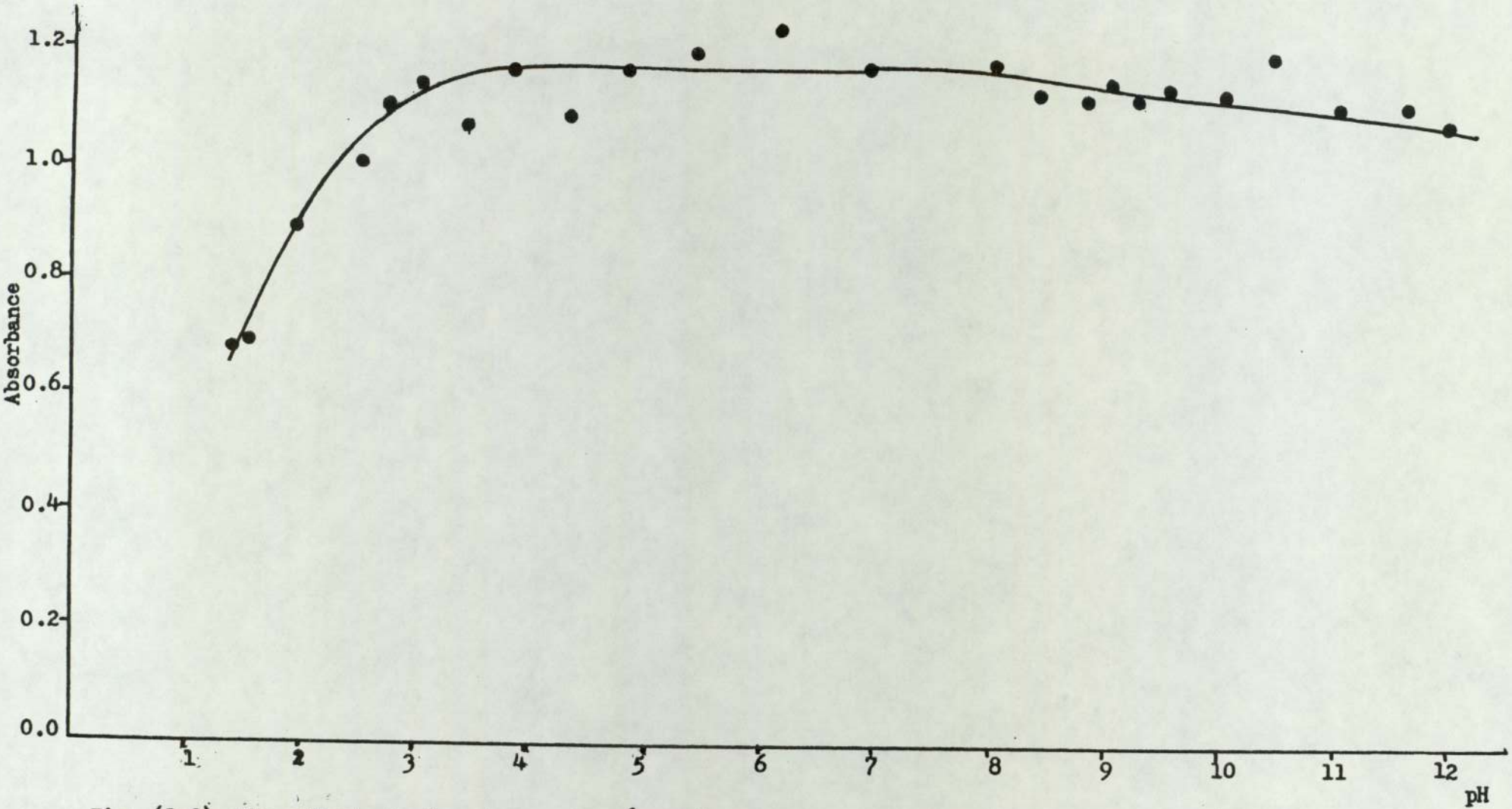


Fig. (3.3) The effect of pH on extraction { Tris(1,10-phenanthroline)Fe(II)-saccharin } in nitrobenzene

Concn. of saccharin 30.0μM, KOH 0.1M - HCl 0.1M, λ_{max} 516 nm

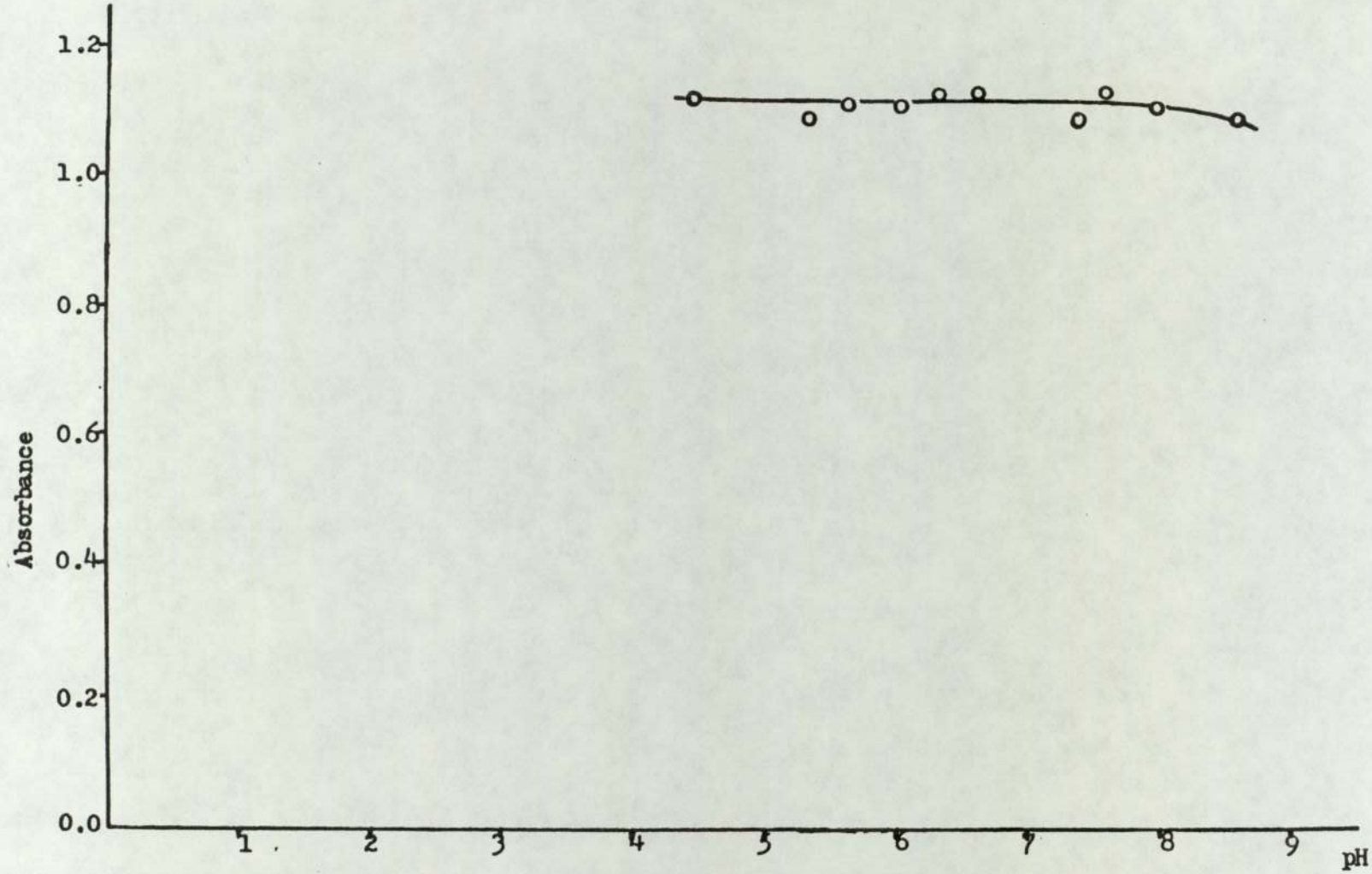


Fig. (3.4) The effect of pH on extraction {tris (1,10-phenanthroline)Fe(II)-saccharin} in nitrobenzene concn. of saccharin 30.0μM, phosphate buffer λ_{max} 516 nm

that phosphate does not interfere.

(3) The Efffect of Shaking Time

All other variables were kept constant, and shaking time of the extraction was varied from 0.5 to 30 minutes. The graph in Fig. (3.5) shows no variation in absorbance with shaking time, and that three minutes was adequate for subsequent experiments.

(4) The Stability of the Colour

Two samples of nitrobenzene containing tris-(1,10-phenanthroline)-iron(II)-saccharin extract, were examined for stability of colour. One sample was exposed to the daylight, and the colour intensity of the nitrobenzene phase was found to be constant for three hours. A slight fading was noticed after 18 hrs. (0.88%). The second sample was kept in the dark, the stability of the colour (as measured by absorbance) was kept constant for three hours. Slight fading was found after 18 hrs. but no further fading over several days. The conclusion is that u.v & visible light does not cause fading.

(5) The Effect of the Number of Extractions

The solution was extracted twelve times using fresh nitrobenzene each time. The absorbance of the first extract was 1.13, the second and the third still had appreciable absorbances, 0.56 and 0.24 respectively. This method may be used as a clean-up method for

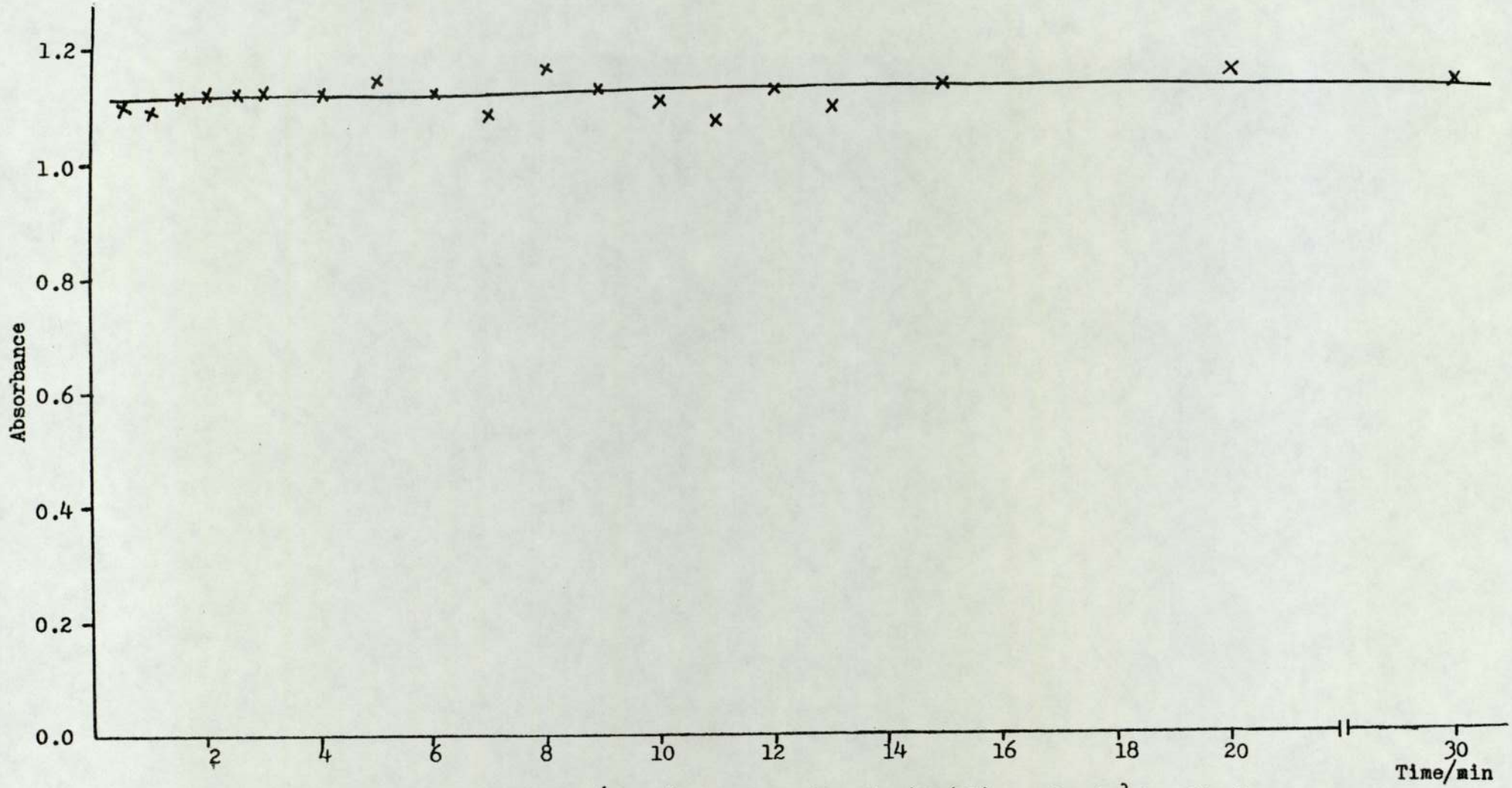


Fig. (3.5) The effect of shaking time {Tris(1,10-phenanthroline)Fe(II)-saccharin} in nitrobenzene

Concn. of saccharin 30.0 μM pH 6.9 λ_{max} 516 nm

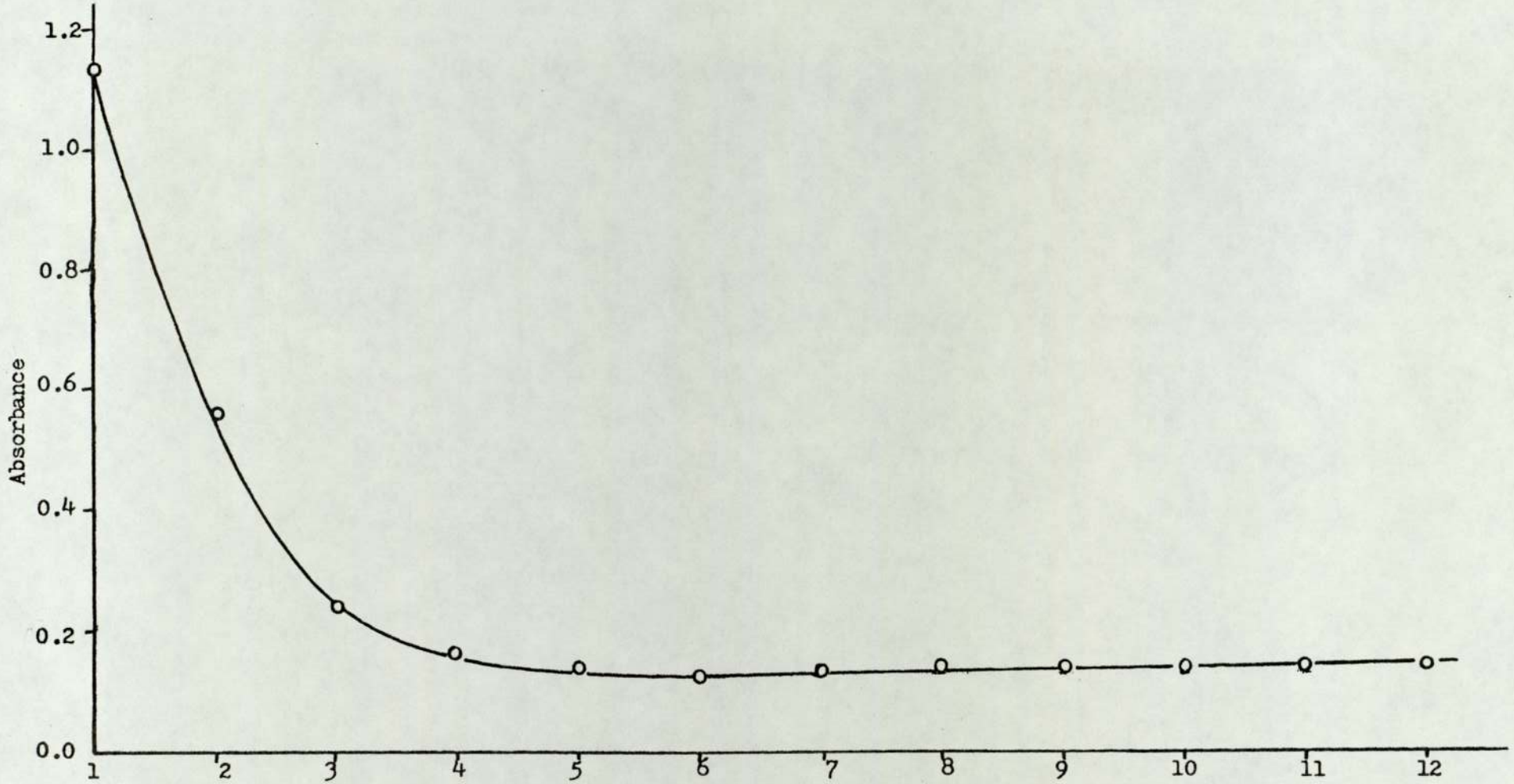


Fig. (3.6) The effect of number of extractions{Tris(1,10-phenanthroline)Fe(II)-saccharin} in nitrobenzene pH 6.9 concn.of saccharin 30.0 μM λ_{max} 516 nm

extraction of saccharin. The graph in Fig. (3.6) shows that after fourth extraction the curve levels out i.e. absorbances for the other numbers of extraction are constant. This indicates that maximum extracted species can be obtained after four extractions.

(6) The Effect of Variation of Mole Ratio 'Tris-(1,10-phenanthroline)-Fe(II) sulphate Concentration to Saccharin Concentration'

In this experiment the total analytical concentration of saccharin is held constant (300 μM & 150 μM), whilst varying the concentration of the chelate cation $\left[Fe(Phen)_3 \right]^{2+}$ other variables being kept constant. The measurements of absorbances were taken at 516 nm where the ion-association complex absorbs. Typical photometric 'titration' curves are shown in Fig. (3.7) from which the following points could be concluded :

i) It is shown that the ion-pair formed between saccharin and $\left[Fe(Phen)_3\right]^{2+}$ is not very stable complex, because the two lines of the curve are linear over small ranges and the slope breaks slowly and not very sharply.

ii) The experimental data indicates how much excess of 'ferroin' reagent is required to completely complex the sacchrin. It is obvious from the graphs that at least the maximum and constant extraction is obtained at a mole ratio, $\left[Fe(Phen)_3 \right]^{2+}$: saccharin about 20:1, i.e. an excess of at least 20-fold (molar) of chelate to saccharin should be used.

iii) Smoother and better curves can be obtained by working at lower ranges of saccharin concentrations (which give absorbance readings between 0.1-0.6). The flat part of the curve is more obvious in such curves.

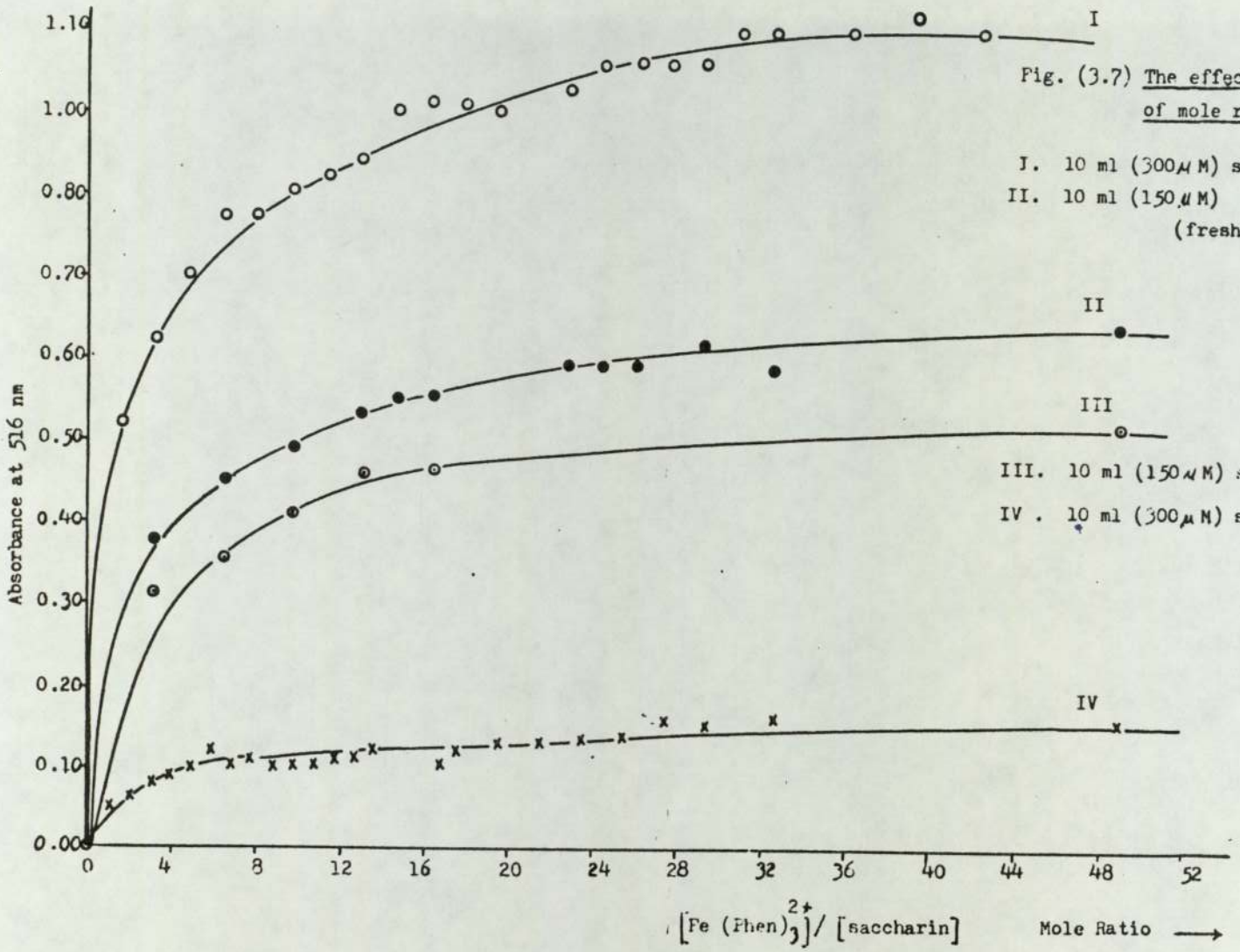


Fig. (3.7) The effect of variation of mole ratio.

I. 10 ml (300 μM) saccharin-pH 6.9
II. 10 ml (150 μM) " -pH 6.6
(fresh ferroin solution)

III. 10 ml (150 μM) saccharin-pH 6.6
IV . 10 ml (300 μM) saccharin-pH 6.9

iv) The plots appear to be continuous smooth curves with no straight-line portions indicate that the complex 'ion-association species' formation is relatively incomplete. Therefore the addition of excess of 'ferroin' reagent in the order of 20-fold is necessary to complete this reaction.

(7) Composition of the Extracted Species, 'Method of Continuous Variations'

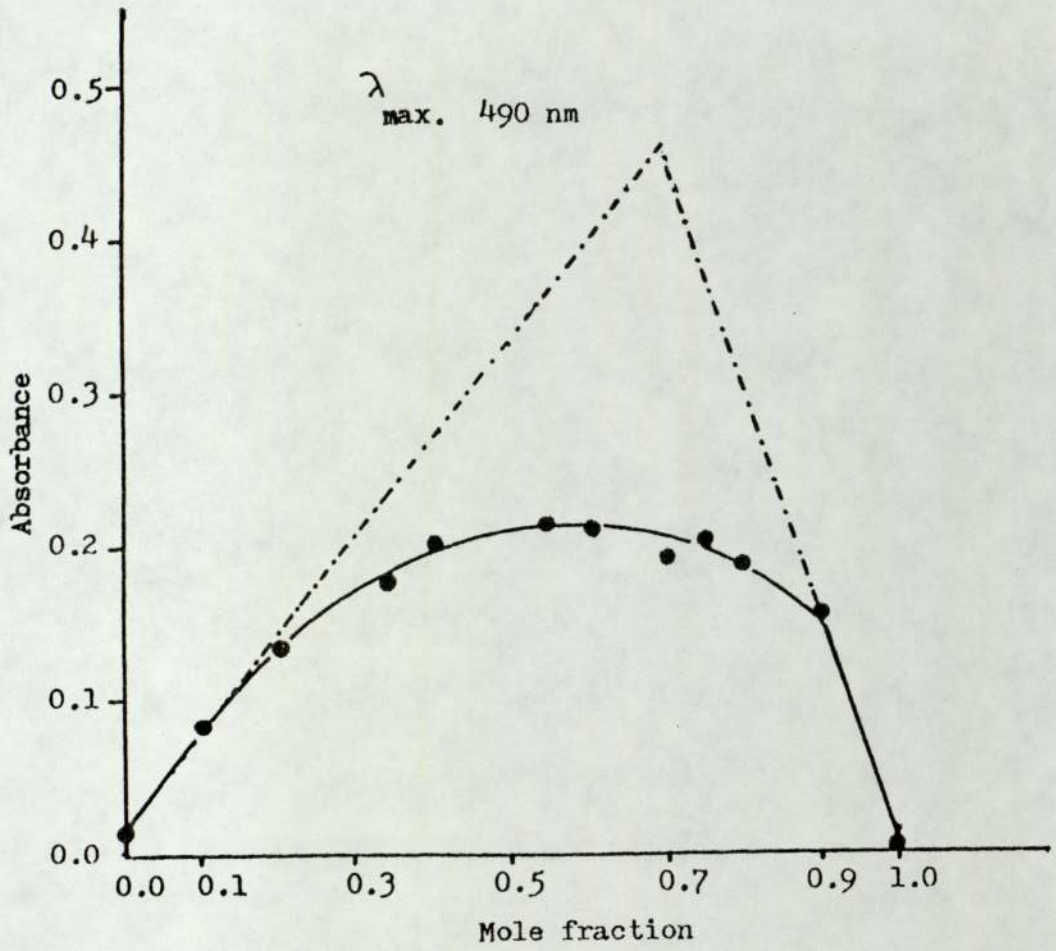
Saccharin and ferrion solutions were prepared in equimolar concentration (10^{-3}M), and mixed in various proportions in such a way as to give the same total volume of each mixture (1.0 ml). The mixtures were extracted with nitrobenzene and absorbances taken at three different wavelengths 450 nm, 490 nm and 516 nm. The results are shown by the graphs in Fig. (3.8) where the plot of absorbance against volume fraction (the same as the mole fraction) of the reacted species is defined as follows :

$$\text{Mole fraction} = V_{Fe}/(V_{Fe} + V_{sacch}) \quad , \text{ or}$$

$$= V_{sacch}/(V_{Fe} + V_{sacch})$$

where V_{Fe} is the volume of the cation $\left[Fe(Phen)_3\right]^{2+}$ and V_{sacch} is the volume of the anion $\left[\text{saccharinate}\right]^-$.

The maximum volume ratio V_{Fe}/V_{sacch} found to be 0.33/0.67 = 0.49 at the wavelength 516 nm and this result suggests that a 1:2 ion-pair is formed between tris-(1,10-phenanthroline)iron(II) chelate cation and saccharinate



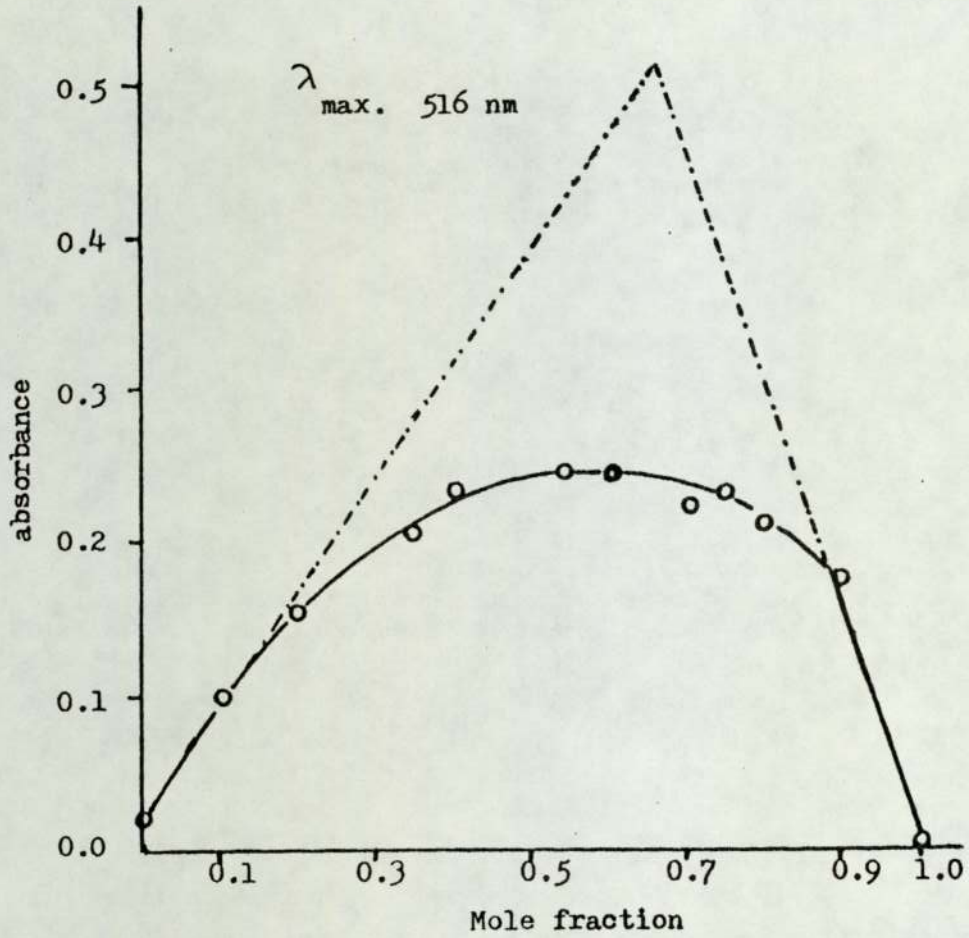
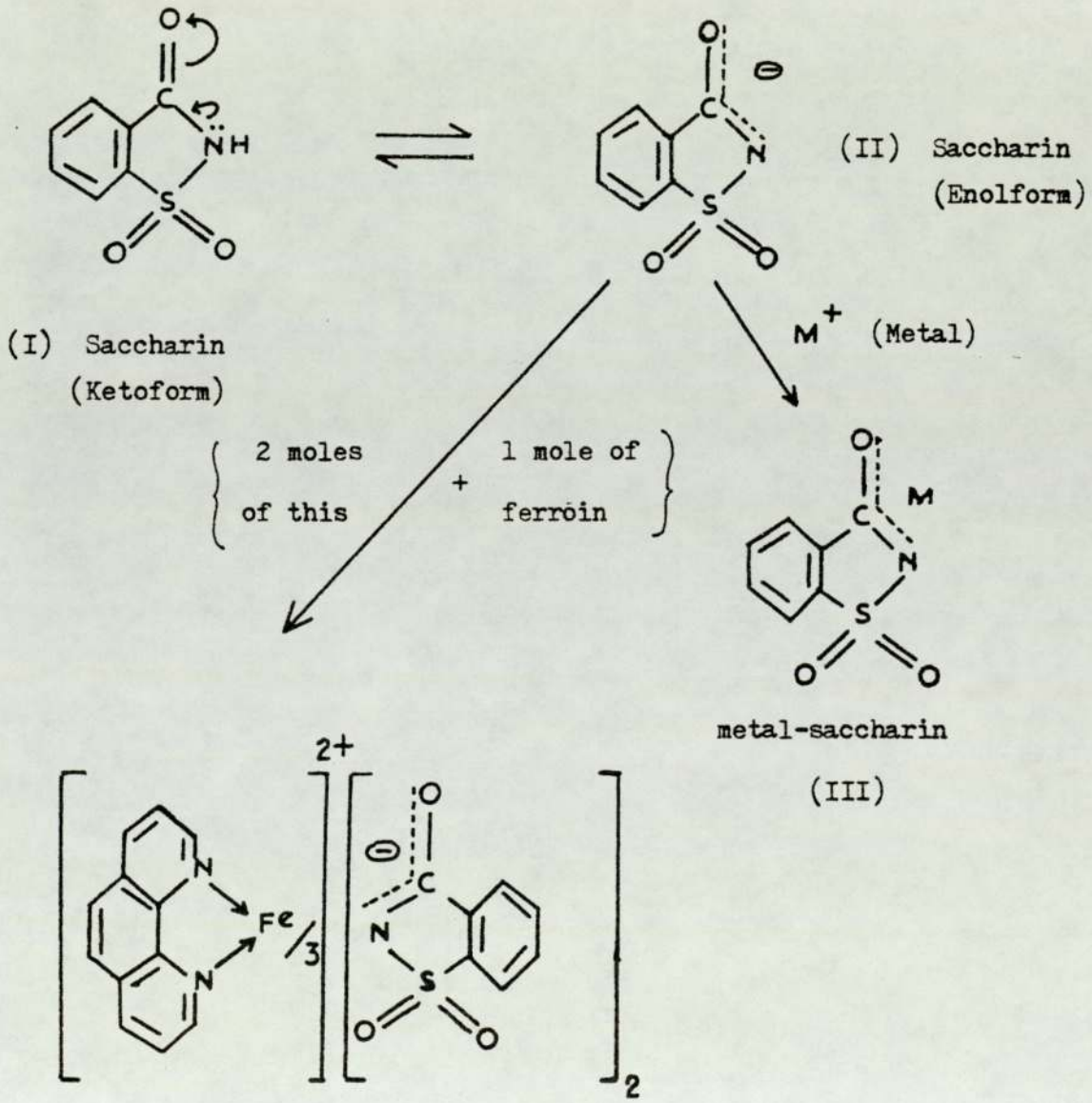


Fig. (3.8) Composition of the extracted species "method of continuous variations"

{ Tris(1,10-phenanthroline)Fe(II)-saccharin } in nitrobenzene (1:2 ion-pair)

anion and the chemical formula of the extracted ion-pair in nitrobenzene is possibly $[Fe(Phen)_3][saccharin]_2$. This experiment also showed from the curvature of the plot that this complex is not very stable. The other value (V_{Fe}/V_{sacch}) at the other wavelength 490 nm is found to be 0.45 and this suggests that not more than one complex is formed between the $\left[Fe(Phen)_3 \right]^{2+}$ and saccharin.

The possible explanation for the ion-association complex in this reaction is illustrated in the following equations :



Ion-Pair

$\left\{ (Ferroin)\ (Saccharin)_2 \right\}$

(IV)

The above illustrations show the formation of the ion-pair (IV) between two moles of saccharin in enol-form with one mole of charged metal chelate $\left[Fe(Phen)_3\right]^{2+}$. In general saccharin anion (II) may react through the nitrogen or the oxygen atoms to form the mono-metal saccharin compound (III). This cloud of electrons distributed between $\overline{O{-}C{-}N}$ gives the possibility of complex formation by "neutralization" with the cationic charge chelate.

(8) The Effect of Other Food Additives

The interference of the other additives, viz sodium cyclamate dulcin, sucrose, glucose (as sweetening agents), citric acid and sodium chloride (as fillers) was examined. Table (3.2) shows the interferences of these food additives in term of 'Relative Error Percent', which indicates the effect of the additive on the absorbance of the extracted species $[Fe(Phen)_3][saccharin]_2$ in nitrobenzene;

$$\text{Relative Error } \% = \frac{\text{Error Value}}{\text{Accepted Value}} \times 100$$

The results which are given in the table (3.2) were for the extraction of 200μM of saccharin, in the presence of dulcin, sucrose, glucose and citric acid as additives. These gave negligible 'relative error' on the absorbance in ratios mentioned. A small amount of cyclamate gave high positive error. Chloride ion has an effect less than cyclamate.

It must be pointed out here that the use of a blank reagent is strongly recommended. The analyst must be careful in using the official method of analysis for extraction of saccharin from soft drinks

Table (3.2) The effect of food additives on the absorbance of $\{Fe(Phen)_3\}\{saccharin\}_2$

No	Additive	μM ratio to saccharin concentration	Relative error %
1	sodium cyclamate	0.007	3.84
		0.067	11.54
		2.667	23.08
		53.333	115.38
2	chloride ion	0.007	3.70
		0.333	3.70
		333.000	3.70
3	dulcin	0.005	0.00
		1.000	
		4.000	
		40.000	
4	sucrose	0.005	0.00
		1.000	
		4.000	
		40.000	
5	glucose	0.005	0.00
		1.000	
		4.000	
		40.000	
6	citric acid	0.005	0.00
		1.000	
		4.000	
		40.000	

(or non-alcoholic beverages). In this method (166), saccharin is extracted from acidic solutions with diethylether. Concentrated hydrochloric acid is used. The chloride ions come from this addition gave a positive error in the determination of saccharin when use the spectrophotometric and AAS methods. More details for the practical consideration of saccharin in soft drink determination will be given in chapter four.

(9) Calibration Curves

Different amounts of saccharin were extracted by the method previously described using the optimum conditions recommended with solutions in concentration range (0-120μM) saccharin. The absorbance of this set of standards was measured at 516 nm using a reagent blank as reference. As shown in Fig. (3.9), Beer's law is obeyed in the range (0-20μM). There is a linear relationship over this range but at higher concentration there is no linearity.

3.3 Applicability of Atomic Absorption Spectrophotometry to Saccharin Determination

An atomic absorption spectrophotometry flame or electrothermal atomizer finish should be easily applied to the spectrophotometry procedure for determination of saccharin. The aim of this work was to investigate this possibility. This hopefully could extend its applicability to both routine and trace analyses. High sensitivity should also be attainable furthermore by using the electrothermal

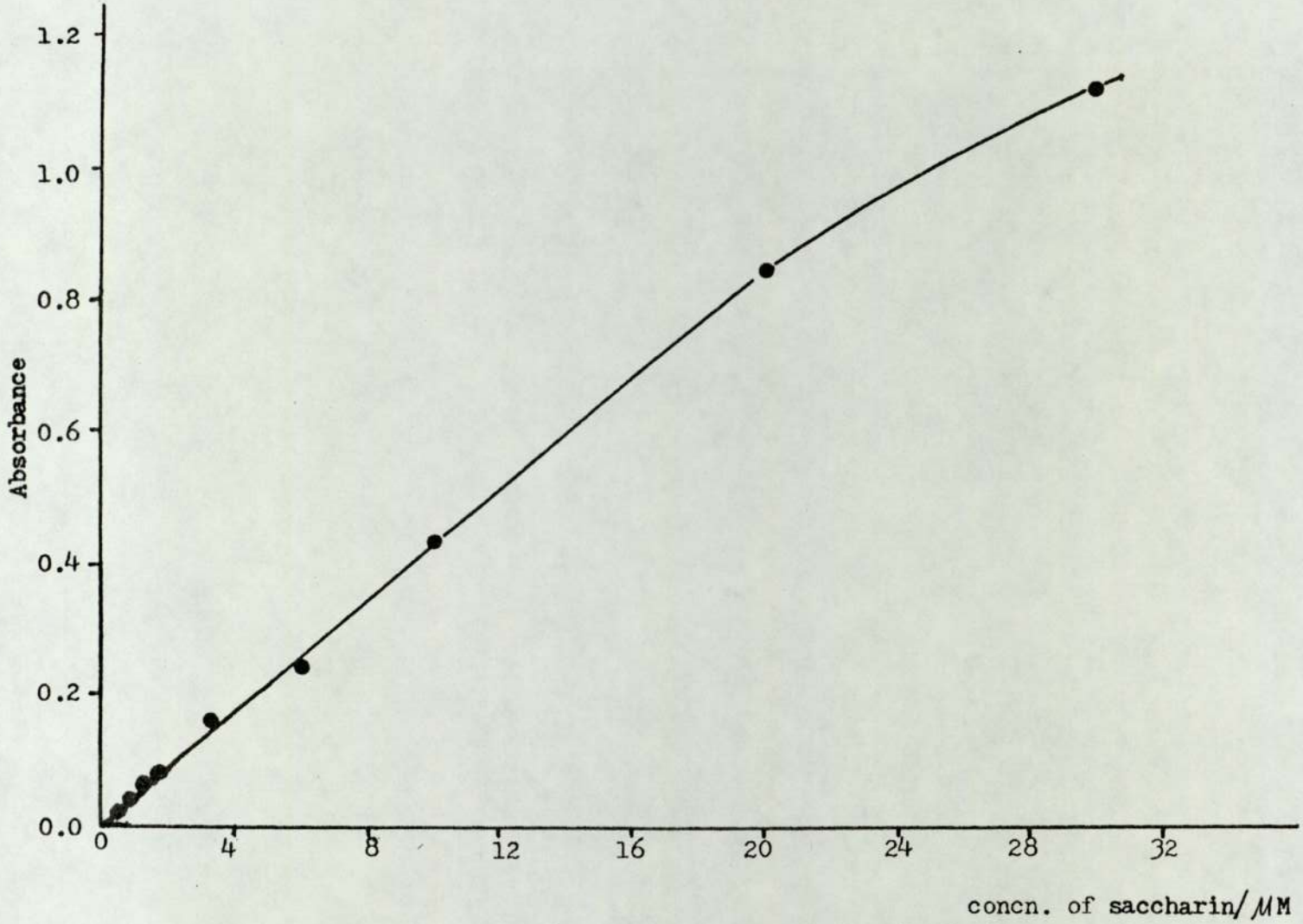


Fig. (3.9) Calibration curve {Tris(1,10-phenanthroline)Fe(II)-saccharin} in nitrobenzene $\lambda_{max.}$ 516 nm

atomizer. The use of AAS as the final stage of analysis for the determination of saccharin is demonstrated in this section, together with the problems associated with this application.

From the continuous variation methods described (see 3.2.3), the chemical formula of the extracted species is assumed to be $\{$tris-(1,10-phenanthroline)iron(II)$\}\{$saccharin$\}_2$, which is the ion-pair stoichiometrically formed between saccharinate anion and the cationic metal chelate $\left[Fe(Phen)_3 \right]^{2+}$. This recommended procedure involves the selective extraction of the ion-association complex into nitrobenzene and subsequent determination of the iron concentration in the extract which gives the amount of saccharin intially present in the aqueous phase.

3.3.1 Experimental Work & Practical Considerations

(i) Apparatus

A Perkin-Elmer Model 303 atomic absorption spectrophotometer and an iron hollow-cathod lamp were used. The flame measurements were made using an air-acetylene. The flameless measurements were carried out using HGA70 graphite furnace atomizer fitted with the main instrument. The experimental conditions for the measurements were as described in the recommended procedures.

(ii) Reagents and Contamination Problems

The same reagents which have been used in the spectrophotometric

method were used for this work, together with carbon tetrachloride used in the 'stripping' technique. The analysis using AAS, particularly with the electrothermal atomizer technique must be carried out with a great care, where it involves measurements less than 1 ng of an element. This increased sensitivity is considered to be the main advantage of electrothermal atomization technique. Accurate results cannot be achieved unless the analyst avoids the sources of contamination. Iron is one of the most common contaminants in the environment, including apparatus, working materials chemicals and instruments. Iron is a contaminant from the production technique of plastics which could be the material of the vessels used or in the micropipettes tips. Careful cleaning is strongly recommended with acid and iron-free water together with dust-free drying and storage. Changing the micropipette tips is one of the problems faced in this work which could involve further contamination of iron.

Nitrobenzene itself is also a source of contamination. A.R. nitrobenzene contains 0.0001 % Fe and some other elements such as Cu 0.00005 % , Pb 0.0005 % and water 0.05 % . This metal content in nitrobenzene is very low but in AAS with electrothermal atomizer technique, the determination of trace metals as low as 10^{-12} g may be measured, and thus these concentrations of metal traces, introduce a difficult problems. The importance of using very pure organic solvents with the flameless technique is thus appreciated. Distillation under vaccum should reduce the metal content further. Further reduction of this content can be achieved by double distillation.

Nitrobenzene was purified in two steps :

(1) Distillation under reduced pressure, the middle fraction was collected

at 150°C.

(2) Purification of the collected nitrobenzene from the previous step could be achieved using either Dithizone or 1,10-phenanthroline compounds as 'Scavenger' reagents. 50 ml of nitrobenzene was shaken for 3 minutes with 15 ml of 1,10-phenanthroline solution (16×10^{-3}M), 10 ml phosphate buffer and 5 ml ascorbic acid (10 %). After leaving for half an hour, the nitrobenzene was filtered through pieces of cotton wool filled the stem of the funnel. The iron(II) traces which may be present in the nitrobenzene will be extracted into the aqueous phase leaving the nitrobenzene, it is hoped, free of iron.

This 'Scavengering' process has been used previously e.g. Bathophenanthroline (4,7-Diphenyl-1,10-phenanthroline) and isoamyl alcohol for extraction of trace amounts of iron from hydroxylamine hydrochloride and buffer solutions prior to their use in the spectrophotometric determination of iron has been reported in the literature (167). An advantage over 1,10-phenanthroline, is there is no need for presence of the perchlorate anion (ClO_4^-) in order to produce an extractable 'ferroin' salt.

(iii) Recommended Procedures

Two ways could be possibly be used for determining saccharin; (a) by determination of iron content in the organic phase which contains the ion-pair extracted from the aqueous solution. The iron content in the extracted species $\{$tris-(1,10-phenanthroline)iron(II)$\}\{$saccharin$\}_2$ is a function of saccharin intially present in the aqueous phase. This iron could be subjected to flame and/or electrothermal atomizer;

alternatively (b) determination of iron content in the aqueous phase after 'stripping' the iron into water, by using another solvent to break the ion-association system and to produce the species of $\{$tris-(1,10-phenanthroline)-iron(II)$\}^{2+}$ in water. Again the final stage of determination could be the flame and/or the carbon furnace technique.

Iron cyclohexanebutyrate, or tris-(1-phenyl-1,3-butanediono)Fe in Xylene or MIBK, are organometallic atomic absorption reagents recommended for preparation of standard iron solutions in non-aqueous phase. However, a method of preparing a non-aqueous iron standards having the matrix $[Fe(Phen)_3][saccharin]_2$ -nitrobenzene was developed in order to evaluate the method and the performance of the instrument.

Procedure (a) : 'Iron content in organic phase'

The same procedure which was used for the spectrophotometric determination (see subsection 3.2.2) was used here. After the filtration of the organic phase through the cotton wool, the absorbance was measured using the 248.3 and 372 nm iron lines. Using the flame technique, and air-acetylene flame from a 10-cm slit burner used with an air flow-rate of 6.0 l/min and an acetylene flow-rate 2.0 l/min was used. For the carbon furnace technique 5-μl aliquot of samples were used; Argon pressure was about 19 lb/in^2; chart speed 0.5 cm/min, the temperature and time programming have been selected for this work as follows :

(1) Drying stage : 100°C - 60 seconds

(2) Pyrolysis stage : 1100°C - 120 seconds

(3) Atomization stage : 2500°C - 10 seconds

Procedure (b) : 'Iron content in aqueous phase-striping method'

In this procedure, 50 ml of the extracted samples in the nitrobenzene phase containing the 'ferroin-saccharin' species was shaken with 5.0 ml distilled water, followed by addition of 5.0 ml of carbon tetrachloride. The layers were allowed to separate, and the iron content measured in the aqueous phase. Sometimes, the traces of organic solvent in the aqueous phase caused some problems. These traces may be removed by centrifuging. An air-acetylene flame from 10-cm slit burner was used with an air flow-rate of 8.5 l/min and an acetylene flow-rate 6.5 l/min for the flame technique. When using the carbon furnace, dilution for the above samples by ten times is necessary, 20 µl sample size are used with Argon pressure about 19 lb/in^2, chart speed 0.5 cm/min. The temperature and time programming were selected as follows :

(1) Drying stage : 100°C - 10 seconds

(2) Pyrolysis stage : 490°C - 30 seconds

(3) Atomization stage : 2500°C - 5 seconds

Procedure (c) : 'Non-aqueous iron standards-iron in nitrobenzene matrix'

In 25 ml -separating funnel add 1.0 ml of standard iron solution (as 5×10^{-4} M ammonium iron(II) sulphate), 1.0 ml 1,10-phenanthroline (16×10^{-3} M), 1.0 ml saccharin solution (10^{-2} M), 5 ml phosphate buffer solution (1/15 M KH_2PO_4 + 1/15 M Na_2HPO_4) and 2.0 ml distilled water. Shake the mixture with 5.0 ml free iron-nitrobenzene and allow to separate into two layers. The aqueous phase is extracted with fresh 5.0 ml nitrobenzene together with 1.0 ml 1,10-phenanthroline solution and 1.0 ml saccharin solution. Repeat the extraction process with another 5.0 ml portion of fresh nitrobenzene. Collect the four extracts of nitrobenzene phase, filter through a piece of cotton wool

into a 25 ml -volumetric flask; add nitrobenzene up the 25 ml mark. Dilution can be made using free iron-nitrobenzene to prepare the desired concentration range of iron. Saccharin (10^{-2}M) is used in this method as a clean-up agent for extraction the iron content in the aqueous phase into the nitrobenzene.

3.3.2 Results & Discussion

In the following discussion, AAS with a Heated Graphite Atomizer (HGA70) has been evaluated in detail for its applicability in the indirect determination of saccharin.

3.3.2.1 Some Practical Considerations

The main difficulty with this determination is that involving the use of nitrobenzene in the carbon furnace (HGA70 Model). The method suffers from 'matrix' and 'background absorption' interferences. Many factors associated with the use of this solvent have to be considered.

It is worth mentioning the advantages and disadvantages of using nitrobenzene in this procedure together with the other factors which govern the choice of the organic solvent.

It is well known that over 50 solvents have been used in analytical flame spectrophotometry and many of these solvents could satisfactorily used in the electrothermal atomizer technique. Ketones, esters, alcohols, ethers, aliphatic hydrocarbons, aromatic hydrocarbons and nitro-compounds; are some of the organic solvents which have been employed in the flame spectrometry.

The 'ideal' solvents for the 'solvent-extraction/AAS flame system' should have the following properties :
Rapid rate of nebulization (low viscosity); high nebulization efficiency; high extraction efficiency; low toxicity; give non-toxic combustion products and low background absorption. Some other factors should also be considered in choice of organic solvent such as; low solubility in the aqueous phase, moderate volatility and ready availability in a suitable pure condition. However, some of the problems associated with the organic solvents in the flame are solved using the electrothermal atomizer technique, where very small aliquots of sample solution used. This eliminates; (a) the problem of toxic combustion products and the other undesirable combustion characteristics, (b) the problem of changing in the nebulization rate and nebulization efficiency which causes variations in the flame, and (c) the problem of atomizer temperature, where the solvent is removed in the carbon furnace prior to atomization process.

Nitrobenzene has toxic characteristics, and in spite of this problem it has still been widely used in analytical flame spectrophotometry and in uv & visible spectrophotometry. There are certain chemical justifications associated with its use. It has a high distribution coefficient for the systems used. It has known that nitrobenzene has a relatively high dielectric constant (34.8) which is suitable for extraction of the ion-pair (159). It has low nebulization rate because its relatively high viscosity. This gives lower mean droplet diameter than that of water, i.e. giving higher nebulization efficiency. However, Yamamoto et.al (168) reported that "the absorbance from the nitrobenzene solution was only very slightly less than that from the

aqueous solution" although the nebulization rate of water was twice that of nitrobenzene.

Problems arising from light scattering and absorption are frequent when using electrothermal atomizers than they are with flames. Because of the relatively high viscosity of nitrobenzene, the smoke presents the most serious problem associated with electrothermal atomizer work. The nitrobenzene matrix condenses after the drying stage to form a smoke or mist. The mist is formed by condensation when the vaporized nitrobenzene matrix reaches the cooler part of the furnace causing errors in the absorption measurements. Whilst the dense smoke comes out of the furnace causing a light scattering and high background absorption. This high background signals which come from the heterogeneously distributed smoke, make the analysis extremely difficult.

The design of the graphite tube or 'standard tube' in this work was found to cause another problem, when using nitrobenzene matrix. This may be described as the spreading of the liquids along the length of the tube. This results in a reduction in sensitivity and precision, together with an inefficient atomization process. It has been claimed that the standard tube is not designed for use with organic solvents with volumes greater than about 20μl because these organic solvents tend to flow toward the ends of the tube, resulting in poor precision if large volumes are to be used. However, in this work 2μl to 10μl of nitrobenzene samples have been used and reduction in sensitivity due to the variations in the rate of atomization has been noticed. It must be pointed out that small volumes of nitrobenzene matrix were used in order to minimize the increased spreading of the nitrobenzene on the graphite surface.

The establishment of the programming selection for the stages; 'drying', 'ashing' and 'atomization' was the intial factor investigated in order to solve the problem of the smoke caused by the nitrobenzene matrix. The temperature and time programming is one of the most significant parameters which can be practically optimised. Mention has been made for the optimum conditions of these parameters in subsection 3.3.1. Different temperatures and time for charring stage have been used in an attempt to choose the optimum charring parameters. It was desirable to select 1100°C and 120 seconds, in order to decrease the possibility of 'molecular absorption' and 'smoke' background interferences which may caused by smoke-producing' nitrobenzene matrix. Increasing the charring time (at the temperature 1100°C) up to 300 seconds did not give much advantage in minimizing the background signals due to smoke. The charring time 120 seconds, however was used. The aim of this selection was achieved when most of the nitrobenzene matrix had been volatilized before the 'atomization stage' for iron.

It was believed that with increasing time and temperature programme for the drying and charring stages would help in elimination or minimizing the 'smoke' problem. It seems, however, that nitrobenzene is a heavy matrix, and because its relatively high viscosity, these conditions have minimized the amount of the smoke but have not eliminated it completely. On the other hand, those conditions appear to offer disadvantages. The life time and the cost of the tube is one of the important factors to be considered. Using temperatures as high as 1100°C for 120 seconds for every sample will shorten the life time of the furnace, will length the time of analysis and will increase the cost of determinations.

It was found possible to use the tubes for a maximum of 170 injections using aqueous matrices. They were not found to age significantly during the use of nitrobenzene matrix, in which they have useful life times of 70 determinations. After this, sensitivity and precision start to decrease with tube age. The cost of the tube is very high nowadays.

Time factor for analysis is very important factor, and using nitrobenzene matrix the time factor is 190 seconds per sample. This is higher than with the aqueous matrix (45 seconds per sample).

The amount of 'smoke' can be so small that it is not visible to the eye but it can give the false results. In the case of nitrobenzene the smoke however, is very visible to the eye.

In order to reduce the viscosity of the nitrobenzene matrix, methyl isobutyl ketone (MIBK) has been used as a diluent after extraction the ion-pair 'ferroin-saccharin' with nitrobenzene in attempt to eliminate the smoke signals. However, this modification did not offer much advantage.

It has been noticed that the tube itself smells nitrobenzene even when it is cleaned by heating up to the maximum temperature. These nitrobenzene matrix impurities are vaporized only slowly and may show 'memory' effect after the atomization stage. The solvent appears to soak deeply into the porous graphite tube walls, besides it is not very volatile matrix. This has caused a shifting in the baseline when using nitrobenzene matrix. It was not practical to increase the temperature and time of charring and atomization stages.

Different flow rates of 'Argon' gas used in order to flush the smoke from the tube during the programme and before the atomization stage did gave not much improvement and the smoke was still produced

during the atomization stage.

Background absorption and scattering effects, which are commonly encountered in electrothermal atomization, are less common at wavelengths above 350–400 nm. The line 372 nm has been used with nitrobenzene matrix. The smoke absorbs less at this wavelength line than at 248.3 nm. This was indicated by the significant reduction of signals appearing during drying and charring temperature stages.

The precision or repeatability when using nitrobenzene could be very much poorer than when using aqueous solutions because very small droplets of nitrobenzene tended to remain behind on the inner walls of the plastic micropipette tips used.

Having outlined the details of the general problems associated with the nitrobenzene matrix within the carbon furnace, it is now appropriate to discuss in more detail the selective steps needed in order to avoid nitrobenzene interference with carbon furnace. It was thought preferable to strip the iron content back into aqueous solutions which could then be subjected to the technique of flame or electrothermal atomizer.

Back-extraction (retrograde extraction), refers to the transfer of a component, from an organic extract into an aqueous phase by shaking the two phases together. The back-extracted species may be desired component to be determined, or undesired foreign component. 'Stripping' is to remove the desired substance completely from the organic phase to the aqueous phase. Water or suitable aqueous solution is used for this kind of extraction. This aqueous phase includes either pH-controlled solutions, or chelating reagents. In work described here another technique has been used. It has been found experimently that water or

acidic solutions were not adequate in removing or stripping completely the species from nitrobenzene to the aqueous phase. However, when carbon tetrachloride is added to the system, the extraction rate is increased.

The probable explanation for this increase in the extraction rate lies in the following facts :

(i) The ion-pair compound $\left[Fe(Phen)_3\right]\left[saccharin\right]_2$ is slightly soluble in water, because its large cation and anion. This limited water solubility makes the partition constant

(P) high ;

$$P = \frac{\left\{\left[Fe(Phen)_3\right]\left[saccharin\right]_2\right\}_{organic}}{\left\{\left[Fe(Phen)_3\right]\left[saccharin\right]_2\right\}_{water}}$$

(ii) Saccharin is insoluble in carbon tetrachloride. This solvent is used to extract benzoates and sorbates from low calorie soft drinks and beverages prior determination of saccharin (169), i.e. to separate saccharin from the other food additives.

(iii) Carbon tetrachloride has low dielectric constant (2.2) compared with nitrobenzene (34.8). Solvents with high dielectric constant are able to extract the chelate ion-pair. The addition of carbon tetrachloride may reduce the dielectric nature in the organic phase system and therefore may reduce the extractability of the ion-pair in nitrobenzene.

(iv) The cation $\left[Fe(Phen)_3\right]^{2+}$ is not extractable into the organic solvent unless an anion part neutralizes the positive charge. However, it is very soluble in water. From these facts one can explain the effect of carbon tetrachloride addition. It causes dissociation of the ion-association system bringing the easily soluble cation $\left[Fe(Phen)_3\right]^{2+}$ in aqueous phase as shown below.

$$\left[Fe(Phen)_3\right]\left[saccharin\right]_2 \rightleftharpoons \left[Fe(Phen)_3\right]^{2+} + 2\left[saccharinate\right]^-$$

(Ion-association system in nitrobenzene) (Ferroin in aqueous phase)

It must be pointed out that, the back-extraction of $\left[Fe(Phen)_3\right]^{2+}$ is often a far slower process than the forward extraction. The addition of the carbon tetrachloride makes the back-extraction process fast and complete. Avoidance of the use of concentrated acids with the organic phase should be taken into account. Also the addition of alkalis is not recommended because of the precipitation of iron in the alkaline medium.

In order to overcome the problems associated with the use of the nitrobenzene, which have been described previously in this section; it was desirable to work with an aqueous solution rather than the organic phase. The determination of the iron content, using the AAS has been carried out from an aqueous phase avoiding the use of toxic nitrobenzene.

3.3.2.2 Analytical Characteristics of the Working Curves

Actually there is no need for the use of standard iron solution for a calibration curve. The only need is a calibration curve of varying amounts of saccharin solution, either extracted with $\left[Fe(Phen)_3\right]^{2+}$ into nitrobenzene or extracted and then stripped into aqueous solution. Sometimes the analyst might need to calibrate some instruments first, using two or three standards iron solutions as check samples.

The use of standard iron solutions in aqueous or organic phases

in this work was carried out in order to assess and determine the performance of the carbon furnace HGA-70 for this determination in terms of some statistical parameters such as range and standard deviation, and sensitivity of the method. These parameters were very useful in giving some ideas for the future work involving saccharin determination.

Iron has got several characteristic lines which could be used for absorption. They vary in relative sensitivity for 1 to 130. The most sensitive absorption is a line at wavelength 248.3 nm. The line at 372 nm is about 5.7 times less sensitive than the first one, but has a maximum precision and better signal-to-noise ratio could be attained (good baseline). The sensitivity reported for "1% absorption" for standard instruments is about 0.1μg/ml at the 248.3 nm line, in air-acetylene flame. The limit of detection is 0.005-0.02μg/ml. Sensitivity using the graphite furnace is reported to be 25 Pg and detection limit is 5 Pg. In this work the line 372 nm was preferable when using organic phase samples to reduce the background absorption. It has been pointed earlier, that, in general, the effect of background absorption is less common at wavelengths above 350-400 nm. This line is not very much less sensitive compared with the 248.3 nm line. However, in working with aqueous phase, the line 248.3 nm is considered to be convenient.

In the following section, the analytical characteristics of the working curves are discussed.

(a) Flame Working Curves

Fig. (3.10)

This plot shows the sensitivity of the two lines used. It is found that the line at 248.3 nm is about 6.4 more sensitive than the

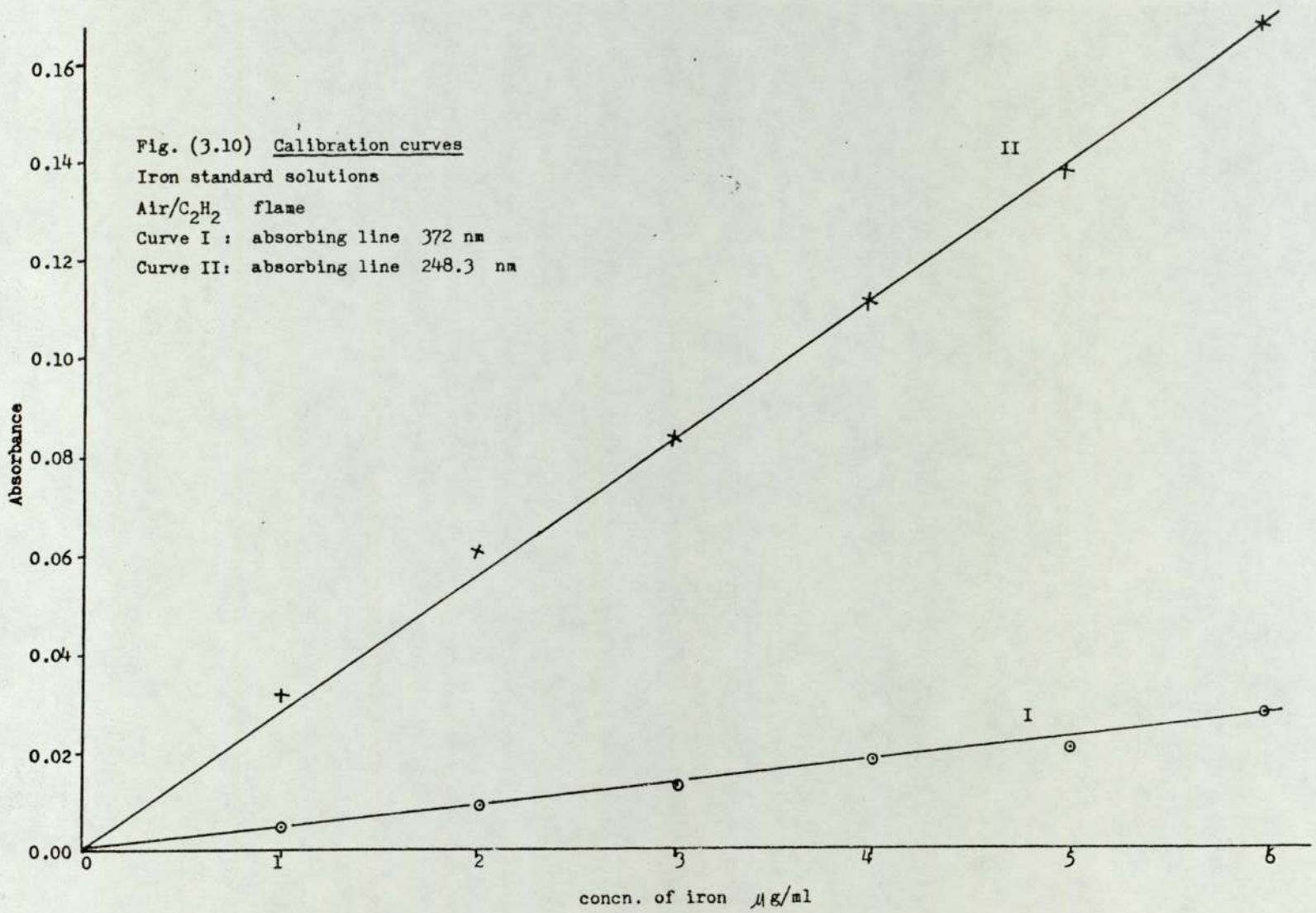


Fig. (3.10) Calibration curves
Iron standard solutions
Air/C_2H_2 flame
Curve I : absorbing line 372 nm
Curve II: absorbing line 248.3 nm

line at 372 nm. Standard iron solutions in water (in concentration range 1-7μg/ml), were used. The graph also shows the linearity of the calibration curve up to 6μg/ml. Air/acetylene flame was used, pressures were 6.5 l/min acetylene and 8.5 l/min air, slit width 3 (0.2 nm) and scale expansion (X1), chart speed 0.5 cm/min. The sensitivity in term of $C_{1\%}$ at line 248.3 nm is 0.16μg/ml of iron.

Fig. (3.11)

The effect of using the scale expansion on the absorbance of iron is shown in this graph. The same conditions were used as in obtaining the previous graph above.

Fig. (3.12)

This is a 'typical flame plot' obtained, by recording the signals response of the varying saccharin amounts in the intial aqueous phase using the 'stripping' technique. A set of standard saccharin solutions were extracted with $\left[Fe(Phen)_3\right]^{2+}$ into nitrobenzene and then the $\left[Fe(Phen)_3\right]^{2+}$ stripped back into aqueous solutions using intially the concentration range (0-$10^3\mu$M). Another set ten times more dilute than the previous solutions (0-100μM) were examined at lines 248.3 and 372 nm. The results showed that :

(i) the curves are linear within a wide range of saccharin concentration; up to (800μM),

(ii) sensitivity at line 248.3 nm is about 5 times higher than at line 372 nm. From the graph, the sensitivity in term of $C_{1\%}$ for 1% absorption, of the flame method for saccharin determination is found to be (11.88μM).

Fig. (3.11) Calibration curves

Iron standard solutions

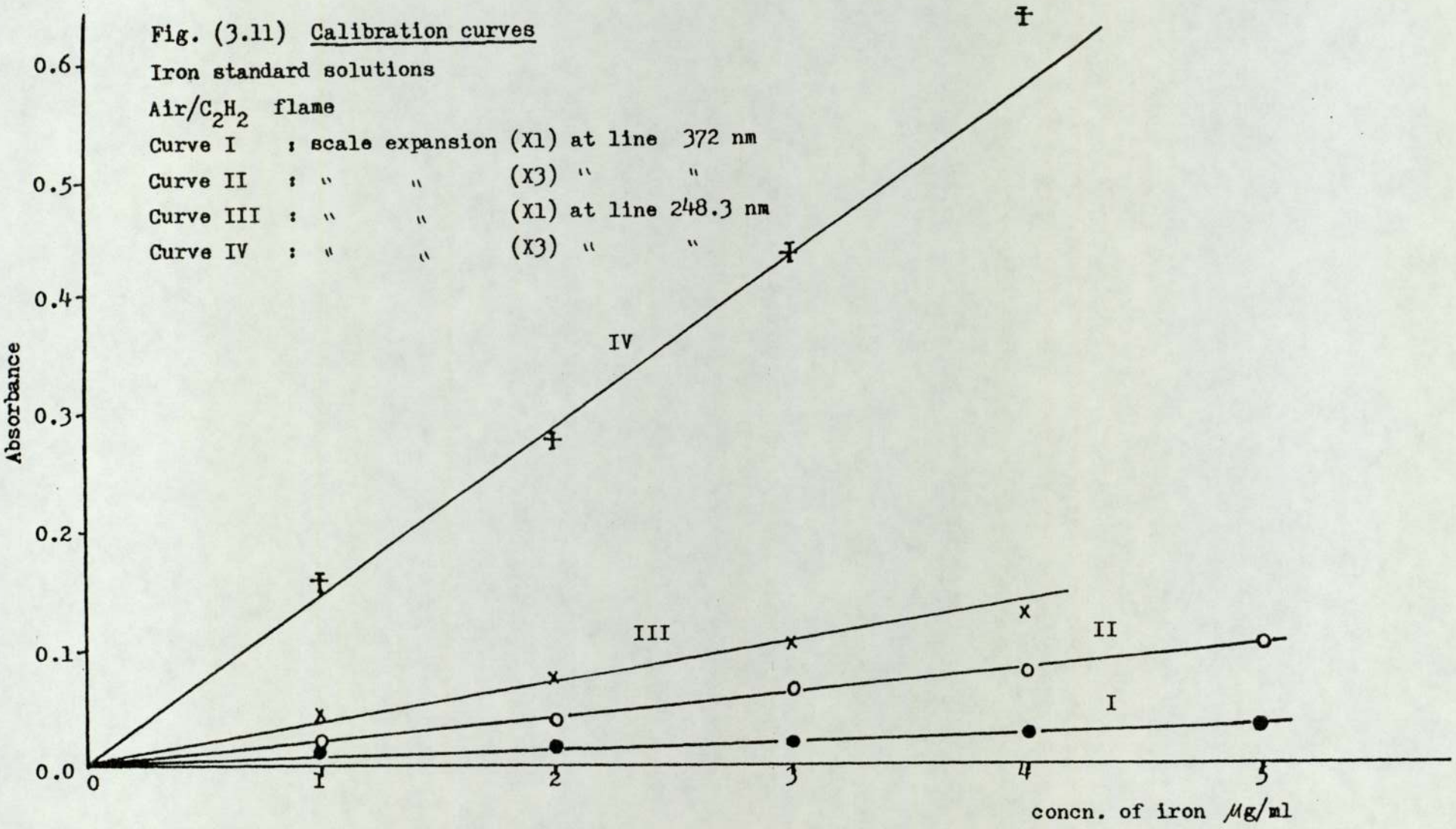
Air/C_2H_2 flame

Curve I : scale expansion (X1) at line 372 nm

Curve II : " " (X3) " "

Curve III : " " (X1) at line 248.3 nm

Curve IV : " " (X3) " "



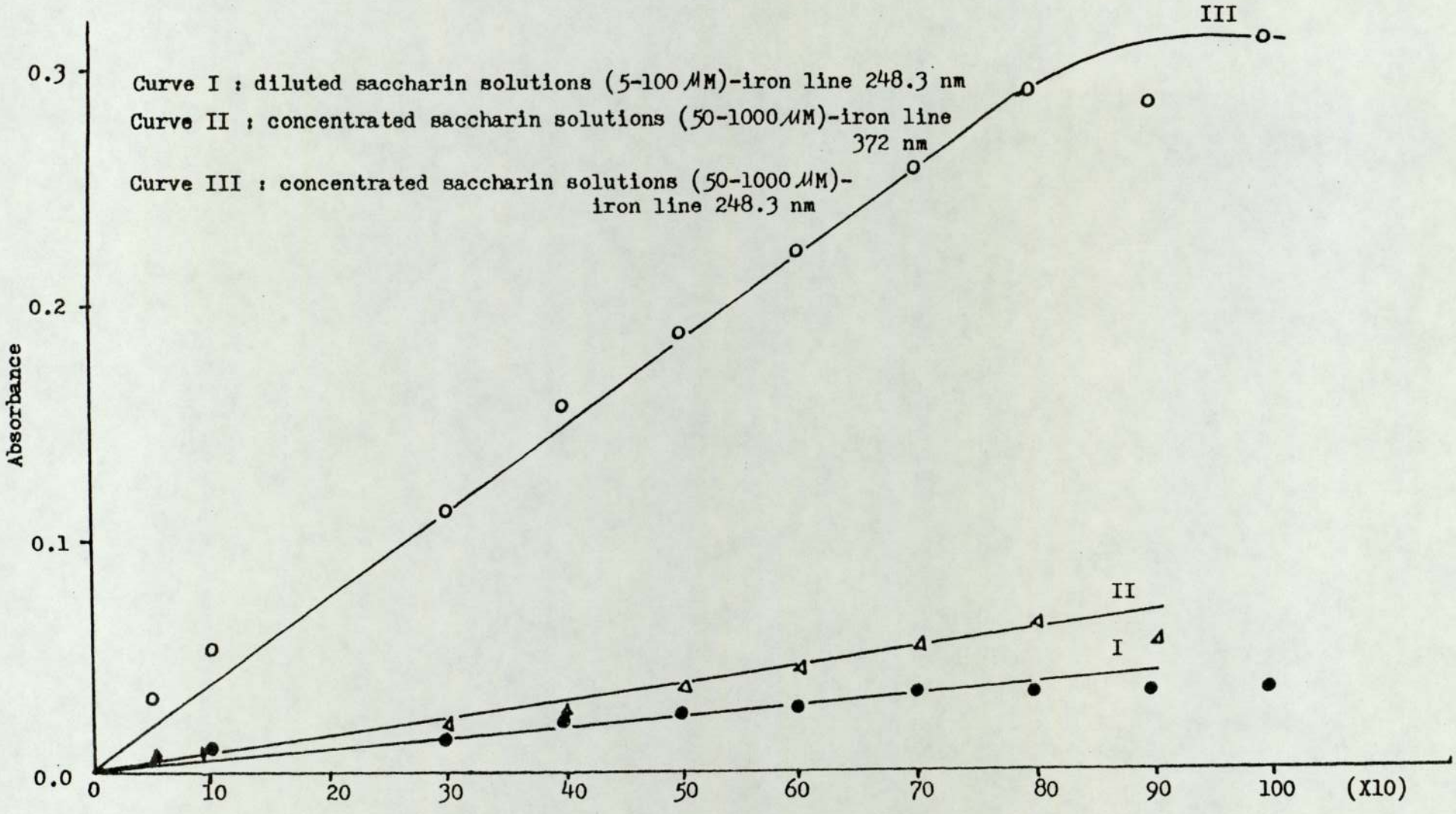


Fig. (3.12) Typical flame plot

Saccharin standard solutions (Stripping method)

Air/C_2H_2 flame

(b) Graphite Furnace Working Curves

Fig. (3.13) & Fig. (3.14)

Standard iron solutions have been used in the concentration range (0-1.0) μg/ml. Two lines were used 372 and 248.3 nm. Other conditions were :

	372 nm	248.3 nm		372 nm	248.3 nm
Slit width (nm)	0.2	0.2	Charring temperature(^{o}C)	230	230
Scale	X1	X1	Atomization "	2500	2500
Noise suppression	2	3	Drying time (second)	30	20
Argon pressure(lb/in^2)	19	19	Charring "	3	3
Sample aliquot(μl)	30	10	Atomization "	10	10
Chart speed(cm/min)	0.5	0.5			
Drying temperature(^{o}C)	100	100			

From these graphs the following points could be concluded :

(i) When using the line at 372 nm, a linear graph is more obtainable than using the line 248.3 nm which tends to be curved at the same range of iron concentration. The calibration graph for line 372 nm is linear up to 0.7 μg/ml of iron, which gives absorbance of less the 0.5 unit.

(ii) The points in the graphs are the mean value of a sets of readings in order to locate the nearest true values. The average 'Range', $\bar{R}$, as a measure of dispersion and the average 'Standard deviation' from the mean value, $\bar{S}$, were found to be :

	$\bar{R}$	$\bar{S}$
at line 372 nm,	0.027	0.016
at line 248.3 nm,	0.047	0.013

(iii) The sensitivity in term of $C_{1\%}$ was :

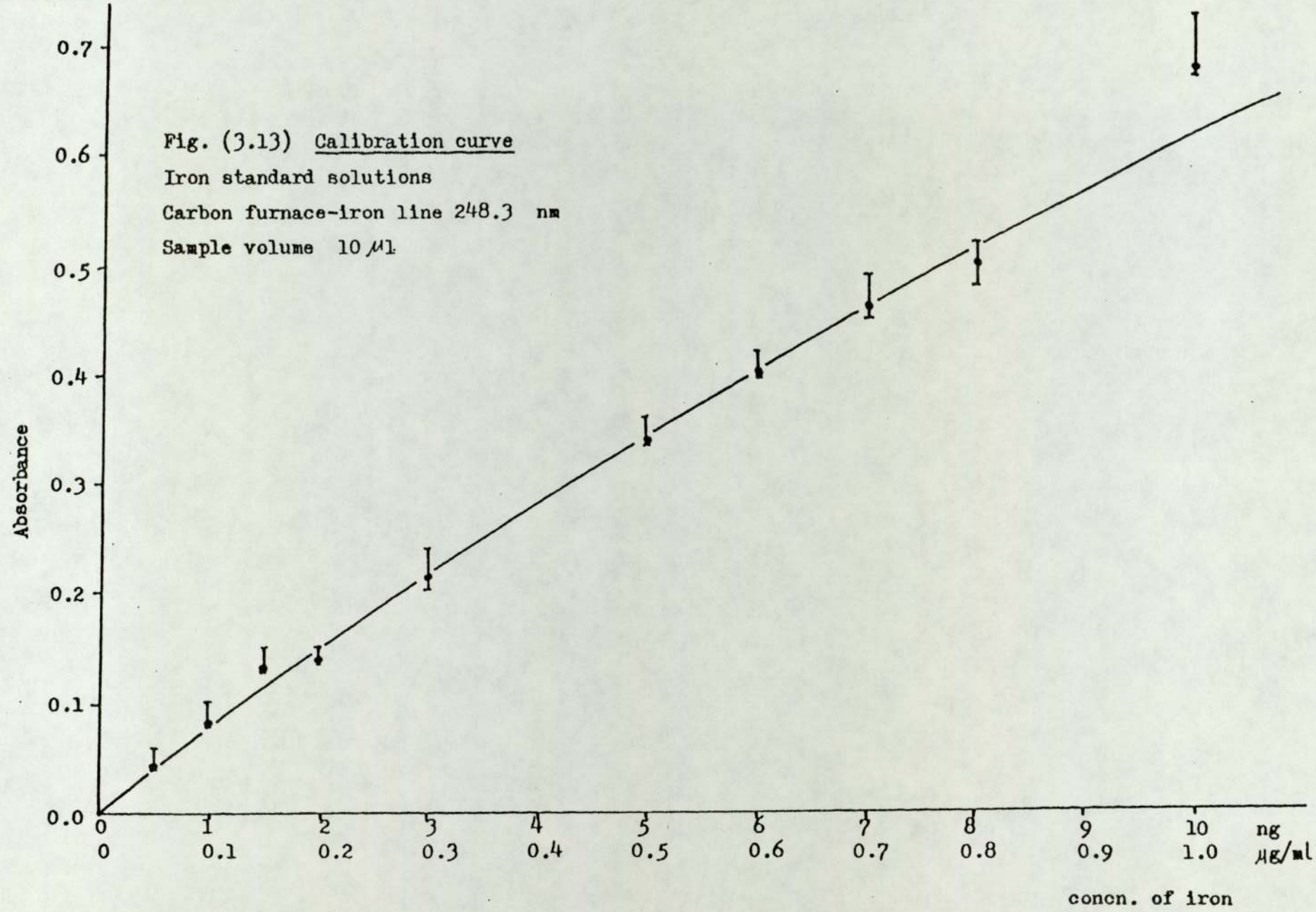


Fig. (3.13) Calibration curve

Iron standard solutions

Carbon furnace-iron line 248.3 nm

Sample volume 10 µl

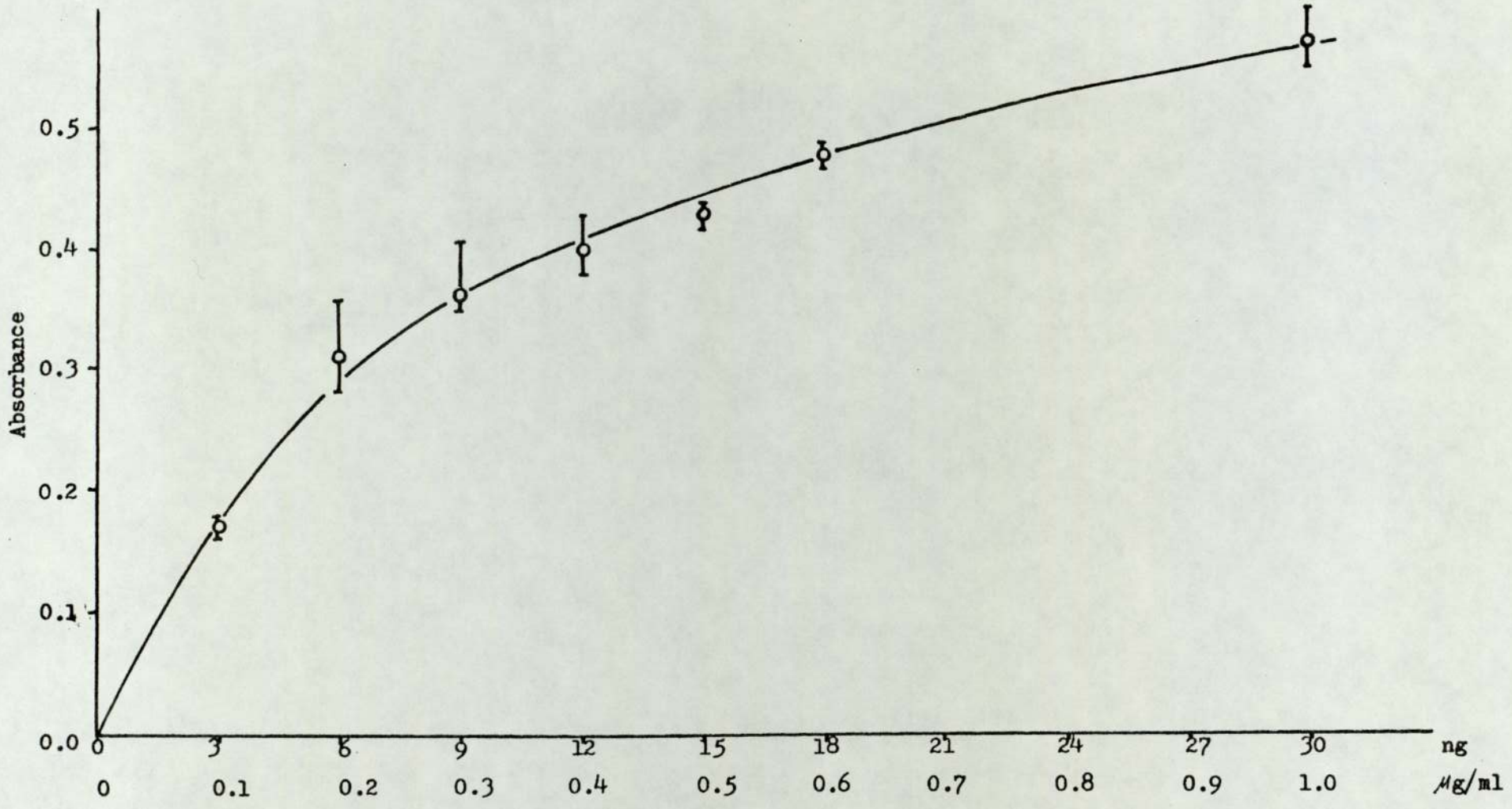


Fig. (3.14) Calibration curve

Iron standard solutions.

Carbon furnace - iron line 372 nm

Sample volume : 30 µl.

at line 372 nm, 0.006 μg/ml iron

at line 248.3 nm, 0.002 μg/ml iron ,

which is about 80 times more sensitive than the flame work.

From the above mentioned points, it is suggested that the absorbance at line 372 nm should be used for maximum precision.

Fig. (3.15)

This graph demonstrates the suitability of the nitrobenzene matrix with the carbon furnace for determination of saccharin. The matrix and molecular absorption effects could be minimized here by using the line 372 nm and high temperature and time programme as follows :

Drying temperature (^{o}C)	100
Charring "	1100
Atomization "	2500
Drying time (second)	60
Charring "	120
Atomization "	10

The thermal destruction of the nitrobenzene matrix is the critical stage in this programme. The higher charring temperature and longer charring time together with selection of the line 372 nm may be very useful in separating the atomization peak from other interfering signals arising from the smoke. The baseline was satisfactory when using these conditions. It may be preferable also to use a small volumes of nitrobenzene matrix injected into the carbon furnace. In this experiment, 5-μl of sample have been used. This volume of nitrobenzene matrix was found to be the optimum volume to be used. This is explained further in the next section (Fig. 3.16). Standard deviation

Fig. (3.15) Nitrobenzene matrix in carbon furnace

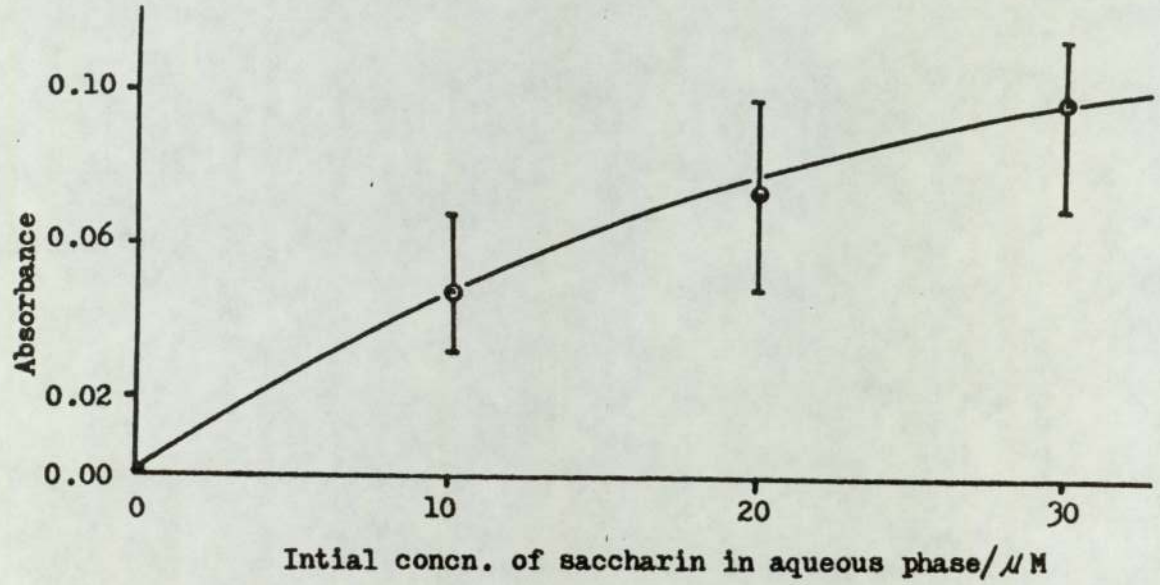
Saccharin standard solutions.

Iron line 372 nm

Sample volume : 5 μl

{ Tris(1,10-phenanthroline)Fe(II)-saccharin }

in nitrobenzene.



was found to be $\bar{S}$ = 0.016 and 'Range' $\bar{R}$ = 0.043 . The graph is curved even for readings under 0.1 value of absorbance. The non-linearty of the curve could be possible explained by the incomplete destruction of nitrobenzene in which some smoke is still formed during the atomization stage.

Fig. (3.16)

When using the HGA-70, the maximum capacity of the graphite tube is 100 μl for the aqueous matrix. The creeping effect of the solution, limits the organic solution volume to no more than 50 μl or sometimes less than 20 μl in some organic solutions.

Using the same conditions as in Fig. (3.15), the absorbance of iron content in the nitrobenzene matrix containing the ion-pair $\{Fe(Phen)_3\}\{saccharin\}_2$ was determined for varying sample aliquots. The plot for the calibration (using fixed concentrations and 30 μM saccharin in the intial aqueous phase) but with varying volumes of nitrobenzene matrix 2, 5, 10 and 15 μl indicates that :

(i) Smoke interference is taking place. This possibility of smoke formation due to nitrobenzene could be determined from the non-linearty relationship shown by the plot.

(ii) The average 'Range', $\bar{R}$, of the observations is found to be relatively very high indeed i.e. 0.1403 and 0.2294 absorbance units when 10 μl and 15 μl sample size respectively have been injected into the carbon furnace. But the 'Range' when using 2 μl and 5 μl volume size was found to be relatively low; 0.0492 and 0.0179 respectively. The standard deviation, $\bar{S}$, was found to increase with increasing of sample volume as 0.0068, 0.0573 and 0.0913 when using volumes 5 μl, 10 μl and 15 μl respectively, i.e. the amount of smoke is increasing with increase

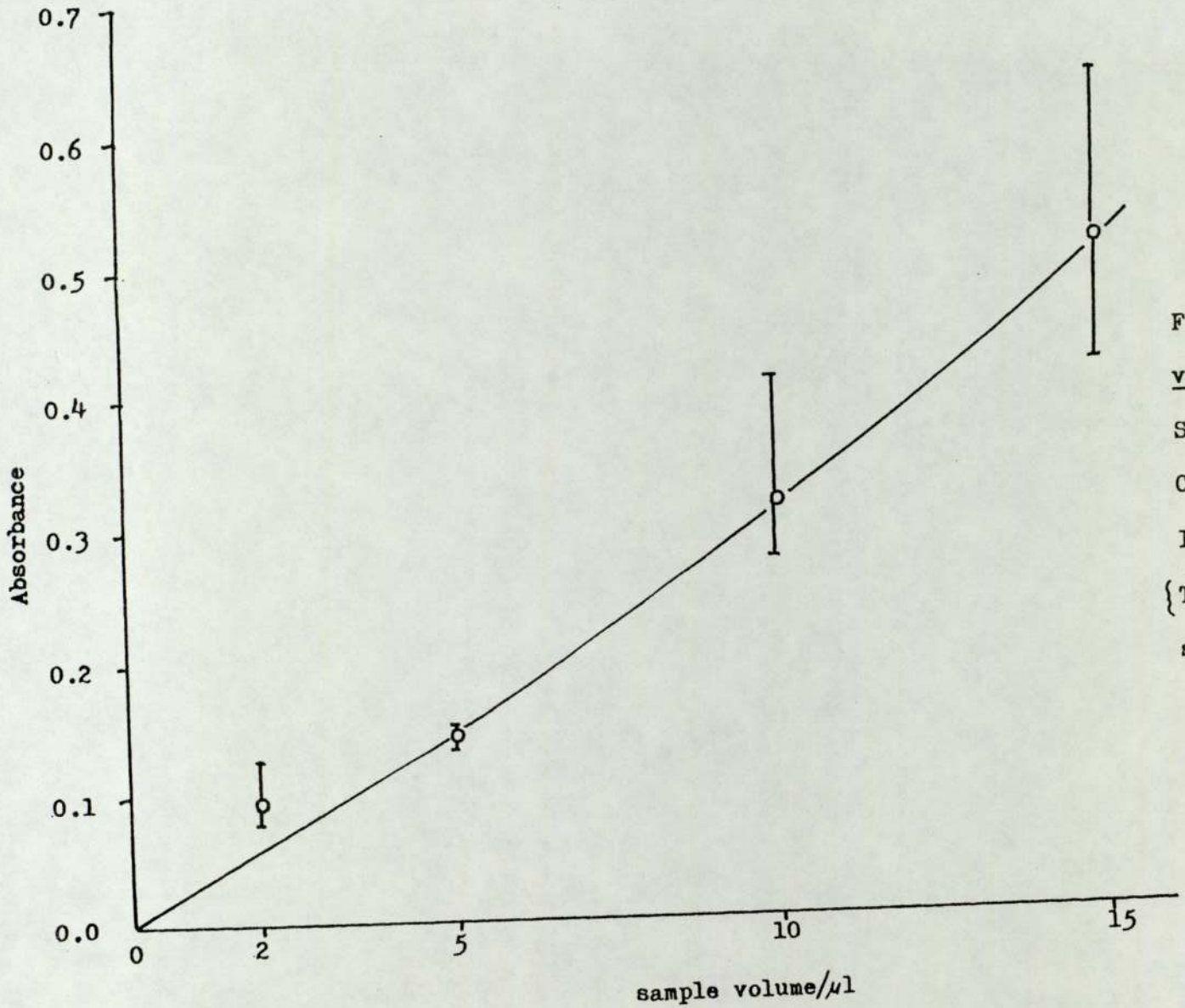


Fig. (3.16) Effect of nitrobenzene volume on absorbance

Saccharin concn. 30.0 µM

Carbon furnace

Iron line 372 nm

{Tris(1,10-phenanthroline)Fe(II)-saccharin} in nitrobenzene.

of the volume size and as expected the creeping effect is much more when using higher volumes. The relatively high results in the range and standard deviation when injecting 2 µl of sample 0.0492 and 0.0205 compared with 0.0179 and 0.0068 using 5 µl of sample may be explained as due to the formation of droplets which tend to remain behind on the inner walls of the plastic micropipette tips. This effect of course may give poor precision when using low volume size. Accordingly, it is suggested under these conditions, that volume size of nitrobenzene matrix more than 5 µl should not be used. Also volumes less than 5 µl are not advisable.

Fig. (3.17)

This is a calibration curve for iron standard solutions using the graphite furnace. The conditions which used in obtaining the graph in Fig. (3.14) have been modified in this experiment as below :

Drying temperature (°C)	100
Charring "	490
Atomization "	2500
Drying time (second)	10
Charring "	30
Atomization "	5
Sample size (µl)	20
Wavelength (nm)	372

The rest of instrumental parameter conditions were the same as in Fig. (3.14). Standard iron solutions in concentration range 0.1-0.6 µg/ml (using 20 µl of each solution) have been injected into the carbon furnace, i.e. the injected samples were contained from 2 to 12 ng iron.

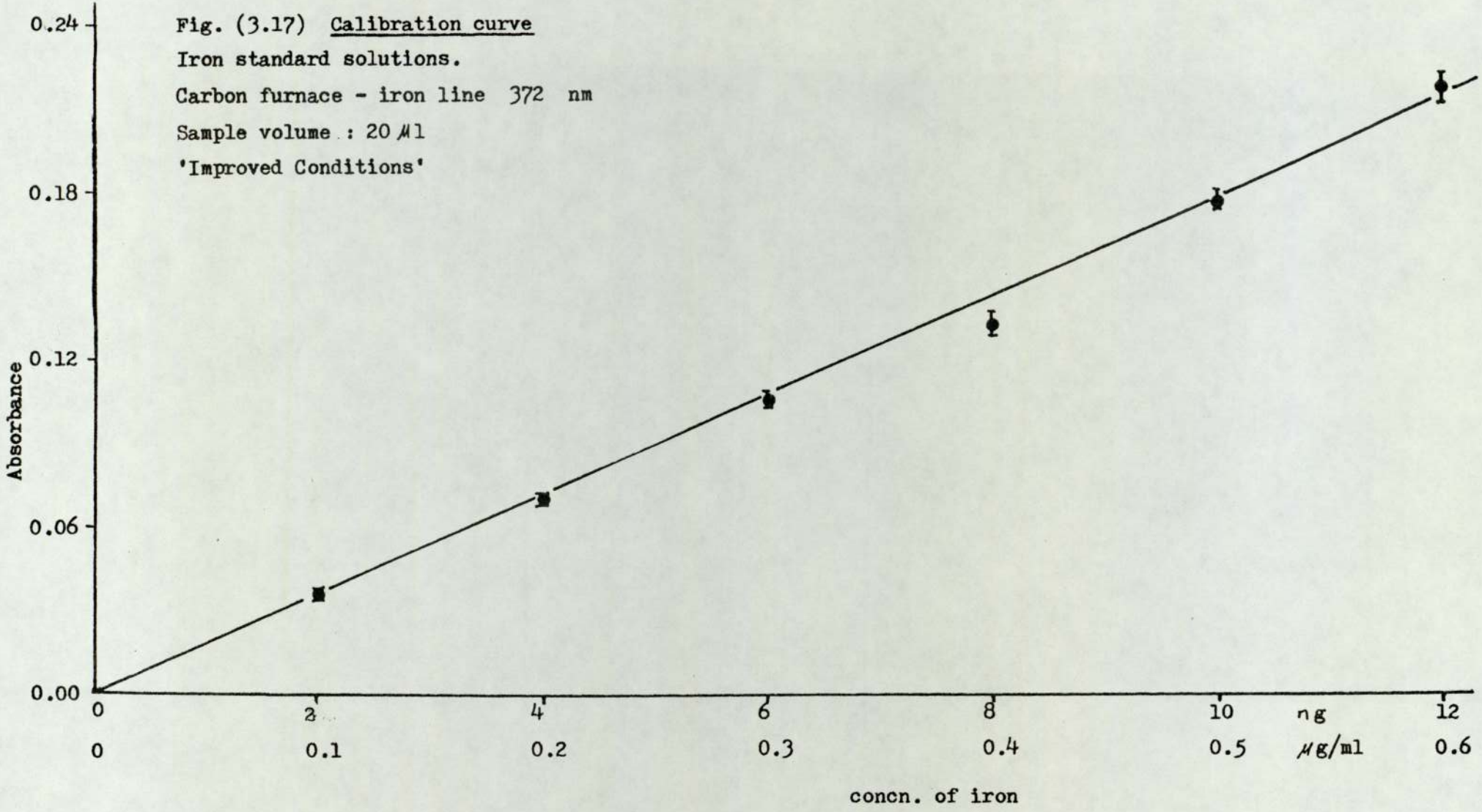
Fig. (3.17) Calibration curve

Iron standard solutions.

Carbon furnace - iron line 372 nm

Sample volume : 20 µl

'Improved Conditions'



The plot was obtained from an average of 5 measurements for each point. The calculated 'Average Range' was found to be $\bar{R}$ = 0.0061 which is much better than the previous conditions in Fig. (3.14) where $\bar{R}$ was 0.0272. The value $\bar{S}$ was found to be 0.0017 compared with $\bar{S}$ = 0.016 in the previous conditions.

Fig. (3.18)

This is a 'typical flameless plot' for the determination of saccharin using the stripping technique. It serves as a calibration curve using the same conditions mentioned with Fig. (3.17). The concentration range of saccharin intially present in the aqueous phase was (5-100μM). The saccharin was extracted first with $\left[Fe(Phen)_3 \right]^{2+}$ into nitrobenzene, followed by iron stripping into the aqueous phase using CCl_4. The curve was found to be linear up to concentrations giving an absorbance of 0.35 unit. This linearity range covers saccharin concentrations up to 60μM in the intial aqueous phase.

The precision and reproducibility were observed to be better in the linear range than in the curved part of the plot. The value $\bar{R}$ taken in concentration range up to 60μM was found to be 0.0175 and $\bar{S}$ was 0.0057. These values were found higher when using saccharin concentrations higher than 60μM in the intial aqueous phase; $\bar{R}$ = 0.0383 and $\bar{S}$ = 0.0122.

3.3.3 Conclusions-Potential and Feasibility of the Indirect Method

In general, the information available on the applications of the flameless AAS as an analytical technique is very limited compared to the wealth of information on the applications of flame AAS. In particular,

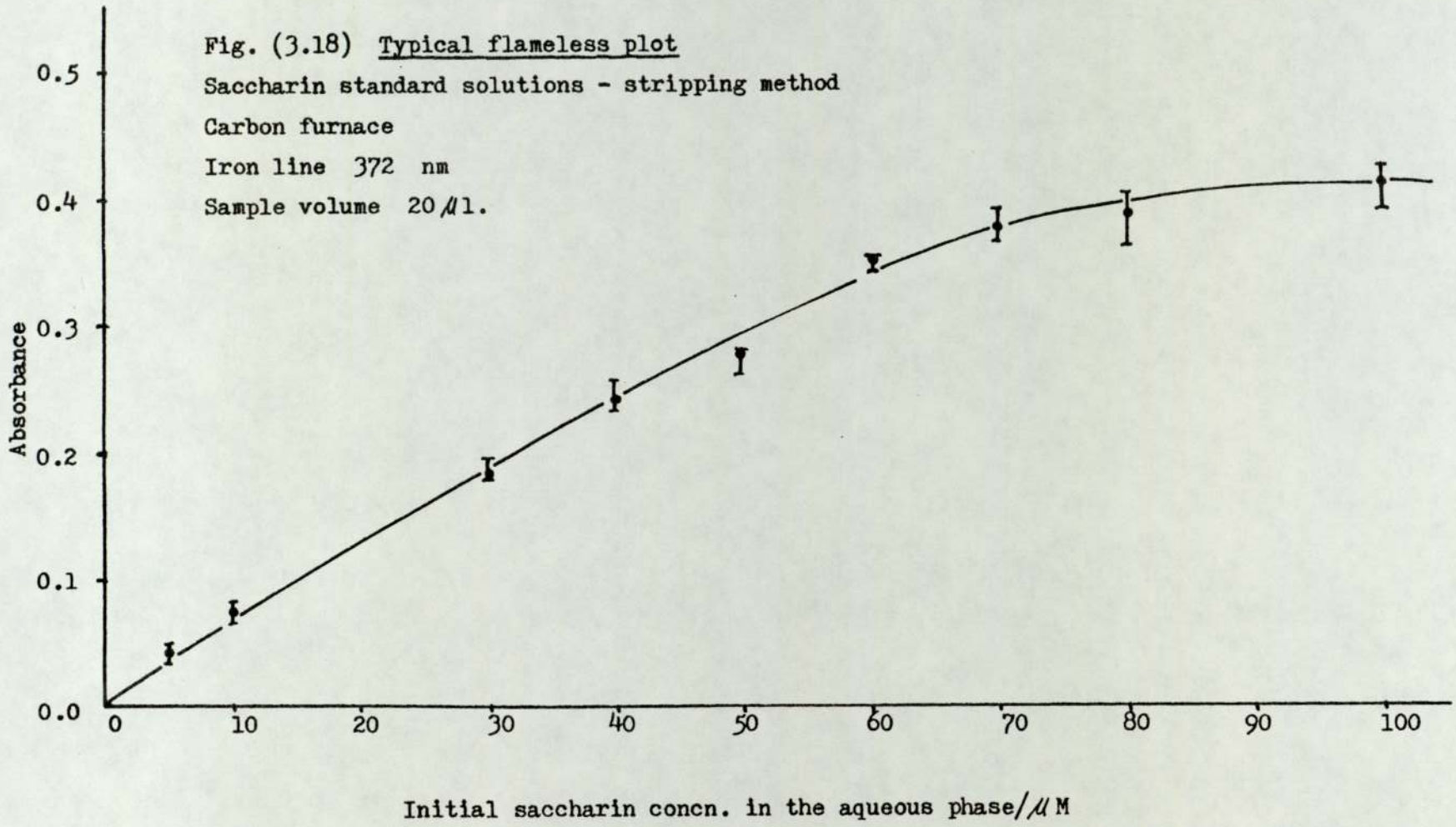
Fig. (3.18) Typical flameless plot

Saccharin standard solutions - stripping method

Carbon furnace

Iron line 372 nm

Sample volume 20 µl.



there is no information available on the application of nitrobenzene matrix within the carbon furnace. Because of this, an investigation involving this solvent was initiated. However, some of the problems associated with this work have been defined together with some practical observations which may be useful in subsequent work.

The Perkin-Elmer HGA-70 has been used in this investigation. It is an early design introduced in 1970, based mainly on the work of Massmann. However, using this old piece of equipment, the sensitivity of the method in terms of ($C_{1\%}$) was found to be 0.748 μM saccharin using iron line 372 nm. This sensitivity was found to be 11.88 μM saccharin using flame technique at iron line 248.3 nm. A mention has been made elsewhere in this chapter that sensitivity for iron determination using line 248.3 nm is 6.4 more sensitive than line 372 nm. Also, sensitivity for iron at 372 nm using flameless technique was 80 times more sensitive than using flame. From these results, one can conclude that saccharin can be determined using the flameless technique with high sensitivity, providing that a more modern instrument is used.

Some alterations could be made in the solvent-extraction system for instance; less volume of nitrobenzene. The nitrobenzene matrix may be used, and the 248.3 nm iron line (which is more sensitive), providing an automatic background corrector is available.

On the other hand , the 'stripping method' may offer some advantages, particularly in superior precision of measurements as it has been discussed previously. It may also reduce the time of analysis, lengthen the useful life time for the graphite tube and subsequently, reduce the cost of analysis by using lower temperatures for less time. The back-extraction may also be used as a more concentration process,

i.e. after extraction of saccharin with 'ferroin' into 5 ml nitrobenzene, iron content may be stripped back into 1.0 ml aqueous solution (water) after addition of suitable amount of carbon tetrachloride. This is a suitable volume to use in the carbon furnace technique where up to 30 μl can be injected with good precision.

Some of the disadvantages of that early design of carbon furnace have been recognised. The temperature control unit is limited to 2-pre-set dry stages which give temperatures 60°C and 100°C; and 5-pre-set ash stages of temperatures 230, 330, 490, 750 and 1100°C.

Although the drying stage is known as the least important stage, it may play a useful role when using an organic solvent such as nitrobenzene. A temperature just below the boiling point of the solvent is required in this low-temperature heating cycle, in order to evaporate any solvents. However, the boiling point of nitrobenzene is 221°C and the drying temperature used 100°C. Also, the ash stage unit using HGA-70 may limit the choice of the optimum temperature.

The limitations also were found with the design of the graphite tube which is of 51 mm length by 8.6 mm internal diameter. This tube has no shallow groove at the centre of the inside surface as in the improved design with other models. This groove is necessary to retain the organic solvent and prevent spreeding effect to take place. The design of the tube has been modified several times since 1970, and is now provided with grooves inside, either in centre or ends of the tube. Pyrolytic coatings are also applied to extend tube life.

Sometimes, when changing the graphite tube, the cones at each end are not well aligned. This causes some corrosion on the edge of the tube and giving a poor electrical contact. This may change the

atomization temperature resulting in poor reproducibility of the determination. This problem is not present with the more modern equipment. where automatic changing of the tube is performing.

The 'stripping method' may be used when using flame technique in order to avoid spraying nitrobenzene into the flame, because of its toxicity and its low nebulization rate. However, the only chemical justification is that, nitrobenzene is a good solvent for extracting the ion-pair formed between ferroin and saccharinate anion.

Finally, purification of nitrobenzene is highly recommended particularly when using the carbon furnace. Centrifuging of the aqueous matrix after stripping may performed to remove the traces of nitrobenzene whcih can cause errors.

CHAPTER FOUR

A NEW METHOD FOR THE DETERMINATION OF SACCHARIN BY DESORPTION OF FERROIN FROM SILICA GEL

4.1 Introduction

4.1.1 Adsorption Chromatography

Adsorption chromatography is today one of the most significant and broadly used techniques available in the chemical analysis. It is considered to be a powerful separation method for various organic mixtures. Its wide applicability in analysis is due to several advantages such as : simplicity of the technique, apparatus and interpretation; great versatitlity and high performance. Many of the adsorbents used in this technique possess ion exchange capability. In these cases, the mixed 'adsorption-ion exchange' systems are much more complex than the 'pure adsorption' systems.

The adsorption chromatography is well known technique and need not to be discussed here.

Two types of adsorption are commonly recognised physical and chemisorption. The first kind of adsorption is similar to phase transformation or mixing processes such as vaporization, melting and dissolution. Normally, the energies involved in this adsorption are small and adsorption and desorption are rapid. The adsorption forces in this kind are Van Der Waals forces which hold nonionic molecules together in the liquid or solid state. In chemisorption an actual covalent or ionic bond is formed between adsorbing molecules and the adsorbent surface. The energy involved here is generally large (i.e. similar in magnitude to bond formation in chemical reaction). The adsorption and desorption processes are slow i.e. a considerable activation energy is involved for adsorption.

In general, an adsorbed molecule X is subjected to interactions on three sides : interactions with the surface of the adsorbent or a surface site, interactions with molecules Y in the adsorbed monolayer and interactions with adjacent molecules Z in the unadsorbed phase. The adsorbate-adsorbent interactions are considered to be of primary importance in the adsorption of sample molecule X. Within these interactions one may define several types of adsorption forces : (i) dispersion or London forces, (ii) induction forces, (iii) electrostatic forces, (iv) hydrogen bonding, (v) charge transfer and (vi) covalent bonding or ion-exchange (chemisorption).

Hydrogen bonding forces have an important role in the adsorption energy of many chromatographic systems. The surfaces of polar adsorbents such as silica and alumina are covered with hydroxyl groups which hydrogen bond to basic or weakly basic adsorbates such as ethers, nitriles, aromatic hydrocarbons. Also, adsorbates with hydrogen donor properties should be capable of hydrogen bonding with proton acceptor groups on the adsorbent surface.

Each adsorbent has a limited adsorption capacity. This capacity can be defined as 'maximum amount of sample which the bed can adsorb from the non-adsorbed phase' (i.e., complete monolayer coverage). It defines the role of the adsorbent in determining the optimum adsorbent/sample ratio. Bed capacity in grams of sample per gram of adsorbent is equal approximately to 0.0003 times the adsorbent surface area (square meters per gram), minus the quantity of water (grams per gram) used to deactivate the adsorbent (170).

$$C = 0.0003A - W \qquad (4.1)$$

where,

C = bed capacity in grams of sample per gram of adsorbent,

A = adsorbent surface area in m^2/g,

W = amount of water used to deactivate the adsorbent in g/g.

Since all the results obtained in this chapter may be interpreted on the basis of the chemistry of silica gel, a short account will first be given of the properties of silica gel.

4.1.2 Properties of Silica Gel

(1) General Chemistry of Silica

At the present time silica is the most widely used chromatographic adsorbent. Adsorbents with the empirical formula $SiO_2 . XH_2O$, described in various terms as silica, silica gel and silicic acid. In this hydrated species, the water is chemically bound in a non-stoichiometric amount. The term silica also includes substances with stoichiometric composition SiO_2. Chromatographic silicas are in the form of amorphous porous solids which can be prepared in a wide range of surface areas and average pore diameters. Variation of solution pH during the acid gelation of sodium silicate yields silicas with surface areas varying from about 200 ($pH \simeq 10$) to 800 ($pH \leqslant 4$) m^2/g.

Several silica derivatives have been prepared by means of surface reactions with an suitable modifier involving organofunctional groups. These groups are chemically bonded at the surface of silica. The surface modification causes an increase in weight sometimes, by up to 30%.

The bulk structure of all forms of silica contain the Si-O

bond, which is the most stable of all Si-X element bonds. The Si-O bond length is shorter (0.162 nm) than the sum of the covalent radii of silicon and oxygen atoms (0.191 nm) (171). This is responsible for the relatively high stability of the siloxane bond. A tetrahedral unit $[SiO_4]^{4-}$ is formed in which each silicon atom is surrounded by four oxygen atoms. The structure of silica gel as shown in fig. (4.1) consists of a network of joined SiO_4-tetrahedrons, where the oxygen atoms are only partially required to make up the Si-O-Si bridges (172). The rest of the oxygen atoms are free to bind ions from the solution out the silica gel.

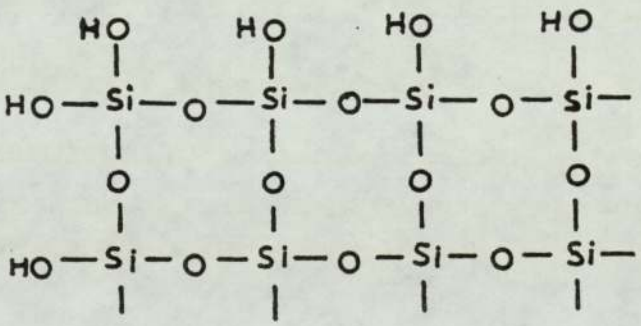
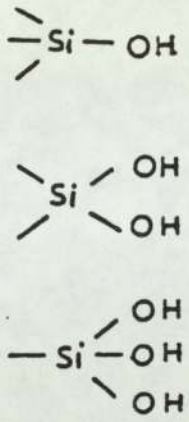


Fig. (4.1) The Structure of Silica Gel.

(2) The Surface Structure of Silica

Considering the surface of silica, one can distinguish two main surface species (173); (a) surface hydroxyl groups, which can be subdivided into different types according to their coordination to the silicon atoms (see figures 4.2 and 4.3) and (b) siloxane groups, formed by dehydroxylation of hydroxyl groups (figure 4.4).

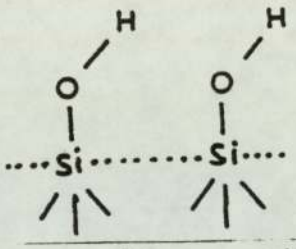


(a) hydroxyl or silanol groups

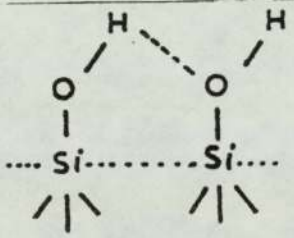
(b) silanediol groups

(c) silanetriol groups

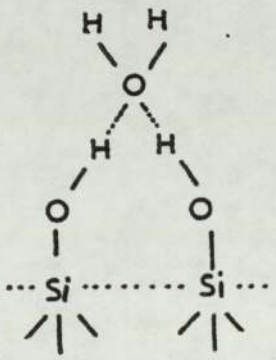
Fig. (4.2) Different types of silanol groups.



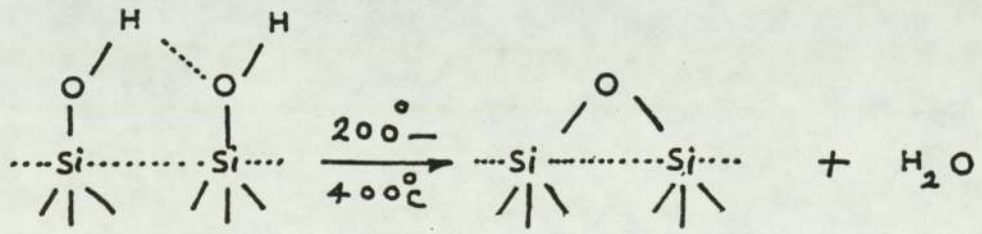
isolated or free hydroxyl groups



vicinal hydroxyl groups (Reactive)



hydroxyl groups bonded to water by hydrogen bonding.



(siloxane)

Fig. (4.3) Arrangement of hydroxyl groups on silica surface.

The amount of physically adsorbed water has to be considered, since this affects the strength of adsorption interaction between the hydroxyl groups and the adsorbate.

The surface of hydrated silica is covered with hydroxyl groups that are attached in various ways to silicon atoms. These hydroxyl groups are involved in a temperature-dependent dehydroxylation-hydroxylation reaction which may be shown as

$$2 \equiv Si\text{-}OH \rightleftharpoons \equiv Si\text{-}O\text{-}Si\equiv + H_2O \qquad (4.2)$$

The total water content of silica is of two types, physically and chemisorbed water, and it is possible to distinguish quantitatively between both.

Physically adsorbed water may be removed at 120°C. When heating the silica surface at the point where adsorbed water is lost, certain surface hydroxyl reactions take place (170). The reactions shown in fig. (4.4) demonstrate that, (i) when heating silica surface at 200°C, dehydration of amorphous silica will be accompanied by dehydroxylation of vicinal hydroxyls. Condensation of reactive (and geminal) hydroxyls takes place to give water and leave siloxane groups, (ii) the condensation proceeds with increasing temperature (>200°C); free hydroxyls begin to migrate about the surface, to form transient reactive groups and then decompose to surface siloxane groups, (iii) at higher temperature, adjacent adsorbent planes also begin to condense, with loss of water, to give particle-particle fusion and permanent loss of surface. Above 500°C, the vicinal hydroxyl groups are completely condensed. The hydroxylation-dehydroxylation process is reversible up

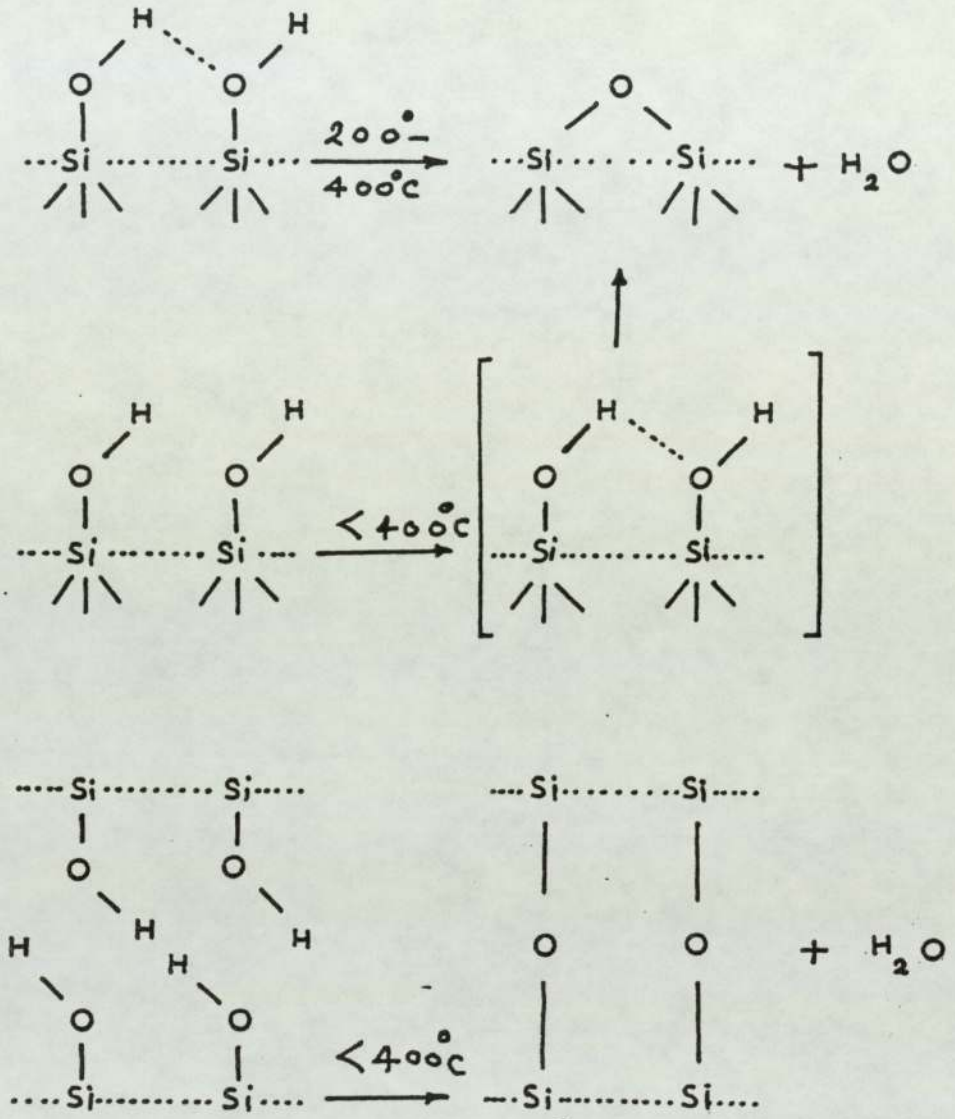


Fig. (4.4) Reactions of hydroxyls on the silica surface.

(i) condensation of reactive hydroxyls.

(ii) " of free "

(iii) " of surface.

to this temperature. It is worth pointing out that the siloxane groups formed are less polar than hydroxyl groups.

The re-addition of water to a dry or partially dehydroxylated silica normally results in adsorption of molecular water upon surface hydroxyl groups. There is a higher possibility of forming multiple hydrogen bonds between reactive (and bound) hydroxyl and adsorbed water molecules. In this hydroxylation process, the dehydroxylated silica surface adsorbs, first, water molecules and secondly, siloxane bonds are cleaved to form hydroxyl groups.

(3) Silica-Water Interactions

The interaction between silica and water is of great importance in understanding the surface chemistry of silica. Three factors involved in this interaction are worth mentioning : (a) dissolution of silica, (b) the stability of the pore structure of silica when it is brought into contact with water, and (c) the ion-exchange properties of silica (a phenomena of the silica surface).

The solubility of silica is a function of a number of factors such as pressure, temperature, structure, particle size and pH of the aqueous solution. As was pointed out in the previous section, the first reaction step between anhydrous silica and water or water vapour is the hydroxylation of the surface layer to form hydroxyl groups. Hydroxylation (hydrolysis) involves cleavage of siloxane bonds by water to give a water-soluble species, monosilicic acid :

$$SiO_2 + 2H_2O \rightleftharpoons Si(OH)_4 \qquad (4.3)$$

The photometric determination of silicon (175) indicates that

the amount of soluble silica is very low (100 ppm for amorphous silica in pure water at room temperature). The solubility of amorphous silica increases linearly with temperature. Hydrolysis accelerates with increasing pH, and the rate of hydrolysis is also influenced by electrolytes and by impurities within the solid silica. The soluble silica remains nearly constant in the pH range 1-9 , but a considerable increase in the solubility is observed when the pH exceeds 9. This is due to the formation of silicate ions in addition of monosilicic acid :

$$SiO_2 + 2H_2O \underset{}{\overset{+OH^-}{\rightleftharpoons}} Si(OH)_4 \qquad (4.4)$$

$$Si(OH)_4 + OH^- \rightleftharpoons [Si(OH)_5]^- \qquad (4.5)$$

Above pH 10.7, silica dissolves mainly in the form of soluble silicates, and the concentration of monosilicic acid simultaneously decreases sharply.

There are some factors affecting the pore structure. These are pH of the aqueous solution, the electrolyte concentration and the temperature.

One of the most significant features for the silica gel is its capacity to act as an ion exchanger. When silica is covered with neutral de-salted water, the pH of the resulting suspension is about 5. This is due to the acidic nature of silica (presence of weakly acidic surface hydroxyl groups). The de-protonation of monosilanol groups may be represented by the following equation :

$$\equiv Si\text{-}OH + H_2O \rightleftharpoons \equiv Si\text{-}O^- + H_3O^+ \qquad (4.6)$$

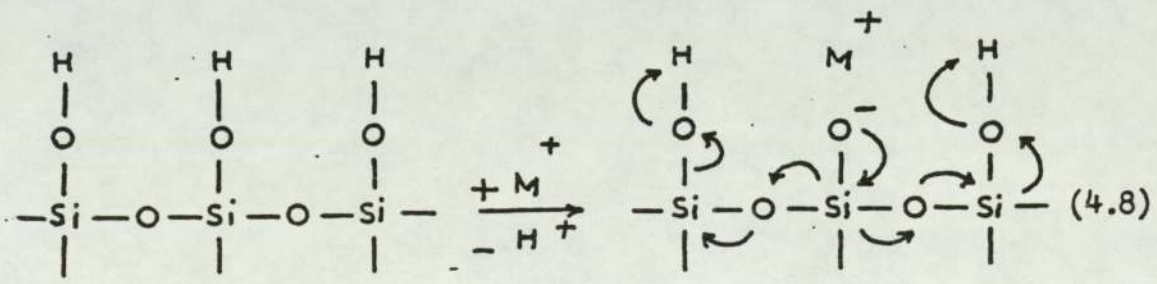
In electrolyte solutions the hydronium ions are exchangeable even in acidic media by cations and in particular by ions that readily coordinate with the oxygen ion to form silicate-metal complexes.

Concerning equation (4.6) at different pH's; the equilibrium shifts to the right-hand side when pH is decreased. At a pH of about 2 the surface groups of silica are completely undissociated (isoelectric state), i.e. the surface is electrically neutral in aqueous solution. Due to this, silica may act as an anion exchanger at lower pH values. On the other hand, in strongly basic solution (pH>9) the silanol groups become increasingly deprotonated and in the presence of metal cations, silicates are formed.

The environment surrounding the silica gel affects the tendency for splitting off a proton from a particular surface silanol group. With regard to the ion-exchange sorption of metal ions M^{n+}, the following exchange reaction takes place :

$$\equiv Si\text{-}OH + M^{n+} \rightleftharpoons \equiv SiOM^{(n-1)+} + H^{+} \qquad (4.7)$$

The following illustration shows the tendency to split off the proton in the presence of the metal ion :



(4.8)

Depending upon the pH, silica acts as anion exchanger (at sufficiently low pH) or as cation exchanger (at higher pH) (177).

The ion-exchange sorption of metal complexes is discussed in section 4.1.3.

(4) Chemical Modification of the Silica Surface

Chemical modification is a process that leads to change in the chemical composition of the surface. It must be pointed out that hydroxylation, dehydroxylation and dissociation of silica surface species in aqueous solutions are involved in surface chemistry of silica. Chemical modification involves a covalent bonding of functional groups to the surface as a result of a chemical reaction between the surface species and a reactant. A large number of chemically modified silicas have been developed in the field of chromatography in which they are specific adsorbents that differ in the type of functional group and in surface polarity. Chemical modification offers some required features in those specific adsorbents, i.e. change in adsorption behaviour or introducing a particular functional groups to cover the surface.

4.1.3 Sorption of Metal Complexes of 1,10-Phenanthroline and Related Compounds on Silica Gel and its Analytical Application and Potential

In the previous section, the ion-exchange sorption of a metal ions has been represented by an equation (4.7). Kohlschuetter and co-workers (178) considered the possibility of hydrolysis during ion exchange. They suggested a three-step mechanism (hydrolytic sorption), as follows :

(a) Hydrolysis

$$M^{n+} + H_2O \rightleftharpoons M(OH)^{(n-1)+} + H^+ \qquad (4.9)$$

(b) Adsorption

$$\equiv Si\text{-}OH + M(OH)^{(n-1)+} \rightleftharpoons \equiv Si\text{-}OH/(OH)M^{(n-1)+} \qquad (4.10)$$

(c) Condensation

$$\equiv Si\text{-}OH/(OH)M^{(n-1)+} \rightleftharpoons SiOM^{(n-1)+} + H_2O \qquad (4.11)$$

The above three equations lead to equation (4.7), which it may written schematically as :

$$n\ HG + M^{n+} \rightleftharpoons MG_n + n\ H^+ \qquad (4.12)$$

Where G is the unreacted gel frame work with its attached functional groups.

Vydra and Stara (179) have represented the ion-exchange sorption of a metal complex on silica according to the equation :

$$m(\equiv Si\text{-}OH) + ML^{n+} \rightleftharpoons \left[(\equiv SiO)_m ML\right]^{(n-m)+} + m\ H^+ \qquad (4.13)$$

where L = ligand.

This mechanism was demonstrated for ethylenediamine complexes of Ag^+, Zn^{2+} Cu^{2+}, Co^{3+} and Co^{2+} (180), for the tris(1,10-phenanthroline)-complexes

of Fe^{2+}, Co^{2+} and Zn^{2+} (181) and for the ammine complex of Co^{3+} (182).

Metal complexes of 1,10-phenanthroline (2,2'-bipyridyl, etc), which are of cationic nature $\left[M(Phen)_m\right]^{n+}$ are very strongly sorbed on silica. The optimum conditions for the quantitative sorption of μg- or tenth μg- amounts of the phenanthroline complexes $\left[Fe\ (Phen)_3\right]^{2+}$ and $\left[Co\ (Phen)_3\right]^{2+}$ on silica gel has been reported by Vydra and Markova (176).

The desorption of these complexes is carried out in 10-20 % aqueous alkaline solution of potassium iodide, or by a saturated methanolic solution of the same reagent. The desorption process is caused by the formation of neutral ion-association complexes such as $\left[Fe\ (Phen)_3^{2+}\ ;\ 2I^-\right]$ and $\left[Co\ (Phen)_3^{2+}\ ;\ 2I^-\right]$. The authors utilised these properties for the development of a method for the determination of traces of iron in nickel, chromium, molybdenum and tungsten prior to the spectrophotometric determination of iron as ferroin.

Vydra and Markova(176,182&183)have observed that the complexes of 1,10-phenanthroline with metals, including iron(II), which are sorbed on silica can be easily desorbed in the form of their complexes $\left[Fe\ (Phen)_3^{2+}\ ;\ 2X^-\right]$, where X is an anion of certain monobasic acids, e.g. monochloroacetic, trichloroacetic, acetic or formic acid.

The sorption of ferroin on silica can be employed as a means of concentrating and determining traces of iron. Bozhevol'nov and Karakovskaya (184) have estimated the iron contents as low as 10^{-7}% by comparison of heights of colour in identical silica columns. The method is based upon treatment of unknown and standard samples of iron with 1,10-phenanthroline to give ferroin at pH 5.5-6.0. Each solution is passed through a separate silica column. The height of the orange-red colour in all columns were compared.

Ferroin has been employed as a clean-up reagent for the extraction of saccharin from food products (174) and saccharin may be determined by spectrophotometric method as it has been discussed in chapter three.

4.1.4 Modified-Surface Silica Matrix for a New Indirect Method-The Present Work

Recently, in the present work, saccharin has been found to desorb ferroin sorbed on silica gel in a quantitative manner.

The desorption is caused by the formation of ion-association system $\left\{(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right\}$, and the amount of ferrion desorbed is one half of a mole fraction of saccharin in solution. This finding has been very useful in the present work and has led to the development of a new rapid method for saccharin determination. The preliminary results obtained from this work have led also to the development of new chemically modified-surface silica matrix.

In the present work, 'ferroin' has been employed as a 'modifier' for the surface of silica gel. The fact that silica gel will function as a weakly acid ion-exchanger and adsorb the metal complexes of cationic nature such as $Fe\ (Phen)_3^{2+}$, have been utilised according to the previous mentioned equation (4.13). The exchange reaction of this chemical modification process can be written schematically as follows :

$$2\ (\equiv Si\text{-}OH) + (Fe\ Phen_3)^{2+} \rightleftharpoons \underset{(Matrix)}{\left[(Fe\ Phen_3)(SiO)_2\right]} + 2H^+$$

(4.14)

The product 'ferroin-impregnated silica' , $\left[(Fe\ Phen_3)(SiO)_2\right]$, is provided as a dried matrix for use. Two techniques have been used for drying the physically adsorbed water; one is a thermal method using the oven for several hours at 120°C and other technique involves washing the matrix with ethanol and drying under vacuum. A stock modified-surface silica prepared by the latter method is subsequently referred to a "dried orange matrix".

When 1-g of this ferroin-impregnated silica matrix is brought into contact with saccharin solution and after shaking for a few minutes, desorption takes place in which the absorbance of desorbed ferroin on silica may be measured in solution using the spectrophotometric method ($\lambda_{max.}$ 510 nm) or by atomic absorption spectrophotometry using the iron lamp and measuring the iron content in solutions.

The possible mechanism for the desorption process is that an ion-association system is formed between saccharin solution and ferroin species on the surface of silica gel according to the equation below.

$$\left[(Fe\ Phen_3)(SiO)_2\right] + 2\ \text{saccharin} \rightleftharpoons \underbrace{\left[(Fe\ Phen_3)^{2+};\ (\text{saccharin})_2^-\right]}_{\text{(Ion-Pair)}} + 2\ \equiv SiOH$$

(4.15)

The presence of organic solvents such as ethanol, methanol and acetone in the medium has shown some interesting results; these have been examined in detail and are discussed in the experimental section.

A thorough investigation has been made in the present work in order to determine the optimum conditions for saccharin determination. The spectrophotometric method developed in this work is found to be

satisfactory for routine chemical analysis. An atomic absorption finish has been sucessfully applied to saccharin determination and this may be considered as a new approach for indirect methods.

The recommended precedures for the conditioning and preparation of modified-surface silica matrix and the saccharin determination are given in the following sections.

4.2 Experimental

Spectrophotometric Method

(i) Apparatus

Absorbance measurements were carried out using 1.0 cm quartz or glass cells and a Pye-Unicam SP 6-400 Spectrophotometer.

(ii) Reagents

Analytical reagent grade chemicals were used throughout :

Iron(II) ammonium sulphate solution (3.3×10^{-2}M)

Dissolve 3.2678 g of the hexahydrate salt in 50 ml of water containing 0.5 ml of Conc. H_2SO_4. Transfer to a graduated flask and make up to 250 ml.

1,10-phenanthroline solution (1×10^{-1}M)

Dissolve 4.9557 g of the reagent in 250 ml of distilled water.

Tris (1,10-phenanthroline)-iron(II) solution (3.3×10^{-2}M)

The ferroin reagent solution is prepared by adding 170 ml iron(II) ammonium sulphate solution (3.3×10^{-2}M) to 170 ml 1,10-phenanthroline solution (1×10^{-1}M).

Standard saccharin solution (2.5×10^{-2}M) *

* Purity > 99%

1.1449 g of saccharin is dissolved in 250 ml of distilled water. Prepare ten times diluted saccharin solution (2.5×10^{-3}M) in order to carry out the subsequent experiments.

Standard food additive solutions

(a) D-Glucose (1 M)

Dissolve 4.504 g Glucose (BDH) in 25 ml distilled water.

(b) Sucrose (1 M)

Dissolve 8.5575 g of Sucrose in 25 ml distilled water.

(c) Sodium Chloride (1 M)

Dissolve 1.461 g NaCl in 25 ml distilled water.

(d) Sodium Bicarbonate (1 M)

Dissolve 0.210 g $NaHCO_3$ in 25 ml distilled water.

(e) Citric Acid (0.1 M)

Dissolve 0.5253 g of citric acid in 25 ml distilled water.

(f) Dulcin (P-Phenetylurea) (0.1 M)

Dissolve 0.4505 g of Dulcin (Eastman organic chemicals reagent).. 17.5 ml acetone and dilute to 25 ml with distilled water.

(g) Sorbic Acid (0.01 M)

Dissolve 0.0280 g of sorbic acid (BDH) in 17.5 ml acetone and dilute to 25 ml with distilled water.

(h) Benzoic Acid (0.01 M)

Dissolve 0.0305 g of Benzoic acid (BDH) in 17.5 ml acetone and dilute to 25 ml with distilled water.

(i) Sodium Cyclamate (0.1 M)

Dissolve 0.5030 g of sodium cyclamate (BDH) in 25 ml distilled water.

(j) Sodium Hydroxide (0.1 M)

Dissolve 4.0 g of NaOH in one litre of distilled water.

Silica Gel

Silica gel, chromatographic grade, 80-200 mesh, was used in this work, which needed to be conditioned before use (see 'Recommended Analytical Procedures' section).

Ferroin-impregnated silica gel matrix 'Modified-surface silica gel', $[(Fe\ Phen_3)(SiO)_2]$

This matrix is prepared in batches, the preparation and conditioning of the matrix has to be followed carefully in order to prepare a uniform distributed ferroin on the surface of silica. This will give reproducible results (see 'Recommended Analytical Procedures' section).

(iii) Recommended Analytical Procedures

(a) Conditioning of silica gel

Weigh out 280 g of silica gel into a beaker, wash with several amounts of 0.1M NaOH to a total volume of 400 ml. Repeat the washing with successive quantities of distilled water to a total volume of about 400 ml. Remove any fine particles of silica gel or any turbidity by decantation. The resulting pH should be 5.2-5.5. Activation of the silica gel surface is carried out by heating in an oven at 120°C for 10-15 hours.

(b) Preparation and conditioning of ferroin-impregnated silica gel matrix

1 : Transfer the activated silica to five-litre beaker (a plastic beaker is preferable), add 400 ml distilled water, followed by ferroin solution (3.3×10^{-2}M) slowly from a burette. Stir the contents continously during the addition. It is observable that the silica gel in the beaker adsorbs

the ferroin added, rapidly first and then more slowly. This is indicated by the colour of the supernatant liquid, which is colourless in the beginning of the process. Carry on adding the ferroin solution slowly with continuous stirring until the supernatant liquid becomes almost intense in orange-red colour. At this point, the silica is saturated with ferroin and the volume consumed in the process should be beyond the amount defined as 'sorption capacity amount' discussed later in section 4.3. Stir for about ten minutes and allow the contents to be settle.

2 : Discard the supernatant liquid (which may be used in preparing another batch of the matrix), and wash the matrix several times with distilled water, in order to remove any excess of ligand or turbidity until colourless and clear supernatant liquid is obtained.

3 : Transfer the orange matrix to large size Buchner funnel fitted with Buchner flask connected to vacuum line. Pass an absolute ethanol (or dry acetone) over the matrix in the funnel, in order to remove the physically adsorbed water from the sites of the gel.

4 : Apply the vacuum to the matrix in the funnel to remove any adsorbed organic solvent. Store the matrix over a suitable desiccant overnight.

5 : Mix the dried matrix well, spread it on a sheet of filter paper, and leave it in contact with air in a free-dust atmosphere for few hours.

6 : Store the matrix in brown coloured dry glass bottle. The amount of matrix thus prepared should be suitable for 275 determinations, but may be further used for more analyses if washed with distilled water and then treated with absolute ethanol or dry acetone several times. Proceeding from step 4 (see also the re-usability of the matrix in section 4.3 for further use).

(c) Recommended procedure for standard calibration curves

Weigh out 1.00 g amounts of the ferroin-impregnated silica gel matrix into a number of 25 ml bottles. Add saccharin solutions of concentrations 1.25 X 10^{-4}M to 5.0 X 10^{-4}M when using distilled water, 7.5 X 10^{-5}M to 5.0 X 10^{-4}M when using 60% ethanolic solution or 80% methanolic solution and 5.0 X 10^{-5}M to 5.0 X 10^{-4}M when using 70% acetone solution. Make up the volume of solutions to 10.0 ml using either of these solvents : distilled water, 60% ethanolic solution, 70% acetone solution or 80% methanolic solution. Make sure that the overall percentage of the organic solvent in the solutions containing saccharin, is as it has been stated above. The pH of this solution should be 4.9-5.2. Shake the contents of the bottle for five minutes using a mechanical shaker. The absorbance of the decanted, and filtered supernatant liquid is measured at 510 nm using 1.0 cm cells and a blank or distilled water as reference.

(d) Procedure for tablet saccharin 'commercial samples'

Two methods can be used for determination of saccharin in saccharin tablets :

(1) Direct method

Weigh a sufficient number of tablets (ten), grind them to powder form. Accurately weigh a portion of the powdered tablets equivalent to 12.5 mg saccharin in small beaker, add 0.80 ml sulphuric acid (2% v/v) solution, dropwise. The pH should be between 4.9-5.2 after a complete dissolution of the tablet (effervescence is involved). Transfer to a 100 ml volumetric flask and dilute to the mark with distilled water. Shake the contents for few minutes and filter through a No 41 Whatman filter paper (the filtration step could be eliminated and the sample solution may be added as it is). 2.0 ml of this solution

is transferred into 25 ml bottle containing 1.00 g of ferroin-impregnated silica gel matrix, followed by 1.0 ml distilled water and 7.0 ml acetone to make the total volume 10.0 ml. Shake for five minutes using a mechanical shaker. Filter through a No 41 Whatman filter paper. Measure the absorbance of desorbed ferroin at 510 nm using distilled water as a reference. Using a calibration curve, calculate the saccharin content and report it as 'milligrame per tablet' taking the average weight of tablet.

(2) Standard-addition method

Prepare the saccharin sample solution as in the method mentioned above (i.e. to give 100.0 ml in a volumetric flask). 1.0 ml of this solution is transferred to each of a number of 10 ml volumetric flasks. One of these solutions is diluted to the mark with 2.0 ml of distilled water and 7.0 ml acetone. To the other flasks, add known amounts of standard saccharin solution ranging from 0.4 ml to 1.4 ml (2.5×10^{-3}M) saccharin, followed by 7.0 ml acetone and dilute to mark with distilled water. Add the content of each to 25 ml bottle containing 1.00 g ferroin-impregnated silica gel. Shake for five minutes using a mechanical shaker. Filter through a filter paper and measure the absorbances of the set of the desorbed ferroin solutions at 510 nm. Three different, accurately known amounts of saccharin are suitable for obtaining a calibration curve. Extrapolation of the plot thus obtained back to an absorbance of zero, gives the saccharin concentration. Calculate the saccharin content as in the direct method.

4.3 Results and Discussion

(1) Preliminary studies

It is worth mentioning the experimental steps which were carried out intially in the present work and which have led to the development of the recommended procedures.

The adsorption-desorption processes for ferroin have been applied in the initial experiments using silica gel and cationic exchanger (Amberlite Resin IR-120). The experiment was carried out using 2 g of each matrix in small bottles, 1.0 ml of 1,10-phenanthroline reagent solution (16×10^{-3}M) was added, followed by 1.0 ml ammonium iron(II) sulphate (5×10^{-3}M) and the volume was diluted to 10 ml with distilled water. After shaking for one minute, the solution was filtered, and the absorbance of unadsorbed ferroin was measured at 510 nm using a spectrophotometer. The following results (table 4.1) were obtained.

Table (4.1) Adsorption of ferroin on cationic exchanger

Absorbance	Silica gel matrix	Resin-Cationic exchanger
A_1	0.390	0.390
A_2	0.050	< 0.005
A_3	0.050	< 0.005

where A_1 = the absorbance of initial solution of ferroin before shaking with the matrix (before adsorption),

A_2 = the absorbance of solution after shaking with 2 g of the matrix for one minute (unadsorbed ferroin), and

A_3 = the absorbance of solution after further shaking time (3 minutes)

The results indicated the suitability of using a cationic exchanger resin which also produce a good blank reading (almost zero). Considering, however, the cheapness of silica gel, it has been used throughout this study.

Three types of apparatus have been cosidered for the preliminary examination using the silica gel; funnel, column and glass bottle. The first two vessels are used for mobile measurements, and the last one is for a static (or batch) procedures. A small funnel was used. It was plugged with a piece of glass wool in its stem and an amount of silica gel was placed above the wool. By passing the reagents; 1,10-phenanthroline and iron(II) ammonium sulphate solution through the funnel, the silica gel adsorbs the ferroin. Following this, different concentrations of saccharin solutions were passed, the desorbed ferroin in each time was collected and absorbance at 510 nm was measured. The results obtained are shown in table (4.2).

Table (4.2) Adsorption-desorption process of ferroin on silica gel using the funnel technique.

Saccharin concentration (M)	Absorbance
4.0×10^{-6} M	~0
2.0×10^{-5} M	~0
1.2×10^{-4} M	0.035
1.0×10^{-3} M	0.130
2.0×10^{-3} M	0.790
3.0×10^{-3} M	0.930

The possible use of a silica gel held in a funnel was investigated because it was hoped that this procedure would enable rapid adsorption-desorption to take place. However, the results were not satisfactory.

It was then proposed to replace the funnel by a column technique which hopefully would give a longer contact time both in the adsorption and desorption parts of the procedure. A glass column of 20 cm in length and 1 cm in diameter, containing 2 g silica gel was prepared for this purpose. The reagents; 1,10-phenanthroline and iron(II) ammonium sulphate were passed through the column. The silica gel bed was washed with distilled water in order to remove the excess of ferroin formed on the surface of silica. This was followed by the addition of saccharin solutions of various concentrations, and the desorbed ferroin was collected for absorbance measurements at 510 nm. The results obtained were not reproducible. The concentration of ferroin adsorbed on the surface of silica gel decreases with each addition of saccharin leading to unreproducible results.

It is not practical to prepare a series of silica gel columns containing the same matrix with identical conditions for each determination of saccharin. Thus, the static measurements were examined using small glass bottles (25 ml) containing silica gel (2 g) and ferroin. The saccharin solutions were added and a mechanical shaking was applied. The absorbance of the desorbed ferroin after filtration was measured at 510 nm. Promising results were obtained as shown in table (4.3).

Experimental :

2.0 g silica gel (without pre-treatment), 3 ml $(Fe\ Phen_3)^{2+}$ (5×10^{-4}M), (0-5.0) ml 40% ethanolic solution of saccharin (5×10^{-2}M),

Table (4.3) Adsorption-desorption process of ferroin on silica gel using the batch technique.

Saccharin Concentration (M)	Absorbance Using Water	Absorbance Using Phosphate buffer
0	0.080	0.045
5.0×10^{-4}	0.075	0.060
1.0×10^{-3}	0.080	0.030
1.5×10^{-3}	0.095	0.040
2.0×10^{-3}	--	0.030
2.5×10^{-3}	0.135	0.050
5.0×10^{-3}	0.185	0.100
1.0×10^{-2}	0.270	0.280
1.5×10^{-2}	0.510	0.420
2.0×10^{-2}	--	0.590
2.5×10^{-2}	0.670	0.710

dilute to 10.0 ml with distilled water or phosphate buffer, shaking for 3 minutes; filter the desorbed ferroin. The water solutions were acidic solutions because of the acidic nature of the silica gel itself, the other solutions using phosphate buffer had pH values 5-6. It was essential thus, from the results shown above to; (a) neutralize the slightly acidic silica gel surface by using 0.1M NaOH followed by washing before use, and (b) determine the maximum amount of ferroin solution which has to be added to silica gel before the desorption process using saccharin is carried out.

Firstly, it was decided to add the amount of ferroin whcih is equivalent to the sorption capacity of silica gel. The sorption capacity is "the amount of sorbed iron in the form of ferroin per 1.00 g of silica gel". This experiment was carried out using 1.00 g of washed silica gel in each bottle, ferroin solution (5×10^{-4}M) was added and the solution was diluted to 10 ml with distilled water. After shaking for 5 minutes, the absorbance of unsorbed ferroin was measured at 510 nm. The results obtained from this experiment are shown in the table (4.4). From fig. (4.5), the sorption capacity of the silica used was shown as 6.8 ml of ferroin (5×10^{-4}M) as an initial concentration added. However, 6.0 ml of ferroin of this concentration was used in the subsequent experiments. Amounts of ferroin from 1.0 ml to 6.0 ml give identical absorbance readings and this absorbance (0.020) is taken as a blank.

Having established this fact, a set of measurements were then made in order to determine the effect of different saccharin concentrations on the ferroin adsorbed on silica gel. Dried silica gel was washed with 0.1M NaOH followed by distilled water. Amounts of 6.0 ml ferroin (5×10^{-4}M) were added to 1 g quantities of the matrix contained in small

Table (4.4) Sorption capacity of silica gel.

(ferroin adsorption)

Ferroin (5×10^{-4} M) mls	Absorbance
0.00	0.020
0.25	0.020
0.70	0.020
1.00	0.020
1.50	0.020
2.00	0.020
2.50	0.020
3.00	0.020
3.50	0.020
4.00	0.020
5.00	0.020
6.00	0.020
7.00	0.090
8.00	0.480
9.00	1.120
10.00	1.600

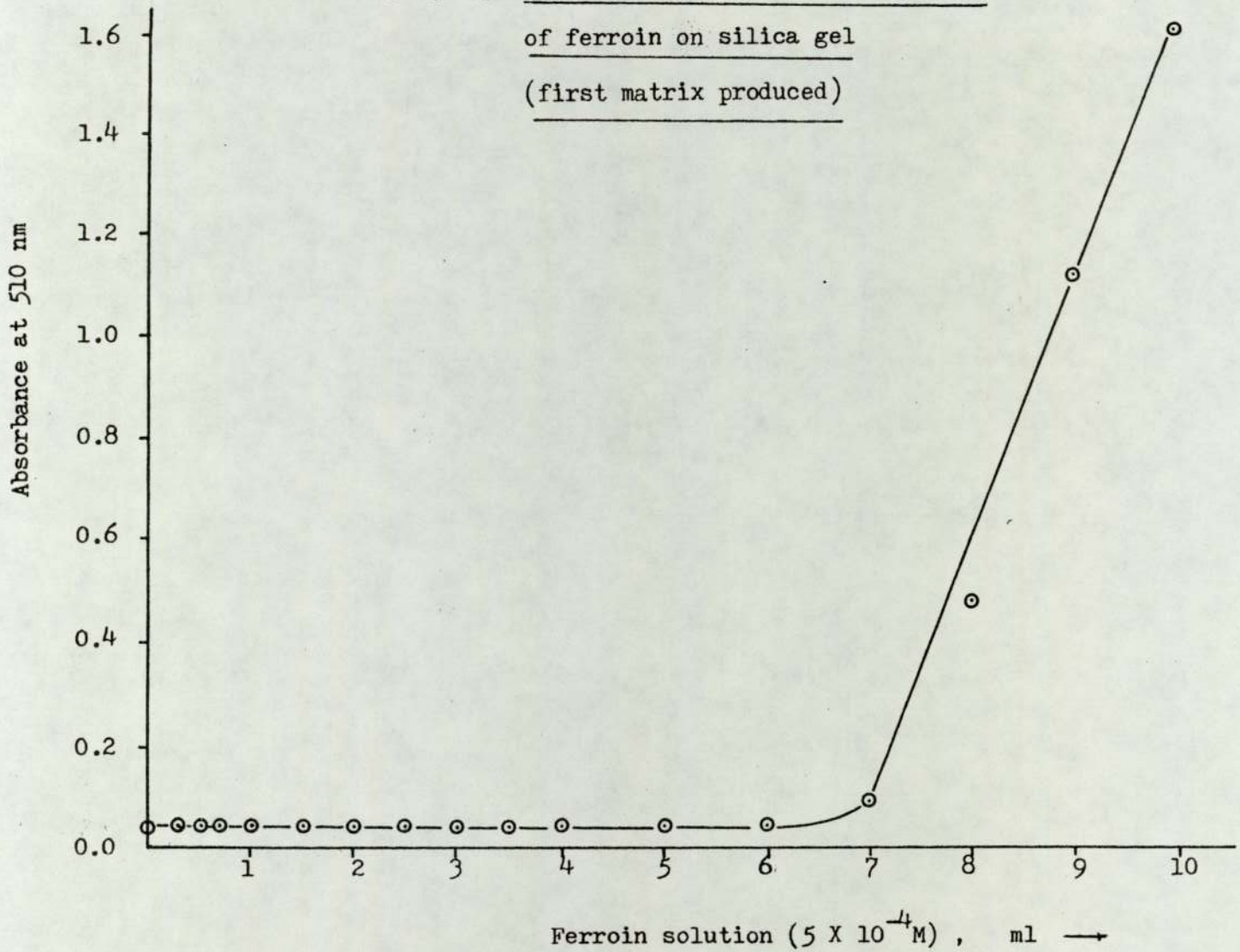
bottles. Different saccharin (40% ethanolic solution) concentrations were added and the solutions were diluted to 10 ml with distilled water. After shaking for one minute, the absorbance at 510 nm was measured. The results obtained were also not satisfactory- the precision and accuracy of the results were poor. This is shown in table (4.5).

Table (4.5) The effect of saccharin solutions on ferroin adsorbed on silica gel.

Saccharin (5×10^{-2}M) ml	Absorbance			
	1	2	3	4
0.0	0.020	0.025	0.020	0.020
0.1	0.070	0.030	0.030	0.050
0.2	0.110	0.065	0.080	0.150
0.3	0.200	0.235	0.350	0.440
0.4	0.370	0.410	0.695	0.530
0.5	0.380	0.420	0.600	0.640
0.6	0.540	0.695	1.080	0.900
0.7	0.910	0.890	0.960	1.000
0.8	0.905	0.990	1.140	0.840
0.9	1.060	0.980	0.950	1.000
1.0	1.260	1.170	0.960	1.100
1.1	1.220	1.160	1.040	0.800

The possible explanation for these results is that; two

Fig. (4.5) Determination of sorption capacity of ferroin on silica gel (first matrix produced)

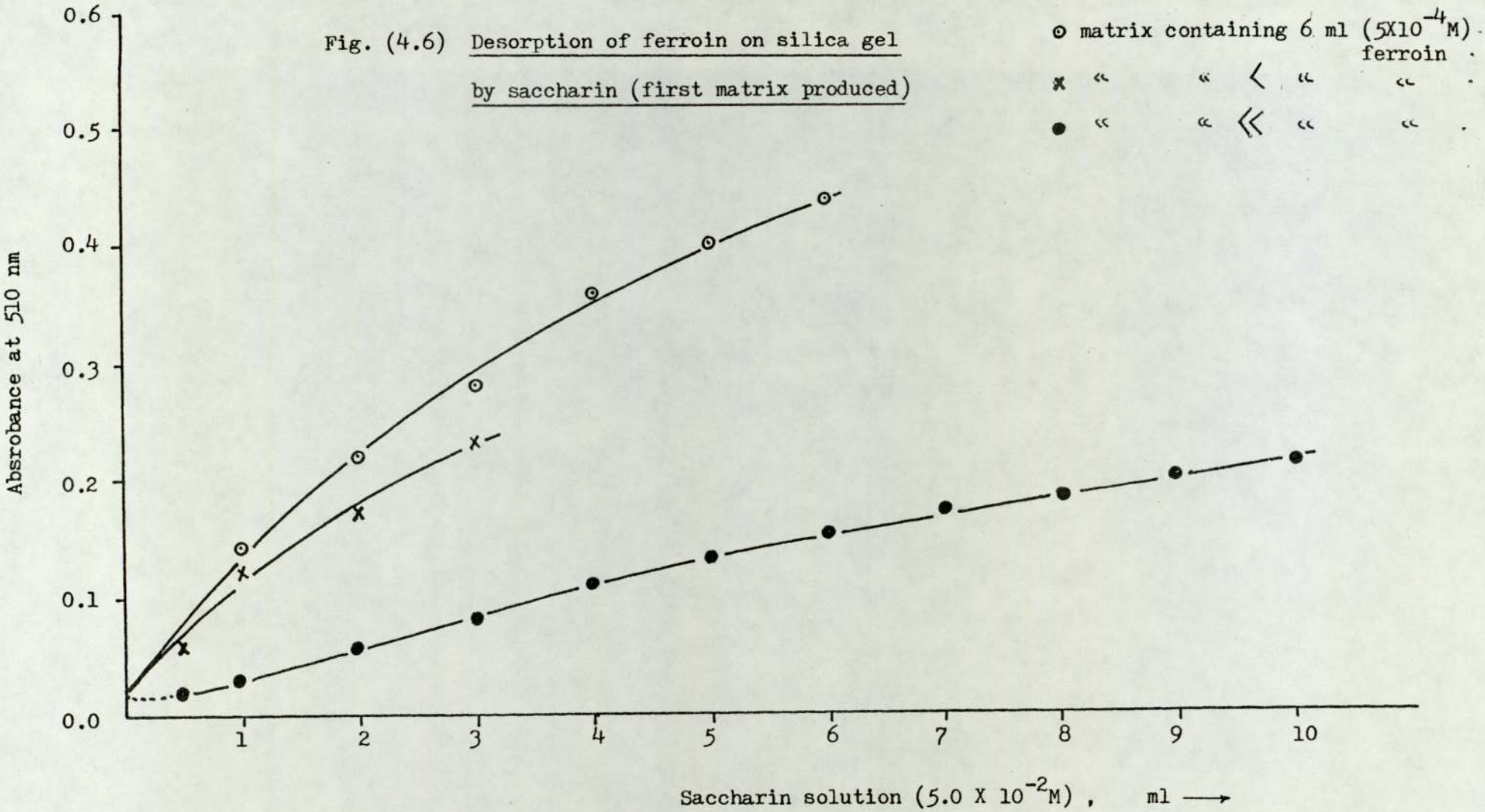


processes are taking place in this method, simultaneously, the sorption of ferroin on silica gel and the desorption of ferroin by the effect of saccharin i.e. the formation of the ion-pair. This may not ensure identical conditions for each determination and gives unreproducible results as just shown in the previous table.

In order to overcome this problem, it was thought that completion of one process at a time might give more precise and accurate results. This was carried out by adsorbing ferroin solution on silica gel, followed by washing with distilled water and drying the matrix in an oven at 110°C for 5 hours. The effect of different concentrations of saccharin on this matrix was examined. One gram of the matrix was placed in small bottle, 0-10 ml of saccharin (5×10^{-2}M) solutions were added followed by diluting to 10 ml with distilled water. After shaking for one minute, the absorbance of the desorbed ferroin was measured at 510 nm. Three different matrices were used, each containing a different amount of ferroin (but less than the amount of total sorption capacity). The results obtained from this experiment are shown in fig. (4.6). The curves show the practicability of using the ferroin-impregnated silica gel in this manner and also show higher absorbance readings with matrices having higher concentrations of ferroin.

A stock of ferroin-impregnated silica gel was prepared in order to determine the best conditions. In this preliminary matrix, 280 g of silica gel (80-200 mesh) was washed with 400 ml of 0.1M NaOH and then 400 ml of distilled water. After filtration, the matrix was dried and baked at 110°C for about 14 hours. Ferroin solution was added; 1680 ml (5×10^{-4}M) with continuous stirring and mixing till a colourless supernatant liquid was obtained. The liquid was decanted, washed with

Fig. (4.6) Desorption of ferroin on silica gel by saccharin (first matrix produced)



distilled water and the matrix was dried at 110°C for 10-15 hrs. This process gave an orange-red coloured silica gel matrix.

Some difficulties were found with the first matrices produced. In order to explain the problems associated with its use, some of the preliminary results are shown in the following paragraphs.

The Perfomance of the Matrices First Produced

The effect of variation of the time for shaking the saccharin solution with the matrix was determined by a series of measurements. From the following table (4.6), five minutes shaking time was adequate to achieve the equilibrium.

Table (4.6) Effect of varying shaking time on the desorption process.

Time (minute)	Absorbance
0.5	0.140
1.0	0.160
2.0	0.200
3.0	0.195
4.0	0.210
5.0	0.205
10.0	0.200
20.0	0.210
30.0	0.210

Experimental : (for table 4.6)

1.00 g ferroin-impregnated silica gel, 3 ml saccharin (50% ehtanolic solution) (5×10^{-2}M), 7 ml distilled water. Absorbance measured at 510 nm.

A quantitative desorption using different amounts of saccharin was observed with good precision. The intial calibration curve obtained was found to be liner in the range (1.00×10^{-2} - 2.75×10^{-2}M) saccharin. The table (4.7) is shown a set of data (see also fig. 4.7).

Experimental : (for table 4.7)

1.00 g ferroin-impregnated silica gel, saccharin, 50% ethanolic solution (5×10^{-2}M), from 0 to 10.0 ml, dilute to 10 ml with distilled water, shaking time = 5 minutes. Absorbance measured at 510 nm.

It was noticed during the experiments that when aqueous saccharin solution containing no alcoholic solvent was used, very little desorption of ferroin occurred. This introduced a new factor into the study i.e. that of solvent effect. This may be referred to as a 'synergic effect' i.e. an increase in desorption by the combined effect of solvent and saccharin. In the initial experiments variations in saccharin content led to variations in the alcohol content i.e. an increase in saccharin concentration gave an increase in alcohol concentration.

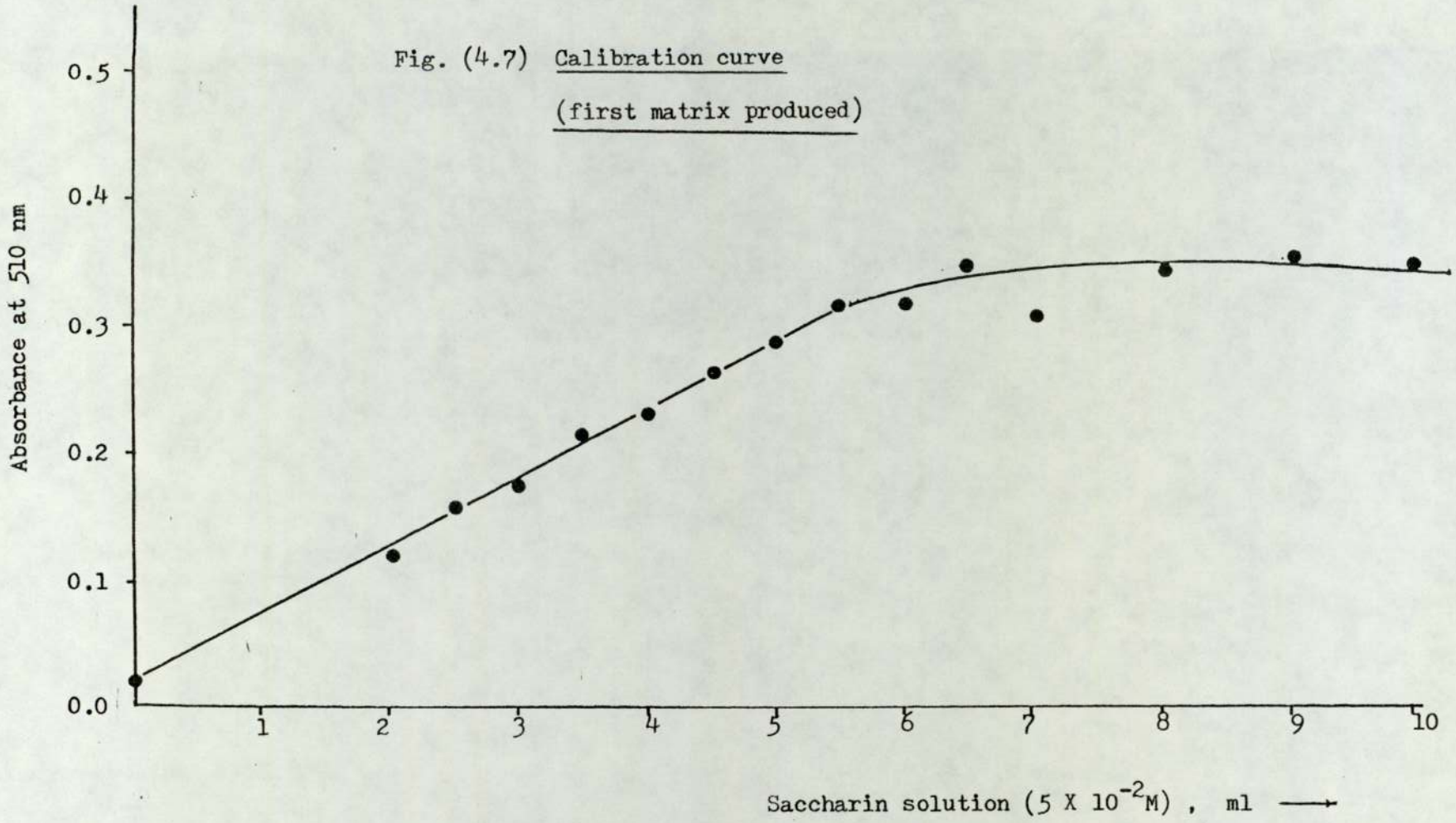
It was thought therefore, that the increase in the absorbance reading of desorbed ferroin with increase of saccharin concentrations was caused by the increase in the amount of ethanol and not the amount of saccharin. But, the effect of some organic solvents on ferroin-impregnated silica gel was examined and the matrix was uneffected by ethanol, methanol and acetone in the absence of saccharin.

Table (4.7) Effect of saccharin solutions on ferroin-impregnated silica gel matrix.

Saccharin ($5X10^{-2}M$) ml	Absorbance	Saccharin ($5X10^{-2}M$) ml	Absorbance	Saccharin ($5X10^{-2}M$) ml	Absorbance
0.0	0.020		0.160		0.310
	0.020		0.170	6.5	0.370
	0.020	3.5	0.200		0.290
	0.020		0.210		0.340
1.0	0.020	4.0	0.225		0.290
	0.025		0.225		0.300
	0.020		0.235		0.300
	0.020		0.225		0.300
	0.020		0.210	8.0	0.330
1.5	0.040	4.5	0.260		0.315
	0.040		0.260		0.335
	0.040		0.270		0.335
2.0	0.110	5.0	0.280		0.340
	0.110		0.280	9.0	0.350
	0.110		0.275		0.360
	0.120		0.280		0.380
	0.115		0.275	10.0	0.345
2.5	0.155	5.5	0.310		0.370
	0.160		0.300		
	0.150		0.310		
3.0	0.170	6.0	0.315		
	0.180		0.305		
	0.185		0.315		

Fig. (4.7) Calibration curve

(first matrix produced)



This led to the study of this "synergic effect" and also to find out the optimum amount of organic solvent present with the saccharin. Some results using two different ferroin-impregnated silica gel matrices and the effect of different percentages of ethanol and methanol are shown in the table (4.8).

Table (4.8) Effect of organic solvent (percent) on desorption process.

Organic Solvent	Absorbance				
	Ethanol			Methanol	
%	G_1	G_2	G_2	G_2	G_2
0	0.020	0.020	0.020	0.020	0.020
20	0.181	0.180	--	0.160	0.140
30	0.256	0.230	--	0.195	0.195
40	0.310	0.275	0.235	0.245	0.245
50	0.323	0.285	0.250	0.270	0.255
60	0.318	0.290	0.245	0.295	0.295
70	0.293	0.270	0.240	0.320	0.300
80	0.247	0.240	0.195	0.340	0.320
90	--	--	0.130	0.310	0.270
100	--	--	0.065	--	0.245

Experimental :

1.00 g ferroin-impregnated silica gel matrix;

G_1 = matrix containing 6.0 ml of 5×10^{-4} M ferroin,

G_2 = matrix containing < 6.0 ml of 5×10^{-4} M ferroin, 4 ml

saccharin solution (5×10^{-2}M), 6 ml ethanol/water or methanol/water. Shaking time = 5 minute. Absorbance measured at 510 nm.

It was obvious from the above table that the absorbance of desorbed ferroin by the same amount of saccharin is changing with different percentages of organic solvent. It was found that the optimum amount of ehtanol is 50-60% in the saccharin solution and for methanol is 80% . As shown in fig. (4.8) the absorbance readings increase to a maximum and then decrease for further increase in alcohol content. The possible explanation is discussed later in this chapter. It may also be concluded from these results that the use of 80% methanolic solution of saccharin may be more advantageous than the use of 50-60% ethanolic solution, i.e. higher absorbance readings for the same amount of saccharin.

Calibration curves were carried out but on a different basis. The same amount of organic solvent in the overall saccharin solutions was used. The dilutions were made using 30% and 50% ethanolic solution, 50% and 70% methanolic solution and 30% acetone solution. The results obtained are given in the table (4.9). Almost constant absorbances were obtained in each set of readings using different concentrations of saccharin except in low concentrations of saccharin (see fig. 4.9).

It ultimately became possible to develop optimum conditions using low concentrations of saccharin. These are described later. It was decided also to change the mole ratio Ferroin : Saccharin (ferroin adsorbed on silica gel). Figure (4.10) represents such type of matrix in which more ferroin was adsorbed on the silica gel.

(2) Preparation and Conditioning of Silica Gel and Ferroin-Impregnated Silica Gel

Fig. (4.8) Solvent effect on the desorption process

(first matrix produced)

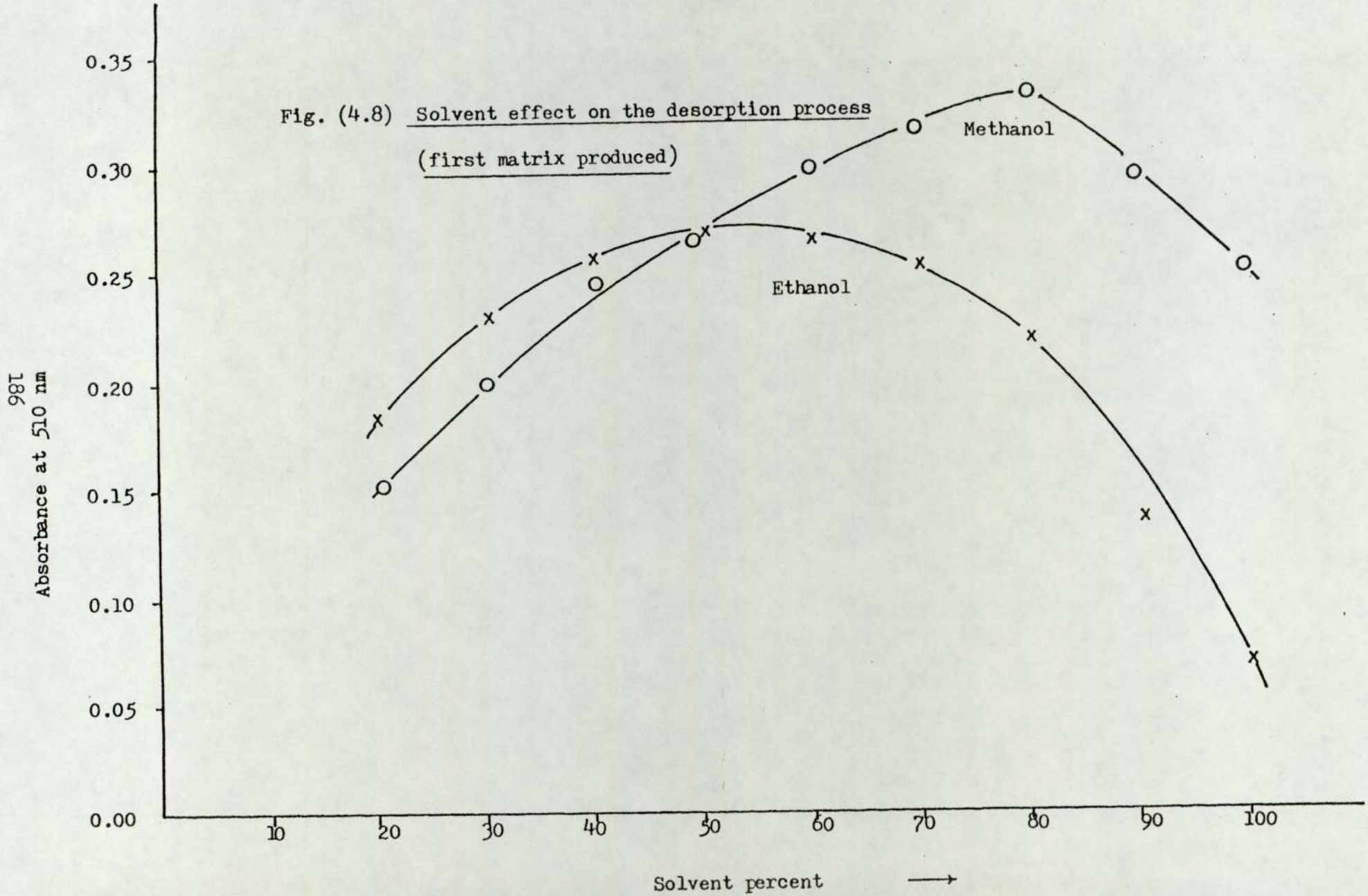


Table (4.9) Effect of saccharin solutions and organic solvents on the desorption process.

Saccharin ($5X10^{-2}M$) ml	Absorbance					
	Ethanolic soln. 30% G_2	Ethanolic soln. 50% G_2	Methanolic soln. 50% G_1	Methanolic soln. 70% G_1	Methanolic soln. 70% G_2	Acetone soln. 30% G_2
0	0.020	0.020	0.020	0.020	0.020	0.020
1	0.195	0.315	0.020	0.025	0.300	0.270
2	0.210	0.340	0.190	0.230	0.310	0.275
3	0.230	0.330	0.210	0.275	0.290	0.260
4	0.235	0.310	0.175	0.320	0.280	0.255
5	0.245	0.310	0.160	0.340	0.270	0.260
6	0.245	0.300		0.330	0.260	0.250
7	0.250	-		0.340	0.245	0.250
8	0.260	-		0.340	-	-
9	0.255	-		0.300	-	-
10	0.270	-		0.235	-	-

Experimental :

As obtained for the calibration curve fig. (4.7).

Samples were diluted with ehtanol, methanol or acetone solution instead of distilled water.

G_1 = ferroin-impregnated silica gel containing 6.0 ml ($5 \times 10^{-4}M$) ferroin.

G_2 = ferroin-impregnated silica gel containing < 6.0 ml ($5X10^{-4}M$) ferroin.

Fig. (4.9) Solvent effect on the calibration curves (first matrix produced)

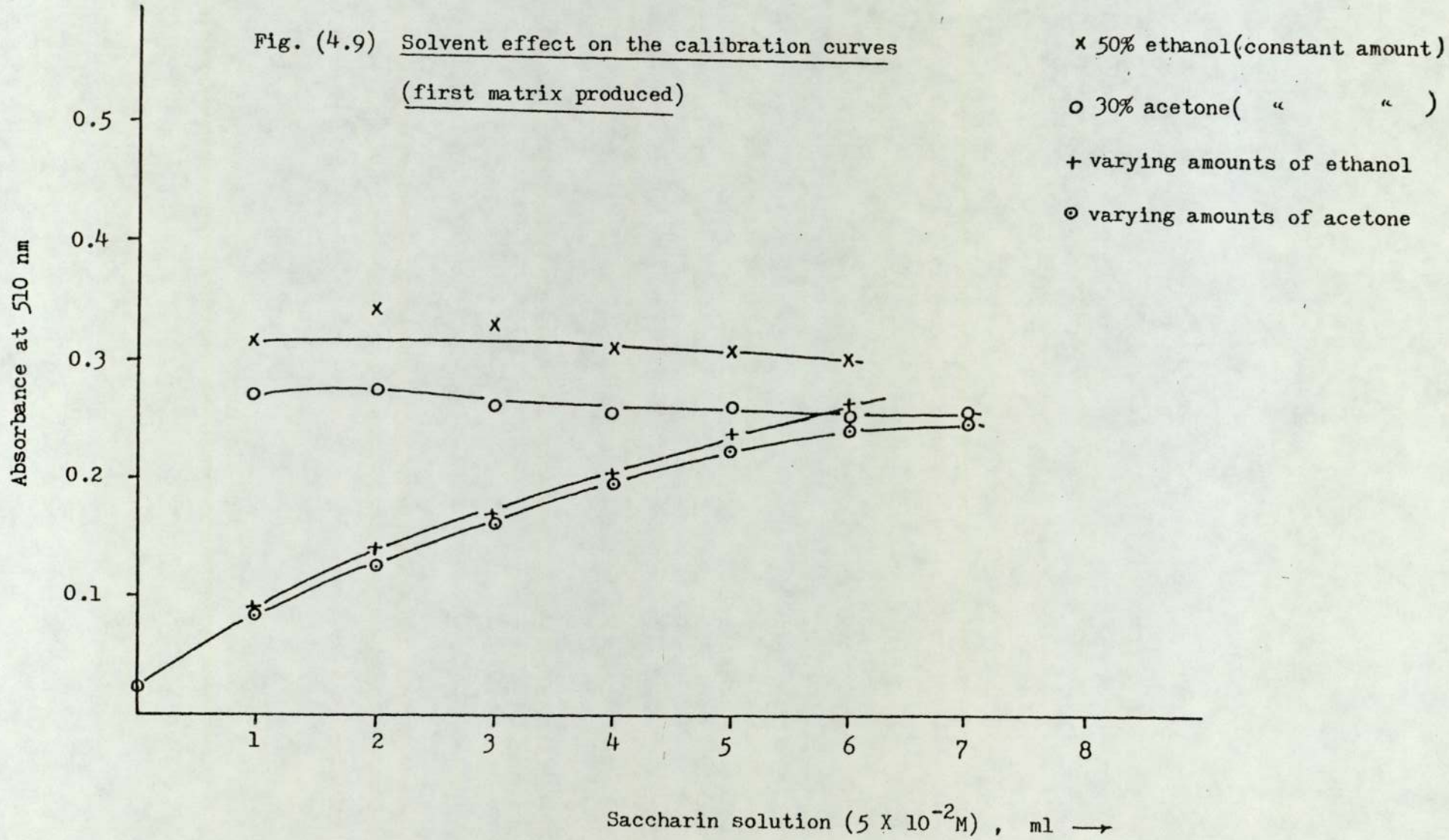
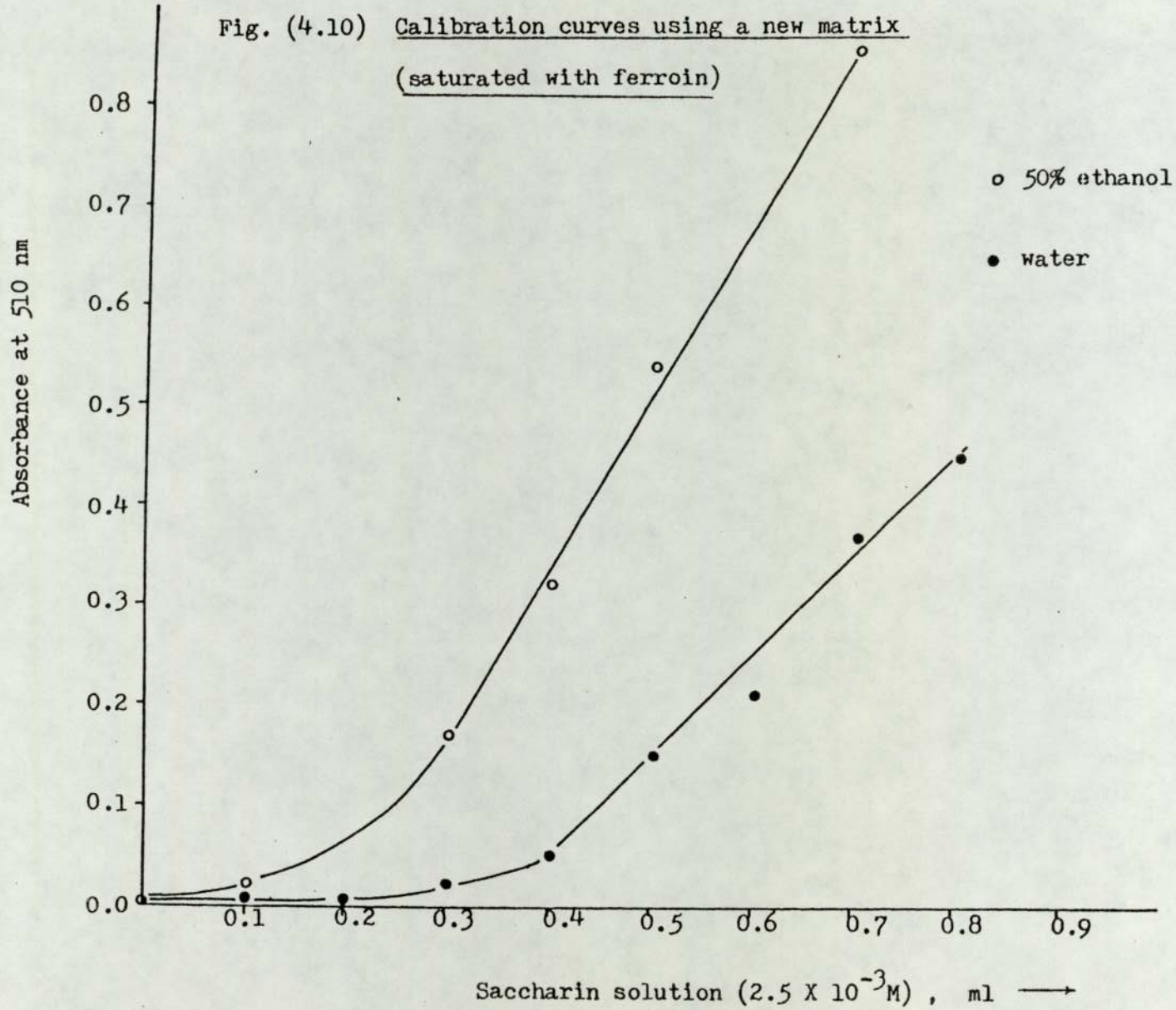


Fig. (4.10) Calibration curves using a new matrix (saturated with ferroin)



The standardized method which has previously mentioned in the 'Recommended Analytical Procedure' section, for the preparation of reproducible silica gel and ferroin-impregnated silica matrix has been developed in this way in order to control the critical adsorbent properties. Among these properties are : adsobent type must be defined (acidic or basic, polarity), adsorbent surface area should be constant and the activity of the surface must be adjusted to the desired level. Moreover, the selective desorption process should be quantitative. This is the most important feature in the preparation of ferroin-impregnated silica matrix.

Silica for chromatography is often contaminated by traces of acid. This acidity is the result of incomplete washing of the original gel, following its formation by the reaction of sodium silicate with an excess of acid. Acid-contaminated silicas can be neutralized by repeated washing of the silica, first with 0.1M NaOH and then with distilled water.

For the selective adsorption onto silica, the surface hydroxyls are responsible. For this, it is generally desirable to use a maximally hydroxylated silica, surface hydroxyl groups, which are the only important adsorption sites.

Activation temperatures of 150-200°C are sufficient to drive off most of the adsorbed water, without significant loss of surface hydroxyls. Higher activation temperatures give reduced surface activity because of loss of surface hydroxyls, so that maximum surface activity occurs for activation temperatures of 150°-200°C. However, 120°C for 10-15 hrs has been used .

The silica gel was therefore prepared as : (a) washing with 0.1M NaOH and then distilled water, to neutralize the acidity of the

gel, (b) thermal activation at 120°C for 10-15 hrs to remove adsorbed (molecular) water and to give the gel of approximately known water content and surface activity, (c) deactivation of silica by adding a fixed amount of distilled water (400 ml).

Following the conditioning of silica gel, the ferroin-impregnated silica matrix was prepared. Two ways of drying this matrix have been performed. After the sorption process of ferroin on silica gel, a portion of the matrix was dried in oven at temperature 120°C for 3 hours and another portion was washed with ethanol and dried under vacuum. One gram of each matrix was placed in a bottle followed by addition of 1 ml saccharin solution (2.5×10^{-3}M) and then the solution was diluted to 10 ml with distilled water. After 5 minutes shaking, the supernatant solution was filtered through a filter paper. The absorbance readings at 510 nm were found as follows :

		Absorbance
(a)	matrix dried at oven	0.060
(b)	matrix dried using ethanol and vacuum line	0.200

(3) Determination of the Sorption Capacity of Ferroin on Silica Gel

This parameter was defined earlier in this chapter as the ratio ferroin adsorbed for each gram of silica gel. Experiments were designed in order to find out the maximum amount of ferroin sorbed which at the same time would produce the lowest blank reading. The batch technique was used in this experiment.

One gram-amounts of silica gel (washed and conditioned as

previously discussed) were placed in 25 ml bottles. Varying amounts of ferroin solutions were added; either (a) 3.3×10^{-2}M ranging from 0.1 to 3.0 ml or (b) 6.6×10^{-3}M ranging from 0.2 to 10 ml. The volume of solution in each bottle was made up to 10 ml with distilled water and the contents were shaken for 20 minutes using a mechanical shaker. The supernatant liquid was filtered through a No 41 Whatman filter paper and the absorbance of ferroin remaining in the solution (unadsorbed) was measured at 510 nm using 1 cm cells. The table (4.10) shows the results obtained from these experiments.

Figures (4.11) and (4.12) represent the sorption capacity curves showing the resulting absorbance of the unadsorbed ferroin in solution plotted as a function of the total amount of ferroin originally added to the silica gel. From these graphs, the amount of ferroin sorbed per one gram of silica was determined by extrapolation to zero absorbance. It was found that 1.12 ml of (3.3×10^{-2}M) ferroin or 6.7 ml of (6.6×10^{-3}M) ferroin is adsorbed per one gram of silica gel.

It must be pointed out here that the value of sorption capacity is taken as a guide for the amount added to the gel to provide a matrix capable of giving reproducible results in quantitative desorption step. It is recommended to add an amount in excess of the sorption capacity.

(4) Absorption Maximum of the Ion-Association Complex

In the spectra shown in fig. (4.13), the maximum absorption for ferroin in water occurs at 510 nm (curve a), while the maximum absorption for the ion-pair $\left[(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right]$ when extracted into nitrobenzene occurs at 516 nm (curve b). This last peak is shifted -

Table (4.10) Determination of sorption capacity of ferroin on silica gel.

Ferroin $3.3X10^{-2}M$ added ml	Absorbance of unadsorbed ferroin	Ferroin $3.3X10^{-3}M$ added ml	Absorbance of unadsorbed ferroin	Ferroin $3.3X10^{-3}M$ added ml	Absorbance of unadsorbed ferroin
0.0	0.020	0.0	0.020	7.6	0.130
0.1	0.020	0.2	0.020	8.0	0.170
0.2	0.020	0.4	0.020	8.2	0.200
0.3	0.020	0.6	0.020	8.4	0.250
0.4	0.020	0.8	0.020	8.6	0.265
0.5	0.020	1.0	0.020	8.8	0.285
0.6	0.020	1.2	0.020	9.0	0.320
0.7	0.020	1.4	0.020	9.2	0.385
0.8	0.020	1.6	0.020	9.4	0.465
0.9	0.020	1.8	0.020	9.6	0.520
1.0	0.020	2.0	0.020	9.8	0.580
1.1	0.065	2.2	0.020	10.0	0.670
1.2	0.080	2.4	0.020		
1.3	0.080	2.6	0.020		
1.4	0.085	2.8	0.020		
1.5	0.140	3.0	0.020		
1.6	0.140	4.0	0.020		
1.7	0.170	5.0	0.020		
1.8	0.215	5.5	0.020		
1.9	0.315	6.0	0.020		
2.0	0.380	6.5	0.020		
3.0	> 2	7.0	0.020		

Fig. (4.11) Determination of sorption capacity of ferroin on silica gel (diluted ferroin solution)

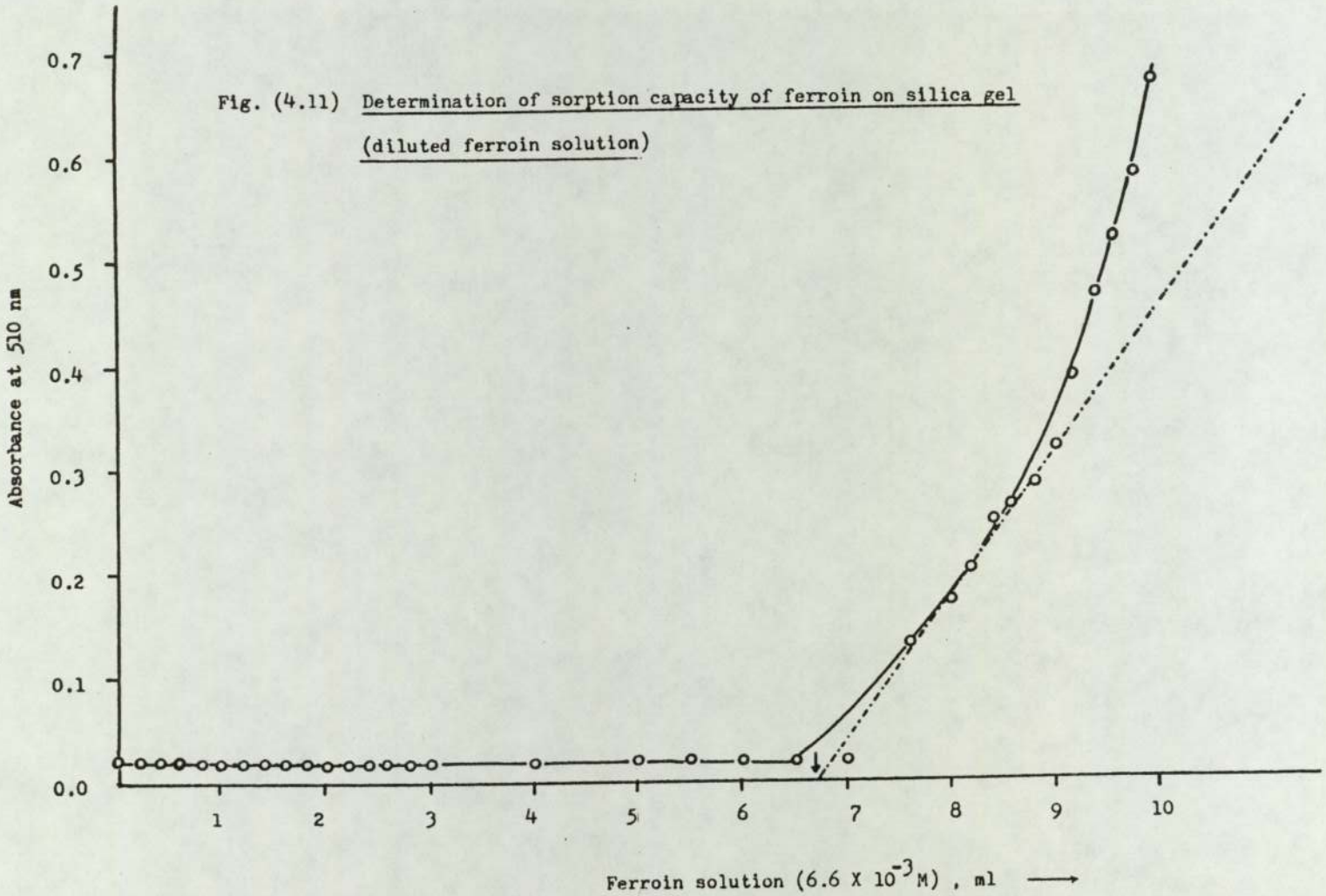


Fig. (4.12) Determination of sorption capacity of ferroin on silica gel (concentrated ferroin solution)

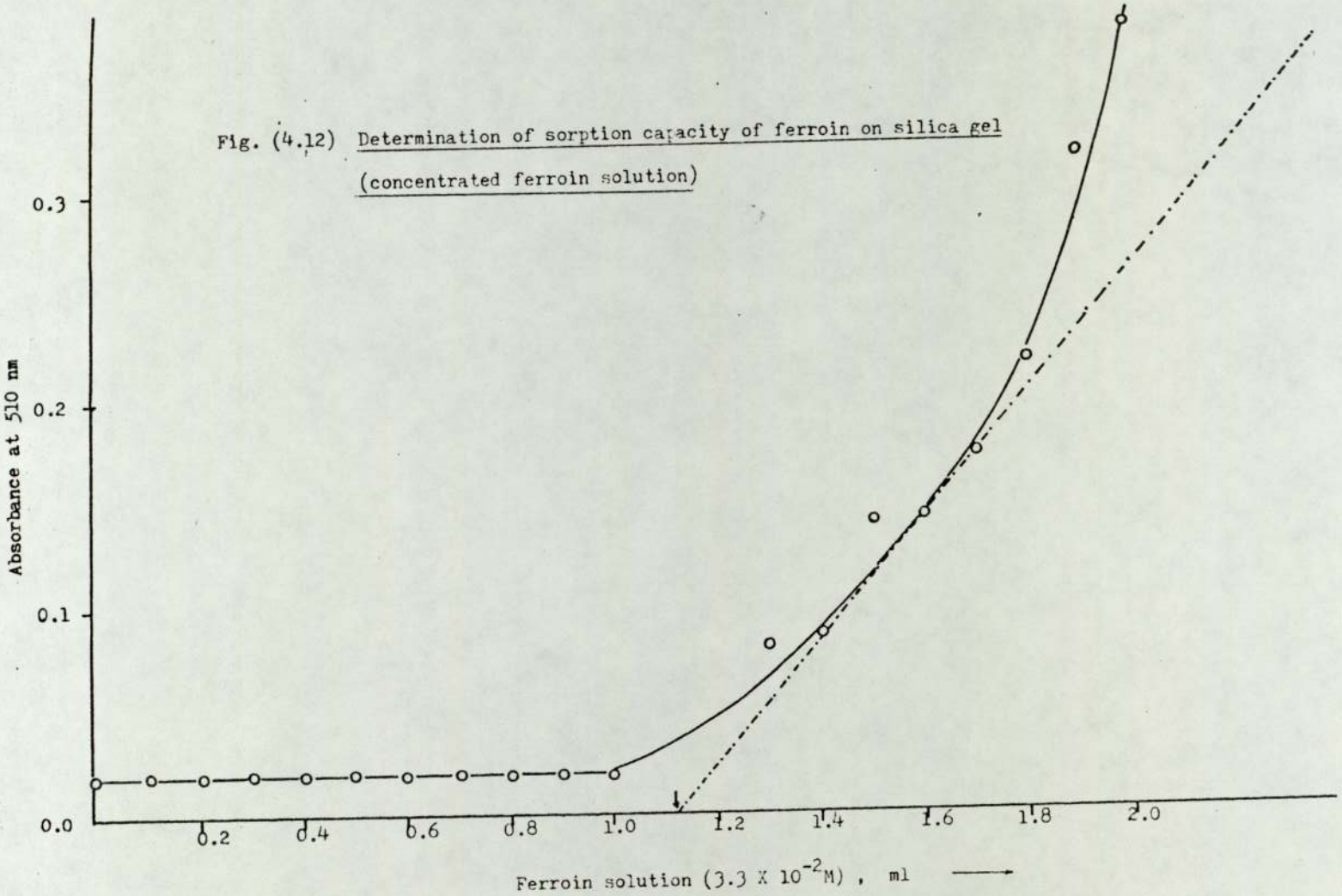
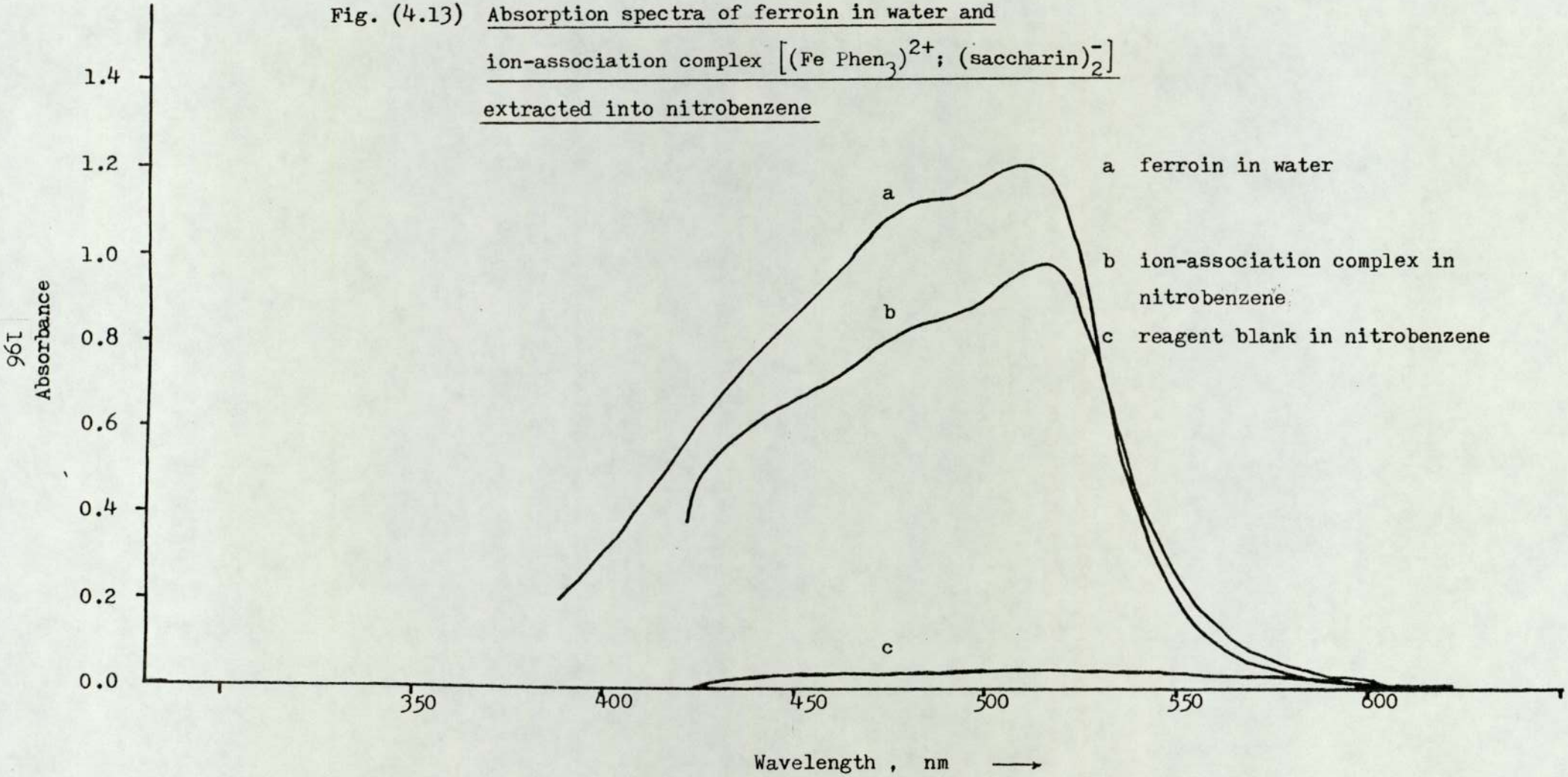


Fig. (4.13) Absorption spectra of ferroin in water and ion-association complex $[(Fe\ Phen_3)^{2+}; (saccharin)_2^-]$ extracted into nitrobenzene



slightly to a lower wavelength (510 nm) when ethanolic, methanolic or acetone solutions are used in the desorption process with ferroin-impregnated silica gel matrix.

Figure (4.14) shows the maximum absorption of the ion-pair which occurs at 510 nm, the same maximum absorption of the ligand $(Fe\ Phen_3)^{2+}$. The saccharin concentration used in obtaining the curves a, b, c and d was kept constant (2 ml of 2.5×10^{-3}M) but different solvents were used; distilled water, 60% ethanolic solution, 70% acetone solution and 80% methanolic solution. The experiment was carried out by shaking the solutions for 5 minutes with 1.00 g of ferroin-impregnated silica.

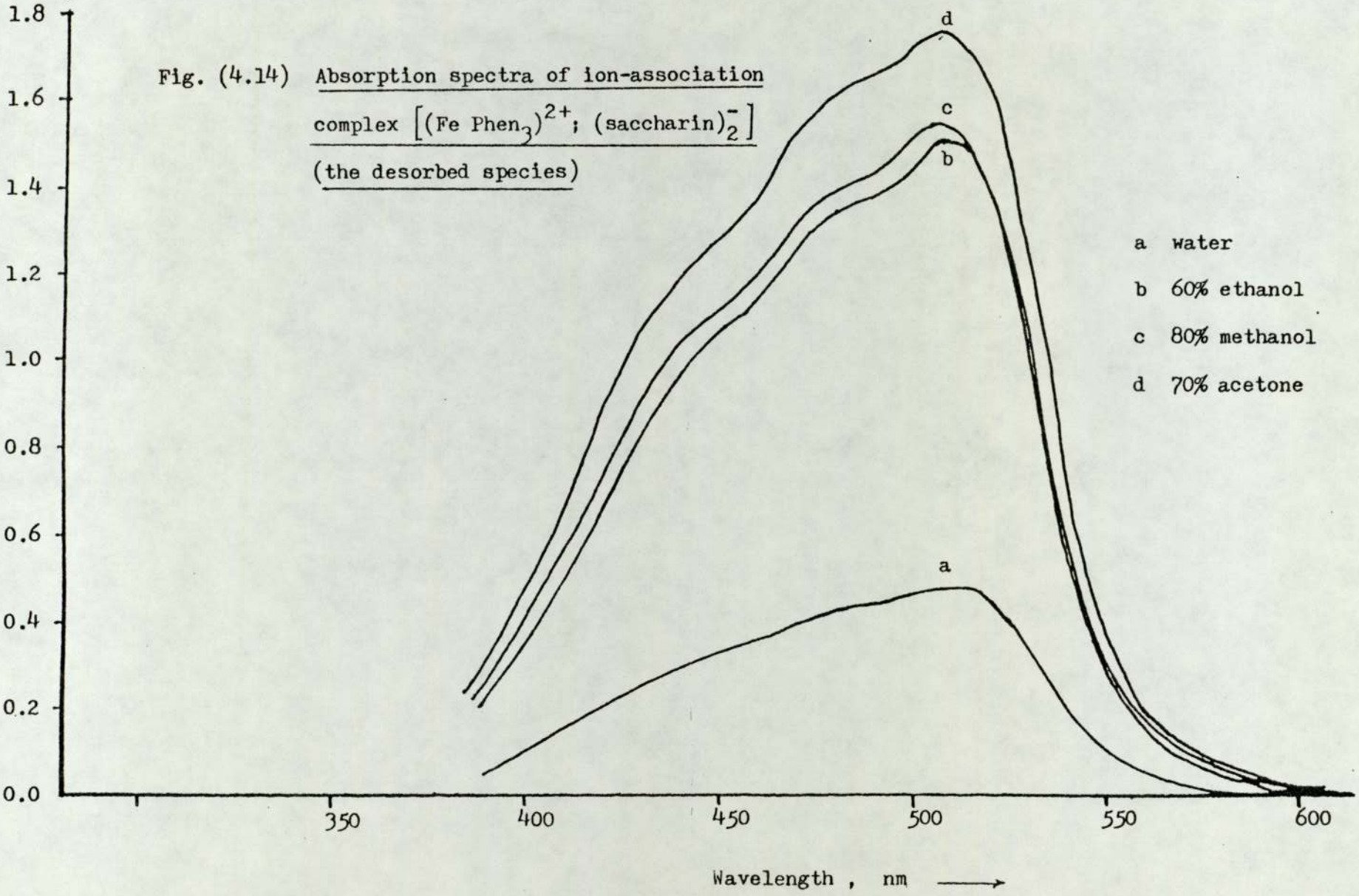
It is noticeable that the shape of ion-association complex spectra with all solvents are similar to the absorption spectra of ferroin. But, differences in absorbance values were noticed when using different solvents as follows (table 4.11).

Table (4.11) Solvent effect on absorbance.

Solvents	Absorbance at 510 nm
distilled water	0.48
ethanolic solution (60%)	1.50
methanolic solution (80%)	1.54
acetone solution (70%)	1.74

From the above results, one can conclude that there is some merit in chosing a solvent which gives a high absorbance for the same amount of saccharin. Acetone has the maximum absorbance. The possible

Fig. (4.14) Absorption spectra of ion-association complex $\left[(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right]$ (the desorbed species)



explanations for these variations are discussed later (section 7).

(5) Effect of Varying the Shaking Time for Desorption Process

The effect of varying the shaking time of the batch desorption process from 0.5 to 20 minutes using a mechanical shaker is shown in table (4.12) and also represented in fig. (4.15). Four sets of experiments were carried out, each set for different solvent. The solvents used in the experiments were distilled water, ethanolic 60%, methanolic 80% and acetone 70% solutions.

The results show a constant absorbance was obtained after 5 minutes for each solvent indicating that the equilibrium between the ferroin-impregnated silica gel and the solution containing saccharin is achieved rapidly.

(6) Precision of the Method

Six samples of saccharin solutions of 2.5×10^{-4} M for each solvent were subjected to the desorption process using 1.00 g amounts of ferroin-impregnated silica matrix. All variables were kept constant, and the mean value of the absorbance of the desorbed ferroin has been determined. Standard deviation values are also given in table (4.13).

The table shows satisfactory results are obtained for the spectrophotometric method and suggests that the ferroin is sorbed and homogeneously distributed over the surface of the silica gel matrix. This confirms that a reproducible batch of ferroin-impregnated silica matrix can be prepared by following the recommended procedure described.

Table (4.12) Effect of varying shaking time on desorption process.

Time (minutes)	Absorbance at 510 nm			
	Water	Ethanolic soln. 60%	Methanolic soln. 80%	Acetone soln. 70%
0.5	0.130	0.415	0.415	0.665
1.0	0.160	0.520	0.615	0.760
2.0	0.180	0.580	0.690	0.810
3.0	0.190	0.620	0.725	0.830
4.0	0.205	0.680	0.740	0.840
5.0	0.220	0.720	0.750	0.850
10.0	0.220	0.720	0.745	0.850
15.0	0.210	0.720	0.750	0.850
20.0	0.220	0.715	0.750	0.850

Experimental :

1.00 g ferroin-impregnated silica gel,

1.0 ml saccharin solution (2.5×10^{-3}M),

6.0 ml ethanol, or 8.0 ml methanol or 7.0 ml acetone,

dilute to 10.0 ml with distilled water, 5 min. shaking.

Fig. (4.15) Effect of varying shaking time on desorption process

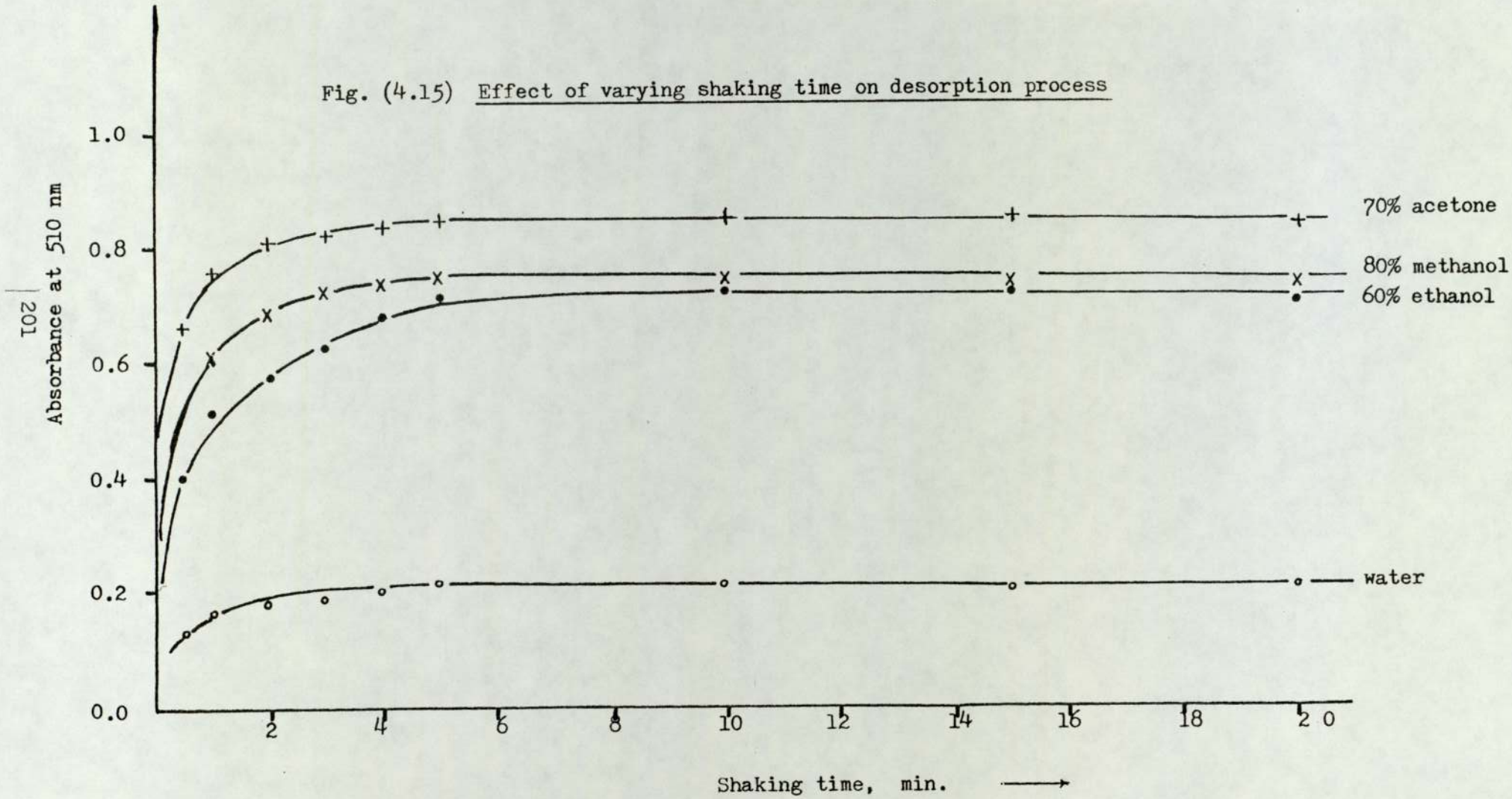


Table (4.13) Precision of the method.

Number	Absorbance			
	Water	Ethanolic soln. 60%	Methaonlic soln. 80%	Acetone soln. 70%
1	0.215	0.740	0.760	0.870
2	0.210	0.725	0.770	0.875
3	0.215	0.725	0.780	0.860
4	0.220	0.740	0.750	0.875
5	0.220	0.745	0.750	0.850
6	0.220	0.745	0.730	0.850
$\bar{A}$	0.216	0.737	0.757	0.863
$\bar{S}$	0.004	0.009	0.017	0.015
$\bar{S}$ %	1.852	1.264	2.311	1.773

$\bar{A}$ = Mean value of absorbance

$\bar{S}$ = Standard deviation (absorbance unit)

$\bar{S}$ % = Relative standard deviation percent.

The results also show that the desorption process is quantitatively reproducible from a set of the same batch.

(7) Solvent Effect on the Desorption Process

An investigation of the effect of solvents on the desorption process by reacting saccharin with ferroin on silica gel surface, has shown some interesting results. It has been shown previously that the shape of absorption curve of the ion-association complex $\left[(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right]$ is the same whether employing water, ethanolic, methanolic or acetone solutions for the desorption or even when extracting the ion-pair into nitrobenzene. The maximum absorption for the spectra using these solvents occurs at 510 nm except in the case of nitrobenzene where the peak is shifted slightly to higher wavelength 516 nm.

The values of absorbance for the desorbed ferroin were found to vary with the concentration of the organic solvent present with the saccharin solution used for the desorption. Table (4.14) shows this effect in which the presence of 60%, 70%, or 80% of ethanol, acetone or methanol respectively gave the maximum absorbance values. The curves obtained from these results are similar in shape (see fig. 4.16).

In order to explain the effect of the solvent on desorption, some facts have to be pointed out. Silica gel is highly polar material which adsorb molecules strongly. It is said to be an active adsorbent. Activity is determined by the overall polarity and the number of adsorption sites. The adsorption sites as discussed before, are the oxygen atoms and silanol groups ($\equiv$Si-OH) which readily form hydrogen

Table (4.14) Solvent effect on the desorption process.

Solvent Conentration %	Absorbance		
	Ethanolic soln.	Methanolic soln.	Acetone soln.
0	0.220	0.220	0.220
10	0.310	0.300	0.290
20	0.490	0.400	0.490
30	0.590	0.490	0.635
40	0.670	0.570	0.780
50	0.725	0.670	0.825
60	0.780	0.760	0.865
70	0.735	0.790	0.910
80	0.695	0.850	0.880
90	0.350	0.760	0.720

Experimental :

1.00 g ferroin-impregnated silica gel matrix,

1.0 ml saccharin (2.5×10^{-3}M),

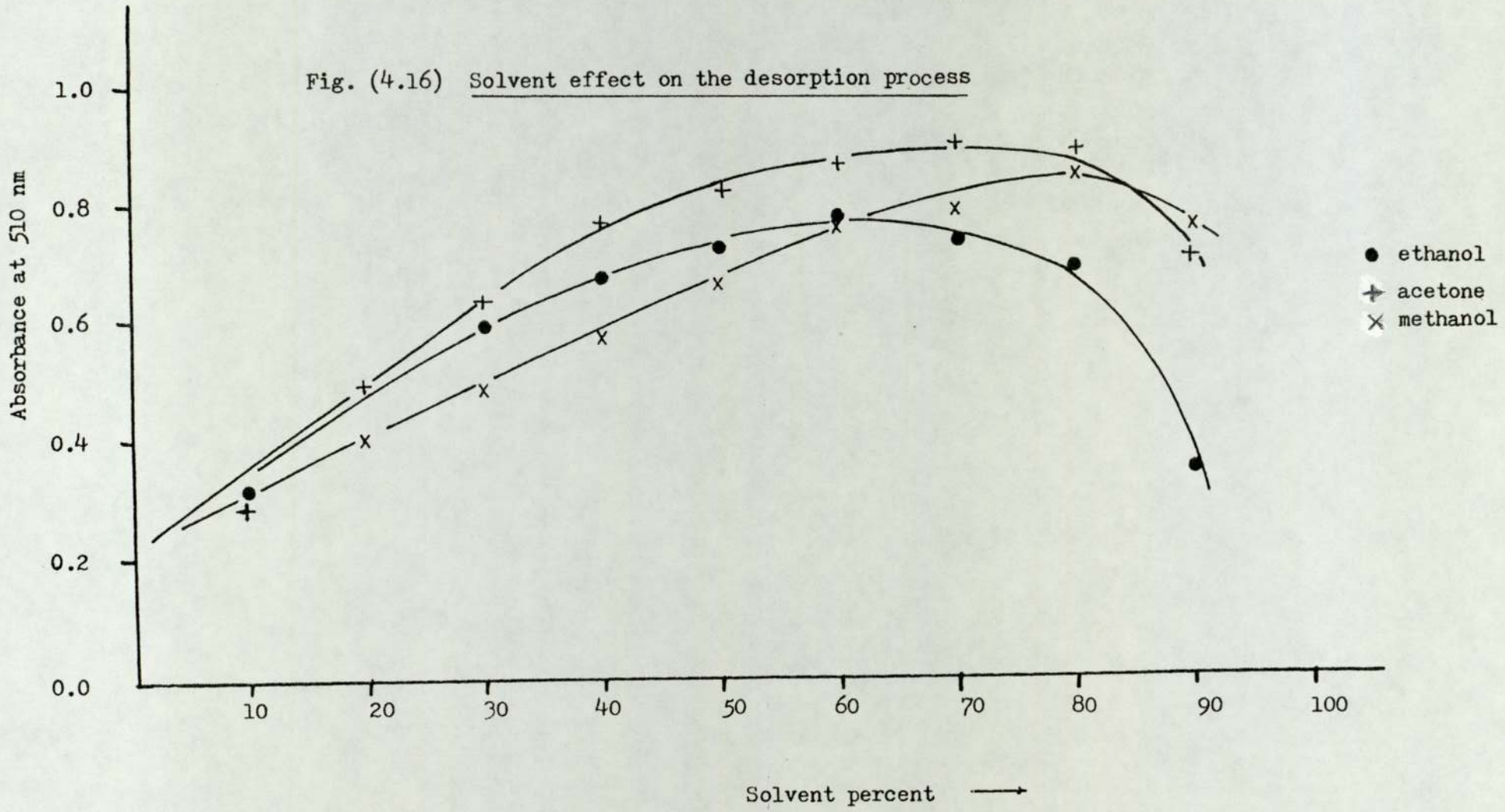
0-9.0 ml of organic solvent,

dilute to 10.0 ml with distilled water,

shaking time : 5 minutes.

Absorbance measured at 510 nm.

Fig. (4.16) Solvent effect on the desorption process



bonds with polar molecules. It has been pointed out earlier in this chapter that silica gel adsorbs strongly the polar ferroin on the surface of the gel to give a neutral surface $\left[(Fe\ Phen_3)(SiO)_2\right]$. In this case, the ferroin-impregnated silica gel matrix has different adsorption properties from the silica gel, including, of course, the surface activity aspect (i.e. the presence of the silanol groups).

Adsorption of ferroin lowers the surface activity, and the coverage of the surface with polar adsorbates such as water or alcohols also causes deactivation. It is thought that the degree of deactivation varies with the concentration of an organic solvent and also with amount of water. The desorption power of the solutions containing saccharin and ehtanol, methanol or acetone were found to be affected when the amount of water content ranged from 20% to 40%.

The degree of solubility of saccharin (at 20°C) in different solvents plays an important role in the desorption of ferroin. It is known that the saccharin solubility (by weight) is as follows :

One part is soluble in 290 parts of distilled water.
" " 25 " boiling ".
" " 12 " acetone.
" " 30 " alcohol.

1,10-phenanthroline is soluble in 300 parts of water but much more soluble in alcohol. The increasing of the absorbance values in the order of water < alcohol < acetone, as solvents in the desorption process may be explained on the light of "solvancy degree" of saccharin. The saccharin is more soluble in acetone than in alcohol, thus the ion-pair

formed is more soluble in acetone than in alcohol.

The "solvancy" of ion-pair depends also upon the dielectric constant of the solvents employed in this determination (185).

Table (4.15) Dielectric constant of some solvents.

Solvents	Dielectric Constant
Water	80.37
Nitrobenzene	34.80
Methanol	33.62
Ethanol	24.30
Accetone	20.00

The fact that the dielectric constant of methanol is higher than ethanol explains the higher absorbance readings of the desorbed ferroin. The ion-pair is more soluble in methanol than in ethanol. In addition, the percentage of organic solvent in the solution is higher, thus the desorbed ferroin is higher. In spite of lower dielectric constant for acetone than for alcohol, the absorbance values are higher. This probably arises because the higher initial solubility of saccharin in acetone rather than any increased solvation of the ion-pair by this solvent.

(8) Re-usability of Ferroin-Impregnated Silica Matrix

It was found in this work that the matrix is re-usable for many determinations of saccharin. In order to determine the number of possible times the matrix could be used for this determination, the following experiment was carried out :

1- 1.00 g of matrix (M1) was placed in 25 ml bottle size and 1.0 ml of saccharin solution (2.5×10^{-3}M) was added, followed by 7.0 ml of acetone, and the solution diluted to 10.0 ml with distilled water,

2- the contents were shaken mechanically for 5 minutes and the supernatant liquid was filtered though a No 41 Whatman filter paper.

3- the absorbance of the liquid was measured at 510 nm, consider this absorbance reading as (A1),

4- the consumed matrix (M2) was washed with distilled water and then acetone for several times, and then it was dried under vacuum,

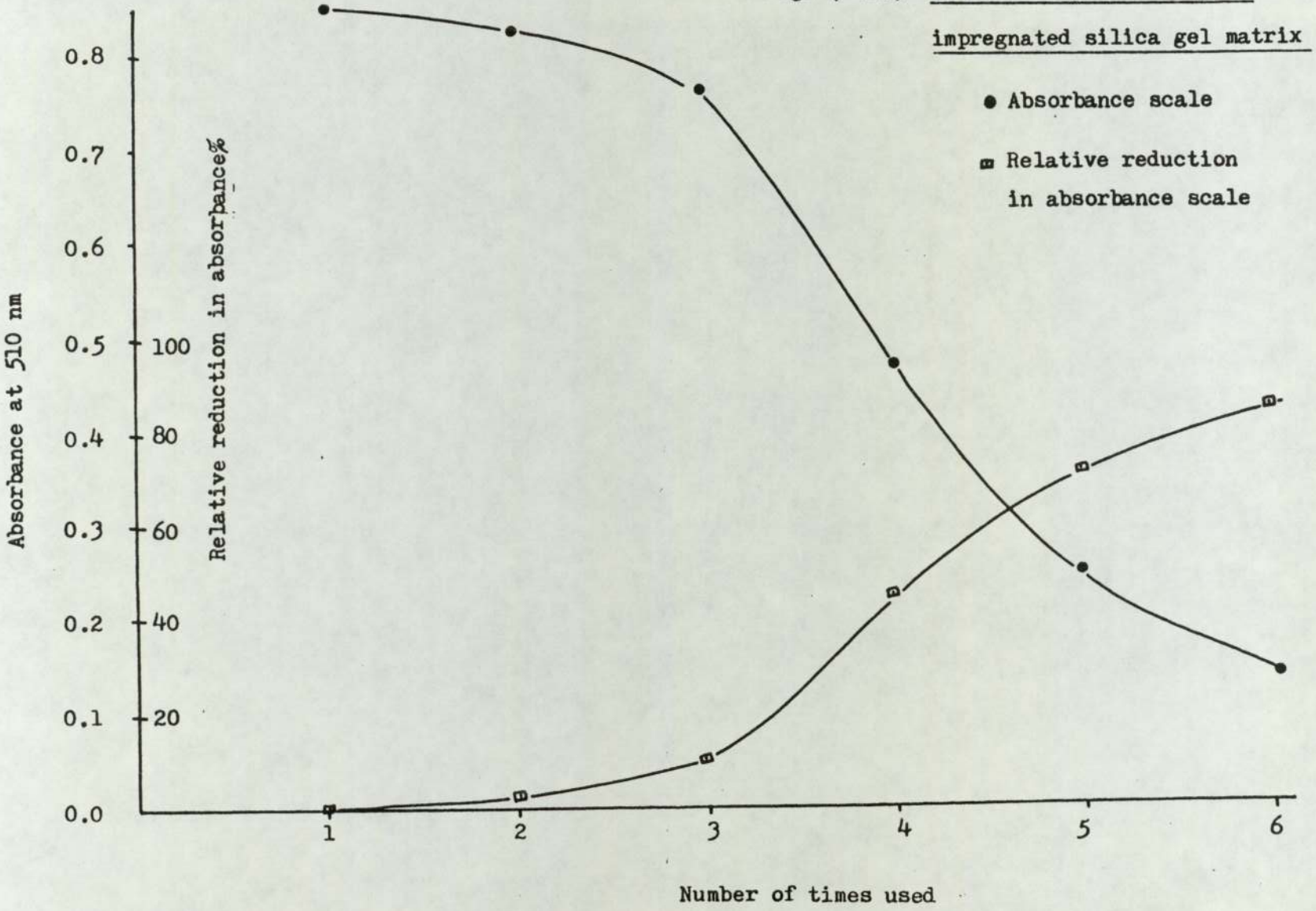
5- the steps from 1to 3 were repeated using fresh saccharin solutions each time to obtain absorbance values (A2, A3, A4, A5 and A6) resulting from the re-use of the consumed matrices (M2, M3, M4, M5 and M6),

6- a plot of number of times of re-use of the matrix versus absorbance readings at 510 nm of desorbed ferroin using 1.0 ml saccharin 2.5×10^{-3}M each time, was obtained, see the set of readings in table (4.16). Figure (4.17) also represents the re-usability of the ferroin-impregnated silica matrix.

It was found that the percentages of reduction in absorbance values (relative to the absorbance A1 obtained from the original matrix) are 2.94% and 10.6% for the consumed matrices M2 and M3 respectively. This indicates that the matrix can be employed more than twice and still give good sensitivity.

In order to employ the consumed matrices M2 and M3 for more saccharin determinations, they are washed after the first use with

Fig. (4.17) Re-usability of ferroin - impregnated silica gel matrix



distilled water and then with acetone several times and finally dried under vacuum and kept in brown bottles labelled as M2 and M3. But in order to use the other consumed matrices; M4, M5, etc. the consumed amount of ferroin on silica gel has to be compensated by adding more ferroin solution to the matrix in distilled water, with continuous stirring until the colour of the supernatant solution shows a red-orange colour. The matrix is washed with distilled water and then with ethanol or acetone for several times and finally dried under vacuum.

Table (4.16) Re-usability of ferroin-impregnated silica.

Matrix No of use	Absorbance at 510 nm	Relative Reduction% (in absorbance)
M1	0.850	0
M2	0.825	2.94
M3	0.760	10.59
M4	0.470	44.70
M5	0.245	71.18
M6	0.135	84.12

(9) Calibration Curves

Calibration curves may be obtained by varying the amount of

saccharin. A plot of absorbance of ferroin desorbed by saccharin solutions of various concentrations is used. The curves in this work were obtained using four different solvent media; distilled water, 60% ethanolic solution, 80% methanolic solution and 70% acetone solution. Experiments were carried out as described in the 'Analytical Procedures' section. The concentration of saccharin used was 2.5×10^{-3}M and the total volume of examined solution shaken with the matrix was 10 ml. The data obtained is given in tables (4.17a) and (4.17b).

As shown in fig. (4.18), each plot consists of two parts, the linear part and the curved part. They were obtained for the same range of saccharin concentrations but with different sensitivities. The curve obtained from acetone as solvent showed highest sensitivity (as represented by absorbance) of all these solvents. The curves obtained from ethanolic and methanolic solutions gave similar absorbance readings.

In fig. (4.19) curves are shown in which the plots gave straight lines over the range of saccharin concentration in the initial solutions as follows :

distilled water	from 1.25×10^{-4}M to 5.0×10^{-4}M
ethanolic solution 60%	from 7.50×10^{-5}M to 5.0×10^{-4}M
methanolic solution 80%	
acetone solution 70%	from 5.0×10^{-5}M to 5.0×10^{-4}M

It is obvious from these results that, using acetone with the desorption process gives higher sensitivity and extends the limits of determination.

Table (4.17a) Saccharin concentrations vs. absorbance of desorbed ferroin.

Saccharin ($2.5X10^{-3}M$) ml	Absorbance			
	Water	Ethanolic soln. 60%	Methanolic soln. 80%	Acetone soln. 70%
0	0.010	0.010	0.010	0.010
0.1	0.020	0.030	0.035	0.045
0.2	0.015	0.070	0.070	0.100
0.3	0.015	0.105	0.125	0.170
0.4	0.025	0.170	0.200	0.245
0.5	0.025	0.250	0.275	0.340
0.6	0.050	0.340	0.360	0.470
0.7	0.080	0.425	0.440	0.580
0.8	0.110	0.490	0.530	0.660
0.9	0.145	0.580	0.600	0.760
1.0	0.200	0.660	0.710	0.850
1.1	0.200	0.750	0.785	0.940
1.2	0.225	0.830	0.855	1.020
1.3	0.250	0.910	0.940	1.140
1.4	0.285	0.980	1.025	1.240

Table (4.17b) Saccharin concentrations vs. absorbance of desorbed ferroin.

Saccharin ($2.5X10^{-3}M$) ml	Absorbance			
	Water	Ethanolic soln. 60%	Methanolic soln. 80%	Acetone soln. 70%
1.5	0.310	1.080	1.110	1.320
1.6	0.335	1.160	1.190	1.420
1.7	0.370	1.250	1.280	1.500
1.8	0.400	1.330	1.350	1.620
1.9	0.420	1.400	1.420	1.690
2.0	0.450	1.480	1.520	1.760
3.0	0.715	1.880	2.000	2.320
4.0	0.880	2.340	--	--
5.0	0.925	2.420	--	--
6.0	0.940	--	--	--
7.0	0.960	--	--	--
8.0	0.950	--	--	--
9.0	0.960	--	--	--
10.0	0.960	--	--	--

Fig. (4.18) Calibration curves for saccharin determination by spectrophotometric method (wide range)

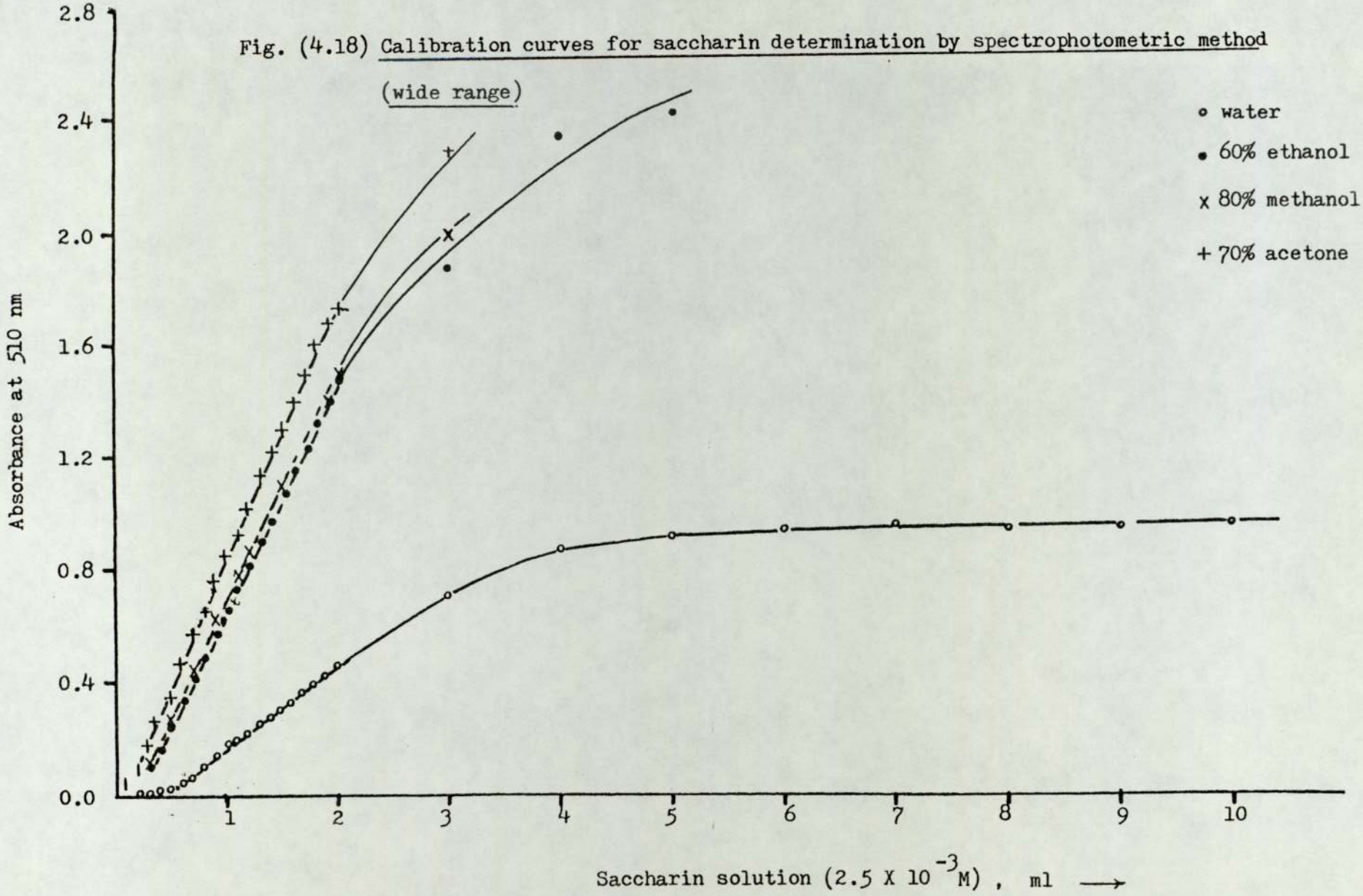
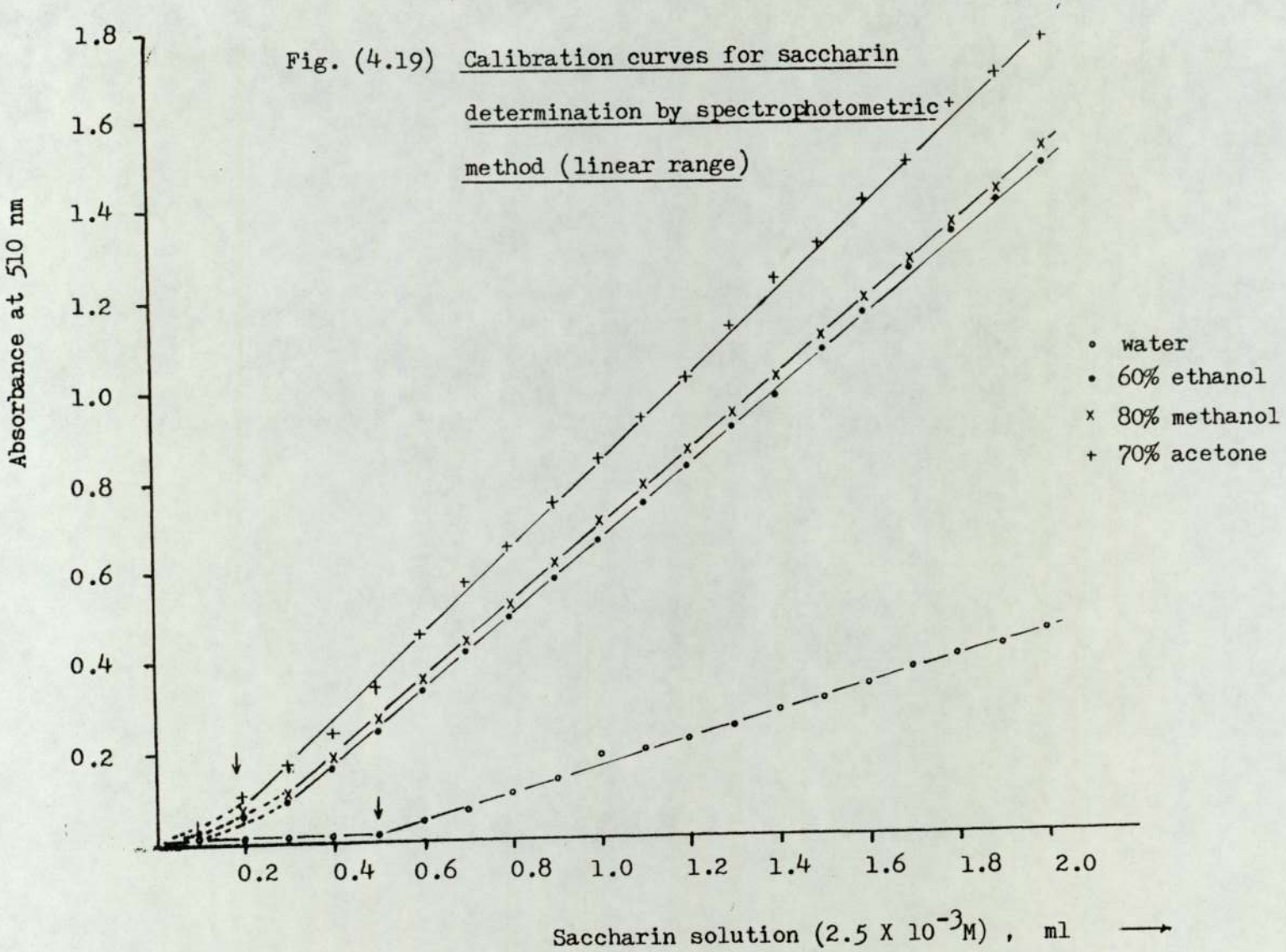


Fig. (4.19) Calibration curves for saccharin determination by spectrophotometric method (linear range)



(10) Interference of the Food Additives

The influence of other food additives such as, artificial and natural sweeteners, preservatives and fillers on the determination of saccharin by desorption process were examined.

The results are given in tables (4.18) and (4.19).

Table (4.18) Influence of admixtures of food additive on the saccharin determination.

No	Food additive	Mole ratio to saccharin	Absorbance at 510 nm	Recoveries %
	0	0	0.340	100.00
1	Glucose	200	0.340	100.00
		400	0.340	100.00
		800	0.340	100.00
		1600	0.340	100.00
2	Sucrose	200	0.340	100.00
		400	0.340	100.00
		800	0.340	100.00
		1600	0.340	100.00
3	Dulcin	4	0.340	100.00
		8	0.340	100.00
		16	0.340	100.00
4	Sorbic acid	0.4	0.380	111.76
		0.8	0.380	111.76
		1.6	0.410	120.59
		8.0	0.505	148.53
5	Benzoic acid	0.4	0.415	122.06
		0.8	0.455	133.82
		1.6	0.475	139.71
		8.0	0.635	186.76

Table (4.19) Influence of admixtures of food additive on the saccharin determination.

No	Food additive	Mole ratio to saccharin	Absorbance at 510 nm	Recoveries %
6	Citric acid	0.8	0.630	185.29
		2.0	0.960	282.35
		4.0	1.500	441.18
		8.0	>2	
7	Sodium chloride	0.8	0.590	173.53
		4.0	0.865	254.41
		8.0	1.060	311.76
		16.0	1.170	344.12
8	Sodium cyclamate	8	0.960	282.35
		16	1.220	358.82
		40	1.480	435.29
		80	1.840	541.18
9	Sodium bicarbonate	2	0.060	17.65
		4	0.040	11.76
		6	0.020	5.88
		8	0.025	7.35
		20	0.030	8.82
		40	0.040	11.76
		80	0.055	16.18

Experimental :

0.5 ml (2.5×10^{-3}M) saccharin

7.0 ml acetone

1.00 g ferroin-impregnated silica gel matrix

+ food additives added as follows :

1. Glucose (1M) 0.25-2.0 ml
2. Sucrose (1M) 0.25-2.0 ml
3. Dulcin (0.1M) 0.05-0.20 ml
4. Sorbic acid (0.01M) 0.05-1.0 ml
5. Benzoic acid (0.01M) 0.05-1.0 ml
6. Citric acid (0.01M) 0.1-1.0 ml

7. Sodium chloride (0.01M) 0.1-2.0 ml

8. Sodium cyclamate (0.1M) 0.1-1.0 ml

9. Sodium bicarbonate (0.1M) 0.1-1.0 ml
(0.01M) 0.25-0.75 ml,

the mixture is diluted to 10.0 ml with distilled water

shaking time : 5 minutes,

filter and measure absorbance at 510 nm.

The results are given in the table show that there is no interference from other sweetening agents glucose, sucrose and dulcin. Sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride gave a positive error. The presence of sodium bicarbonates resulted in very low recovery for saccharin.

(11) Applications

Saccharin tablets (187) may be prepared by mixing a mixture of saccharin and sodium bicarbonate, or saccharin sodium followed by compression to form the tablets. Unless otherwise specified, tablets each containing the equivalent of 12.5 mg of saccharin are supplied.

The content of saccharin as reported in the standards is to be in the range 90.0-110 % of the prescribed or stated amount calculated as $C_7H_5NO_3S$. Sodium saccharin tablets (soluble saccharin) usually contains 15, 30, 60 mg of saccharin sodium.

Two samples of saccharin tablets (A) and (B) from different manufacturers were examined in order to check the validity of the proposed procedure for saccharin determination. The saccharin content of each of ten tablets was determined in order to find out the degree of

variation in saccharin content from tablet to tablet. Some statistical parameters were determined. The correlation between the amount of saccharin found and the amount declared on the label served as a guide to the applicability of the proposed method.

A study was made to ascertain the optimum pH at which ferroin is quantitively desorbed by forming the ion-pair $\left[(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right]$ It has been found in this work that adding 0.80 ml of H_2SO_4 2% (v/v) to each tablet was sufficient to eliminate the bicarbonates and adjust the pH on the range 4.9-5.2. The same pH value is used for the saccharin standard solutions. The same pH at which ferroin does not desorbes when the matrix (ferroin-impregnated silica gel) is shaken with amount of distilled water.

In tables (4.20) and (4.21), the correlation between the amount of saccharin found and the amount of declared on the lable is shown. The results were obtained by using the recommended procedure for tablet saccharin (direct method).

Some statistical parameters were calculated from the results and are shown in table (4.22).

Experimental : (for tables 4.20 and 4.21)

2.0 ml of saccharin sample solution (one tablet/100 ml),

1.0 ml of distilled water,

7.0 ml of acetone,

1.00 g ferroin-impregnated silica gel matrix,

shaking time = 5 minutes,

filtration through filter paper (Whatman No 41)

Absorbance measured at 510 nm-Absorbance values for these solutions range from 0.355 to 0.520.

Table (4.20) Analysis of saccharin tablets - sample (A) *

Number	wt. of tablet (mg)	Saccharin found (mg)	Claim%
1	59.4	12.720	101.60
2	61.8	11.220	89.76
3	59.5	14.200	113.60
4	59.4	12.590	100.72
5	59.9	12.365	98.92
6	59.7	13.510	108.08
7	59.1	13.970	111.76
8	60.7	13.510	108.08
9	57.5	15.115	120.92
10	61.6	13.970	111.76

* Sample (A) = Thornton & Ross Ltd.

The saccharin claimed = 12.5 mg per tablet

Wt. = weight.

Table (4.21) Analysis of saccharin tablets - sample (B) *

Number	wt. of tablet (mg)	Saccharin found (mg)	Claim%
1	60.06	11.793	94.34
2	59.10	11.908	95.26
3	59.40	13.969	111. 75
4	60.10	11.335	90.68
5	60.00	12.595	100.76
6	59.00	12.137	97.10
7	57.60	12.137	97.10
8	60.70	11.221	89.77
9	58.70	13.740	109.92
10	59.80	11.221	89.77

* Sample (B) The Wallis lab.

The saccharin claimed = 12.5 mg per tablet

Wt. = weight.

Table (4.22) Statistical data for saccharin tablets analysis

Number	Statistical parameter	Sample (A)	Sample (B)
1	Mean weight of tablet(mg)	59.860	59.500
2	Mean saccharin found (mg)	13.300	12.206
3	Mean claim (percent)	106.520	97.640
4	Standard deviation found (mg)	1.120	0.976
5	Standard deviation found (percent)	8.350	7.996
6	Standard deviation from the theoretical value (declared value) (mg)	1.400	1.024
7	Standard deviation from the theoretical value (declared value) percent	11.200	8.195

The results reported above showed a variation in content of saccharin from tablet to tablet but this variation is within the permitted amounts as has been mentioned previously in this section. This demonstrates the applicability of the method for saccharin tablets.

The method of standard-additions is also applicable in which has the advantage of compensating for variations caused by physical and chemical interference in the sample solution. Another advantage of this method that it is a confirmation method since at the same time calibration graph is obtained. The advantage of the direct method is that it is quicker for a large number of samples for routine analysis hence only one calibration curve is needed.

The proposed method can be adapted to the determination of saccharin in soft drinks after removal of the interfering materials. However, these substances can be eliminated by a preliminary treatment, for example, the low calorie soft drinks and beverages containing benzoates and sorbates as preservatives can be removed by pre-extraction twice with carbon tetrachloride (169). Chlorides can be eliminated by passing the sample through a column of anion exchange resin before the extraction of saccharin by diethyl ether.

4.4 Feasibility of Atomic Absorption Spectrophotometry to Saccharin Determination Employing the Desorption of Ferroin from Silica Gel

Some problems are encountered in the method employing nitrobenzene solvent extraction technique for saccharin determination with ferroin. These were discussed in chapter three (see 3.3.2.1). The replacement of nitrobenzene was not possible, as also shown previously

in chapter three, but the stripping of ferroin back to the aqueous phase was found more practical.

As an alternative approach; the technique discussed in the previous sections has proven to be a suitable and a convenient means for preparing the sample for indirect AAS determination. The use of organic solvents such as ethanol, methanol and acetone, makes the determination by AAS more feasible and increases the sensitivity of the method since the orgaic solvent is nebulized more efficiently than nitrobenzene and smoke problems are eliminated.

Thus, by using the technique of desorption of ferroin on silica gel, one can use less toxic and more suitable solvents.

In this section, the feasibilty of AAS to saccharin determination using the desorption technique of ferroin on silica gel is examined. The suitability of solvents ethanol, methanol and acetone employed for this determination is discussed.

Experimental

(i) Reagents

The same as that in the spectrophotometry method (section 4.2)

(ii) Apparatus

The atomic absorption measurements were made using a Perkin-Elmer atomic absorption spectrophotometer model 460 and an iron hollow-cathode lamp with the following instrumental conditions : wavelength 248.3 nm; slit width 0.2 nm; air pressure 30 Psi and acetylene pressure 8 Psi.

(iii) Analytical Procedure

The same samples prepared previously for the calibration curves for the spectrophotometric method, were been used in the AAS measurements (see the Recommended Analytical Procedures section). Solutions of desorbed ferroin using varying amounts of saccharin are aspirated into the flame.

Results and Discussion

(1) Calibration Curves

Under the above mentioned conditions, the calibration curves were found to be linear for the range of saccharin concentrations in the solutions tested as follows :

distilled water		from $1.25X10^{-4}$M to $5.0X10^{-4}$M
ethanolic solution	60%	from $7.5X10^{-5}$M to $5.0X10^{-4}$M
methanolic solution	80%	from $7.5X10^{-5}$M to $4.0X10^{-4}$M
acetone solution	70%	from $2.5X10^{-5}$M to $5.0X10^{-4}$M

From the graphs shown in figures (4.20), (4.21) and (4.22), the following points may be noted :

(1) the range of linearity is about the same as that in the spectrophotometric method;

(2) with acetone as solvent, the limits of determination has found to be extended more than in the spectrophotometric method (from $5.0X10^{-5}$M to $2.5X10^{-5}$M saccharin concentration);

Fig. (4.20) Calibration curves for saccharin determination by flame-AAS method (wide range)

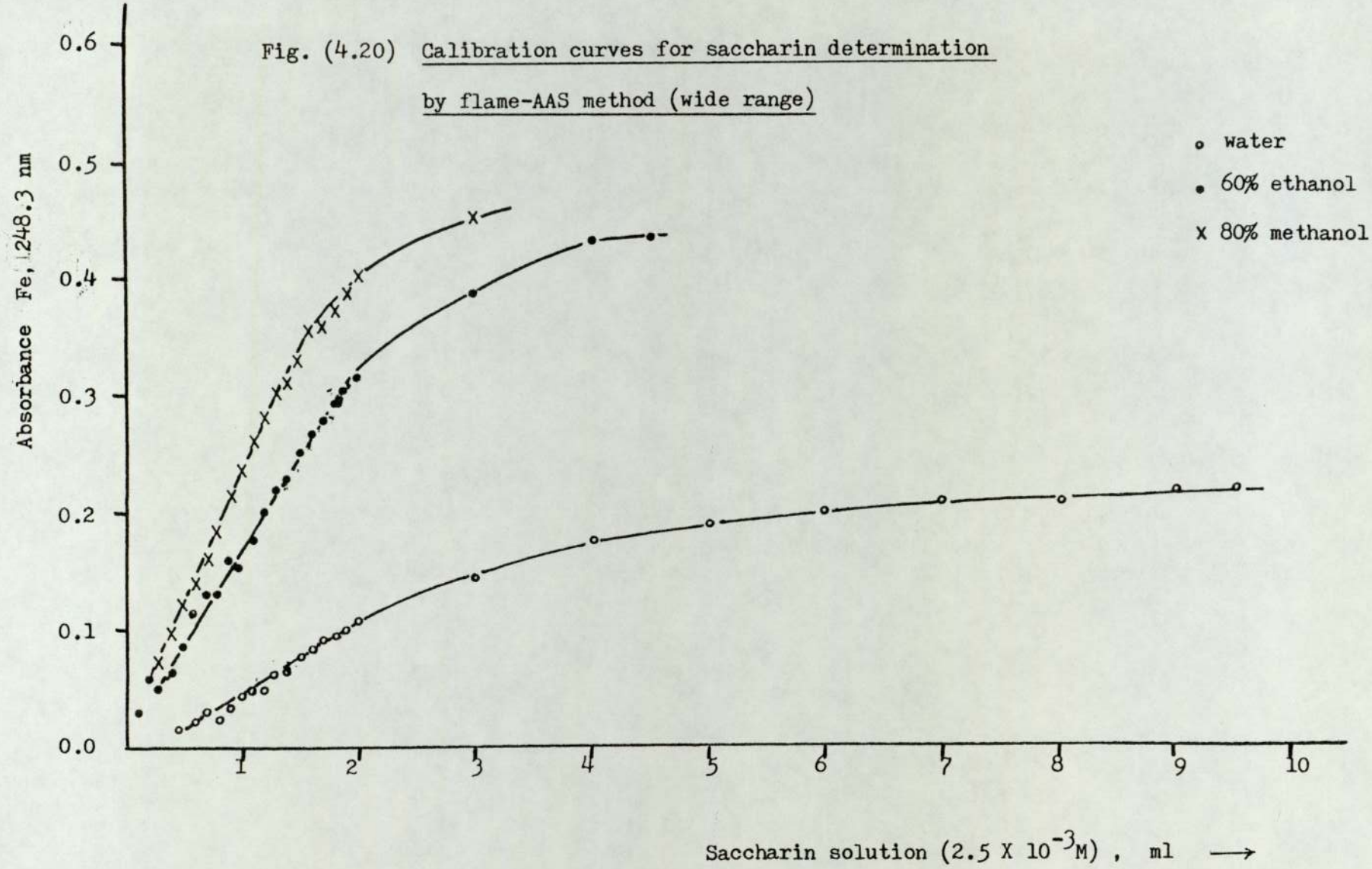


Fig. (4.21) Calibration curves for saccharin determination by flame-AAS method (linear range)

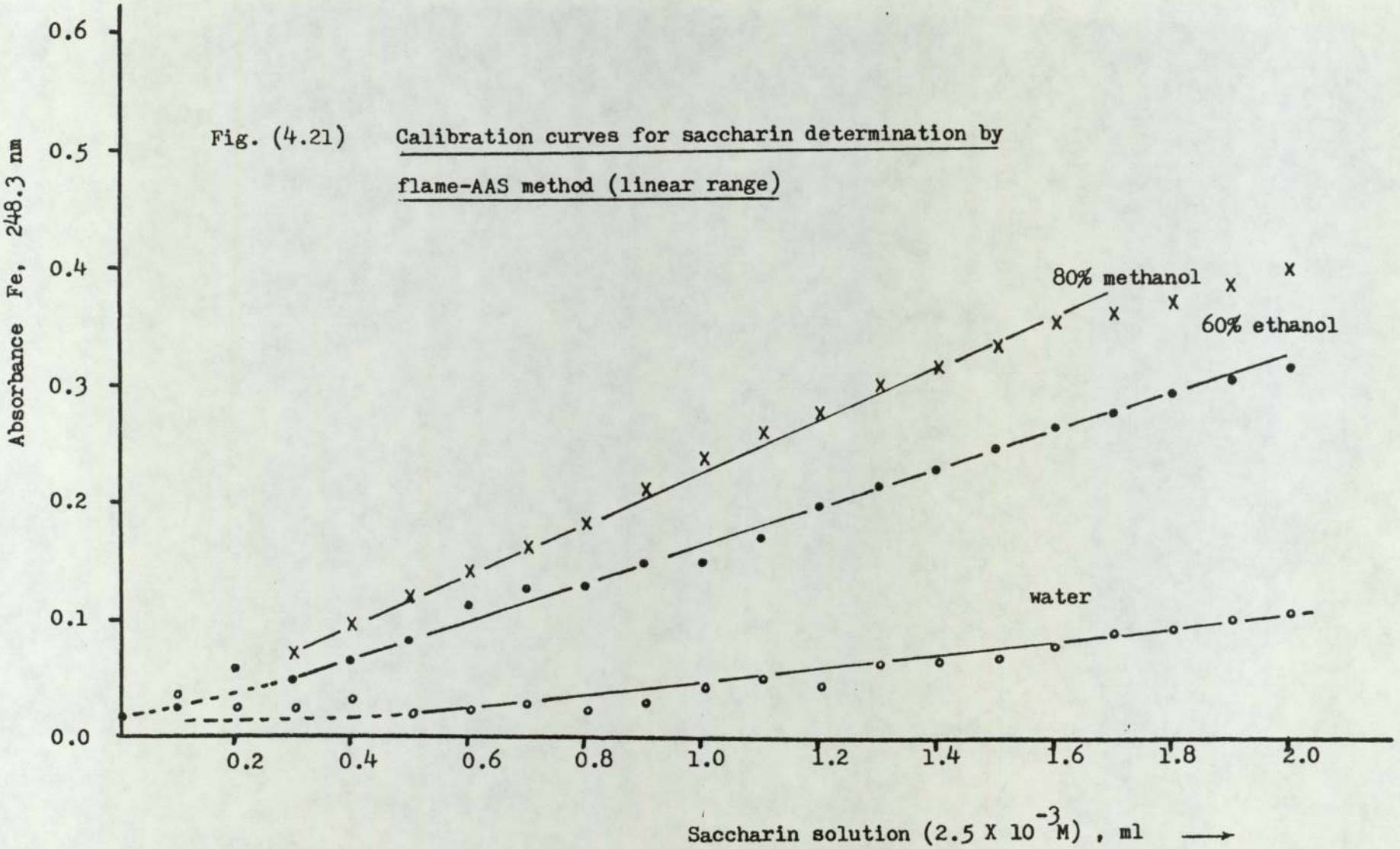
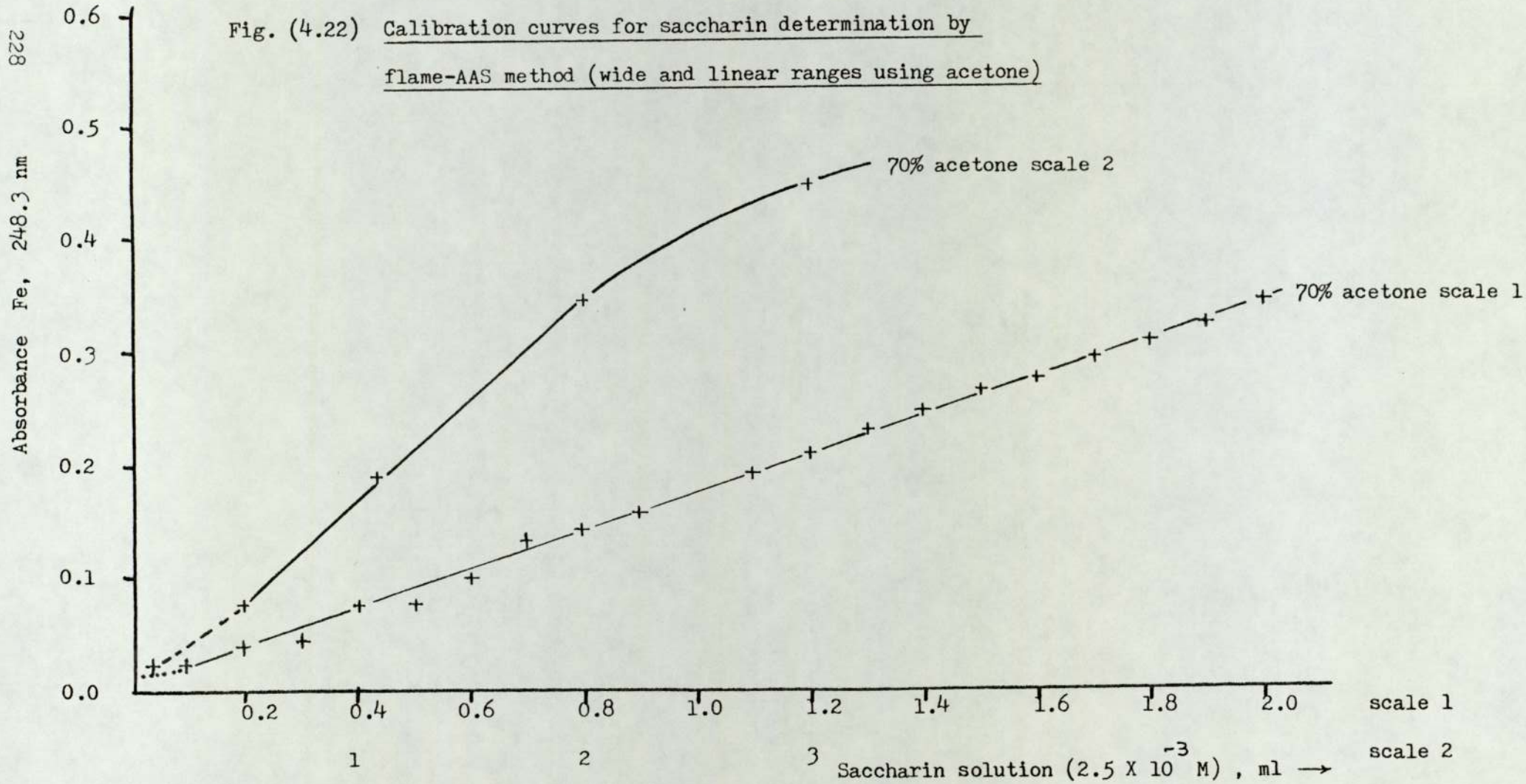


Fig. (4.22) Calibration curves for saccharin determination by flame-AAS method (wide and linear ranges using acetone)



(3) with methanol as the solvent, the graph tends to be curved at absorbances higher than 0.35 absorbance units;

(4) the absorbance values when using the methanol, are higher than that of ethanol and water;

(5) the absorbance values when using acetone are higher than the ethanol values but lower than the methanol vlaues.

Tables (4.23a) and (4.23b) show the absorbance values of iron using varying amounts of saccharin.

(2) Solvent Enhancement Effect on Flame-AAS Measurements

The use of organic solvents that are miscible with water, to enhance an elements response, was at one time in common use. Industrial methylated spirits, iso-propylalcohol and acetone were some of the most commonly used. The effect of such a solvent is entirely physical. The nebulization efficiency is improved, so that a greater proportion of small dropletes and hence absorbing species is carried to the flame. Ketones and alcohols are commonly added to aqueous solution to obtain improved nebulization rate and/or efficiency and hence higher sensitivity.

There are two factors to be considered when examining the significant part played by solvents. Firstly, the effect of solvent on the nebulization of solutions; secondly, the effect of the introduction of a solvent on the properties of the flame, i.e. the temperature and composition of the products of combustion. Considering the second factor, organic solvents introduced into flame should be regarded as an additional source of fuel gas. The most important factor is increased nebulization rate for solvents with a viscosity lower than water and

Table (4.23a) AAS measurements-calibration curves.

Saccharin solution ($2.5X10^{-3}M$) ml	Absorbance iron 248.3 nm line			
	water	ethanolic soln. 60%	methanolic soln. 80%	acetone soln. 70%
0.0	0.031	0.020	0.020	0.021
0.1	0.041	0.033	0.035	0.018
0.2	0.027	0.063	0.076	0.038
0.3	0.027	0.050	0.073	0.046
0.4	0.038	0.065	0.099	0.077
0.5	0.021	0.087	0.124	0.076
0.6	0.025	0.117	0.143	0.100
0.7	0.034	0.133	0.165	0.135
0.8	0.025	0.132	0.188	0.146
0.9	0.033	0.156	0.217	0.159
1.0	0.049	0.152	0.240	--
1.1	0.053	0.176	0.263	0.193
1.2	0.049	0.205	0.283	0.212
1.3	--	0.221	0.303	0.235
1.4	0.069	0.233	0.317	0.250

Table (4.23b) AAS measurements-calibration curves.

Saccharin solution ($2.5X10^{-3}M$) ml	Absorbance iron 248.3 nm line			
	water	ethanolic soln. 60%	methanolic soln. 80%	acetone soln. 70%
1.5	0.072	0.252	0.333	0.268
1.6	0.085	0.268	0.357	0.278
1.7	0.098	0.282	0.363	0.297
1.8	0.098	0.297	0.373	0.312
1.9	0.105	0.306	0.389	0.324
2.0	0.113	0.319	0.404	0.346
3.0	0.149	0.387	0.454	0.446
4.0	0.180	0.435	--	--
5.0	0.190	0.434	--	--
6.0	0.205	--	--	--
7.0	0.215	--	--	--
8.0	0.210	--	--	--
9.0	0.222	--	--	--
10.0	0.222	--	--	--

smaller droplet formation. This provides a higher nebulization efficiency and improved atomization efficiency. In general, high nebulization efficiency is favoured by low viscosity and high density. Characteristics of some solvents used for nebulization solutions are shown (186), in table (4.24).

Table (4.24) Characteristics of some solvents.

Solvent	Density (g/cm^3)	Boiling point (^oC)	Coefficient viscosity P(20^oC)	Surface tension (dyne/cm)	Droplet diameter (μ m)
acetone	0.79	57	3.3	23.7	13.1
methanol	0.79	65	5.9	22.6	13.8
ethanol	0.79	79	12.0	22.8	15.3
nitrobenzene	1.73	211	19.8	42.6	16.4
water	1.00	100	10.0	73.0	19.0

Severe toxicity problems are associated with the use of nitrobenzene. As discussed early in chapter three, nitrobenzene is a good extractant for the ion-pair $\left[(Fe\ Phen_3)^{2+};\ (saccharin)_2^-\right]$ because of its high dielectric constant and its immiscibility with aqueous solutions. However, there are organic solvents with high dielectric constant but because they are miscible with water they are not employed in solvent extraction of the ion-pair. Organic solvents such as ethanol, methanol

and acetone are employed in the present work using the desorption technique of ferroin on silica gel. These solvents have advantages such as; miscibility with water in various proportions, high in dielectric constant i.e. good solvents for the saccharin and hence for the ion-pair, very inexpensive and the health hazards associated with these solvents are not particularly severe.

Experiments in this work has established that the use of organic solvents ethanol, methanol and acetone mixed with water (60, 80, 70 % respectively) are superior to aqueous solutions from the point of view of sensitivity of measurements. The sensitivity for 1% absorption ($C_{1\%}$) for saccharin is found to be as in the table (4.25).

Table (4.25) Sensitivity of the indirect AAS method.

Solvents	Sensitivity ($C_{1\%}$)
water	20.46 μM
60% ethanolic solution	6.60 μM
70% acetone <<	6.06 μM
80% methanol <<	4.62 μM

It is noticeable from the above results, that there is enhancement in sensitivity in the order of water < 60% ethanolic solution < 70% acetone

solution <80% methanolic solution. In spite of the lower viscosity of acetone, compared with the value of methanol, the results show decreasing absorbance values and hence a decrease in the sensitivity for acetone. This result may be explained by the concentrations of the organic solvents present in these solutions in which the enhancement increases with increasing proportion of organic solvent.

CHAPTER FIVE

N-NITROSAMINES: CHEMICAL, ENVIRONMENTAL AND ANALYTICAL ASPECTS INCLUDING POSSIBLE INDIRECT AAS METHODS FOR N-NITROSAMINE DETERMINATION

5.1 Introduction

(1) Environmental Aspects

The term 'food additives' normally relates to chemicals which are added to any food during the course of manufacture and which serve some well-defined technological or nutritional purpose. On the other hand food contaminants, are compounds not deliberately added to food and may be either of natural or of technological origin. The first type of contamination may be compound; naturally occurring in food (e.g. alkaloids) or, arising from natural contamination by micro organisms or arising directly from environmental cross-contamination (as trace metals and polynuclear aromatic hydrocarbons). The second type of contamination may arise from :

(a) deliberate treatment during production (agricultural pesticides),

(b) incidental treatment during processing and distribution (solvent residues, trace metals),

(c) delibrate addition during preparation and processing (smoke constituents, preservatives, antioxidants, colours, flavours), and

(d) secondary contaminants arising from the previous kind of contamination (c) (e.g. nitrosamines from nitrite). The terms: 'intentional food additives' and 'unintentional additives' are also in use.

In chapters three and four, analytical methods have been developed for the determination of saccharin as 'food additive'. In the present chapter, N-nitrosamines are taken as an example for a compound of the 'food contaminant' class.

N-Nitrosamines have become a recent problem for humans since

recent reports have demonstrated that these kind of chemicals are widely distributed in the environment : soil, water, and urban air. They are also formed by chemical reactions in the human stomach and during cigarette smoking. The carcinogenic and other biological actions of N-nitrosamines have become of current interest. This has increased the need for a reliable and simple analytical method for detection and determination of trace level of these compounds.

The N-nitrosamines are considered to be among the most potential hazardous of all chemical carcinogenic agents (188) to man. Recently, great concern has arisen over these compounds in human food supply and their possible link to cancer in man,table (5.1) shows the ranges of N-nitrosamine concentrations in some foods (189).

Table (5.1) N-Nitrosamines in some foods

Food	N-Nitrosamine (μg/Kg)
Fish	
smoked herring	0.5-9.5
kippers & do	0.5-40
smoked haddock	15
" mackerel	0.6
Meat	
smoked sausage	0.8-2.4
Bacon	0.6-6.5
smoked ham	5.7
Mushrooms	
different varities	from 0.4 to 30

N-Nitrosamines are beleived to produce tumors in many species of animals and almost all organs are susceptible to at least one nitroso compound (188). Environmental factors are thought to be a major cause of human cancer, and it is natural to suspect chemicals as being among the prime agents responsible.

Magee and Barnes (188) have given a comprehensive review of the chemical and biological properties of the nitroso compounds. Many N-nitroso compounds have been tested for carcinogenicity in animals and it has been found that 80% of them have produced tumors of one kind or another.

The initial research on nitrosamine toxicity and carcinogenicity was stimulated by human poisoning in an industrial situation (190). It was not until the 1960's that consideration was given to other possibilities for contact with nitrosamines, when an out-break of liver disease in sheep was caused by 30-100 ppm of dimethylnitrosamine present in a herring meal used in the feed (191). This raised the possibility of nitrosamines occurring in human food, particularly at concentrations low enough to produce no obvious toxic effects yet great enough to produce cancer.

Foods which are processed with nitrite have alerted world wide concern in the food industry. This is due to the possibility that nitrites (used as preservative) could react during curing, storage, or cooking, with amines occurring naturally in certain foods, to form nitroso compounds. The nitrosation reaction could also occur in the stomach and during digestion at a more rapid rate (see fig. 5.1).

$$RR'\text{-}NH + HNO_2 \longrightarrow RR'\text{-}N\text{-}N{=}O + H_2O$$

Fig. (5.1) Reaction of nitrite with a secondary amine to form a nitrosamine.

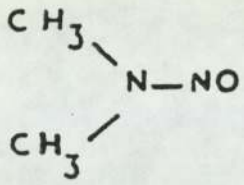
Research is now directed towards a study of the ditributions of nitrates, nitrites, amines and nitroso compounds in the human environment. Nitrite is used for curing meat and fish, to preserve colour, enhance flavour and protect against the danger of botulism. It is also widely spread in environment; in vegetables such as spinach, beets, celery and lettuce and in the well-water supplies. There is also a possibility that nitrosamines could be formed during the burning of tobacco (192). Other possible sources of nitrosamines for human exposure come from the nitrogen compounds in polluted air.

(2) Chemical Aspects

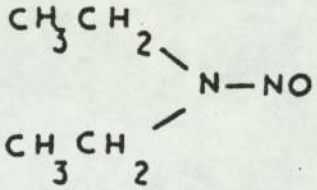
(i) Classification, Structure and General Properties

According to Magee (193), N-nitroso compounds can be divided generally into two groups. One group includes the dialkyl, alkylaryl and diaryl nitrosamines and the other includes alkyl and aryl nitrosamides. These are classified into several groups (194), some examples are given below for N-nitroso compounds.

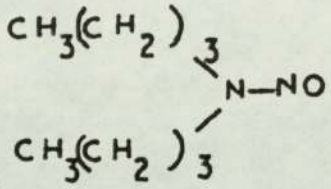
(a) Symmetrical dialkyl (aryl) nitrosamines



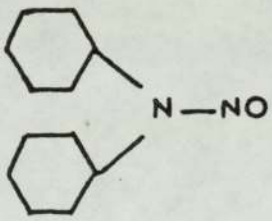
N-nitrosodimethylamine
(DMNA)



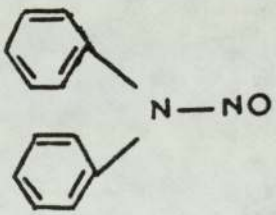
N-nitrosodiethylamine
(DENA)



N-nitrosodi-n-butylamine
(DBNA)

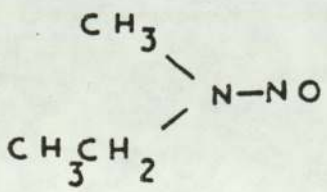


N-nitrosodicyclohexylamine

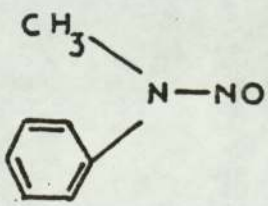


N-nitrosodiphenylamine

(b) Asymmetrical dialkylnitrosamines

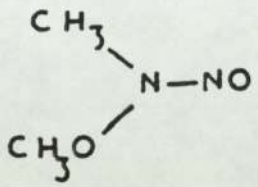


N-nitrosomethylethylamine

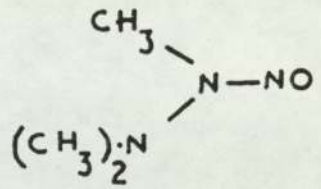


N-nitroso-n-methylphenylamine

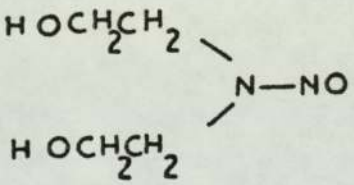
(c) Nitrosamines with functional groups



N-nitroso-N-methyl-O-methyl-hydroxylamine

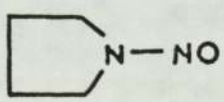


N-nitroso.N,N',N'-trimethylhydrazine

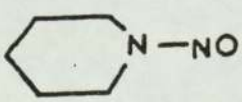


N-nitrosodiethanolamine

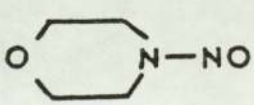
(d) Cyclic nitrosamines



N-nitrosopyrrolidine

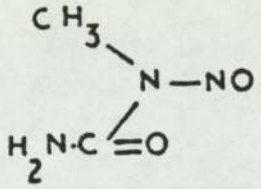


N-nitrosopiperidine

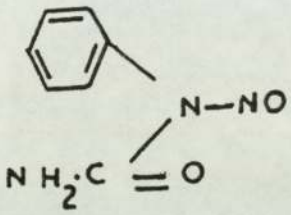


N-nitrosomorpholine

(e) Acylalkylnitrosamines



N-methyl-N-nitrosourea



N-nitrosophenylurea

A wide variety of physical properties are found for nitroso compounds. Whilst N-nitrosodiethylamine is an oily liquid at room temperature and miscible in water, other nitrosamines may be liquid or solid and soluble in water or organic solvents. In general, N-nitrosamines are photosensitive and ultraviolet light will split off the nitroso group to give various products. This property has been analytically utilized in order to determine the N-nitrosamine (see later in this chapter). The boiling points of many nitrosamines are between 150-220°C.

Many of the compounds are partially soluble in water, the degree of solubility varying according to molecular weight. They are readily soluble in organic solvents. They show weakly basic properties; a hydrochloride of N-nitrosodimethylamine is formed by passing hydrogen chloride into an ethereal solution, and the salt is completely decomposed in the presence of ethanol or water.

The simple aromatic nitrosamines, such as N-nitrosomethyl-phenylamine, are low-melting solids or yellowish oils. They are insoluble in water and can be distilled under reduced pressure.

Another important property is the dipole moments of N-nitroso-dialkylamines which indicate considerable polarity in the molecule, but the value is reduced by introduction of phenyl groups (195).

The methods of preparation (196) of N-nitrosamines may be divided into three principal groups :

(1) nitrosation of secondary amines in an acidic medium, (metal nitrites with acid salts of secondary amine). This is the classical reaction for the preparation of N-nitrosamines and may be represented generally by the reaction in fig. (1);

(2) nitrosation with the aid of NOCl, N_2O_3 and N_2O_4;

(3) nitrosation by the cleavage of tertiary amines .

Aliphatic nitrosamines are highly reactive. This high chemical reactivity is due to the possession of nitrosamino-group with four lone pairs of electrons, which make these compounds potential Lewis bases. The occurrence of p-π conjugations with the withdrawal of the electron cloud towards the oxygen atom is responsible for many interesting reactions of nitrosamines.

(ii) Chemical Reactions

Inorganic acids (hydrolysis)

In general, N-nitrosodialkylamines decompose on heating with hydrochloric acid into dialkylamine hydrochloride. This is the reversal of the method of formation.

$$R_1R_2\text{-N-NO} \underset{H_2O}{\overset{HCl}{\rightleftharpoons}} R_1R_2\text{-NH.HCl} + HNO_2$$

The methods of nitrosation and denitrosation have been used as a ready means of preparing pure specimens of secondary amines in ether or toluene solution. Gaseous hydrogen chloride is a more effective denitrosating agent. Also, bromine/sulphuric acid and hydrogen bromide in glacial acetic acid are used as denitrosating agents.

Fan and Tannenbaum (197) examined some N-nitroso compounds and their cleavage by the effect of acids. Their kinetic study, led to the hypothesis that the splitting off of the nitroso group is preceded by protonation, subsequent formation and splitting off of the nitrosonium cation. An equilibrium between nitrosamine and its protonated form is established before separation of the nitroso group :

$$RR_1\text{-}N\text{-}N{=}O + H^+ \rightleftharpoons RR_1\text{-}\overset{+}{N}H\text{-}N{=}O \rightleftharpoons RR_1\text{-}NH + NO^+$$

Denitrosation takes place more easily in hydrochloric acid than in sulphuric or perchloric acid media. The following nucleophilic reaction explains this :

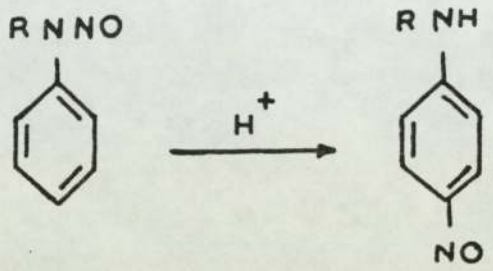
$$RR_1\text{-}\overset{+}{N}H\text{-}N{=}O \xrightarrow{Cl^-} RR_1\text{-}\overset{+}{N}H\text{-}N\begin{matrix}Cl\\O^-\end{matrix} \longrightarrow RR_1\text{-}NH + NOCl$$

The hydrolysis takes place also when N-nitrosodimethylamine is heated with methyl iodide (198) according to the reaction below :

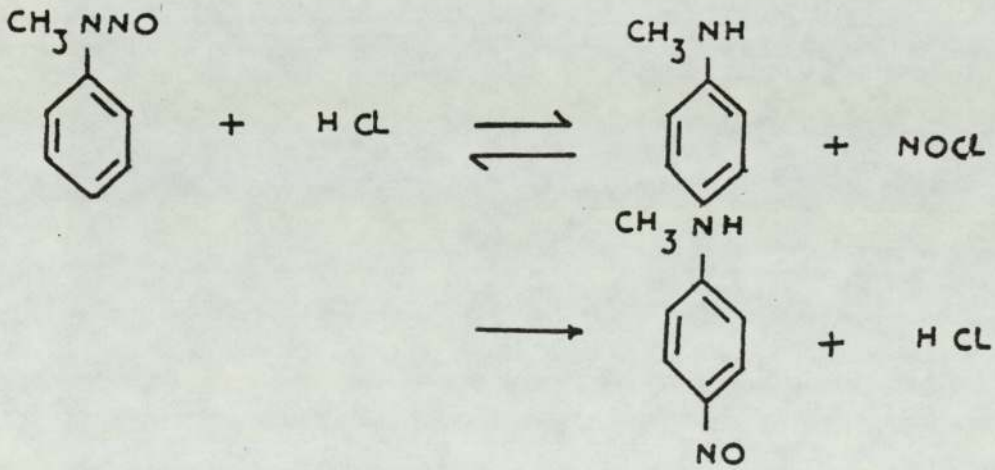
$$(CH_3)_2N\text{-}N{=}O + 4\,CH_3I \longrightarrow 2\left[(CH_3)_4\overset{+}{N}\overset{-}{I}\right] + 2\,NO + I_2$$

The reaction of acids with the aromatic nitrosamines has been

studied (199). It is well-known now that N-nitroso aromatic amines rearrange in acid solution to form the C-nitroso isomer. In most cases that have been recorded the para isomer is formed, viz.



This rearrangement of aromatic nitrosamines on treatment with acids to give ring-substituted isomers is known as the 'Fischer-Hepp rearrangement'. This process involves a reversible de-nitrosation forming the secondary amine and nitrosyl chloride, followed by C-nitrosation in para position as shown in the reaction below :

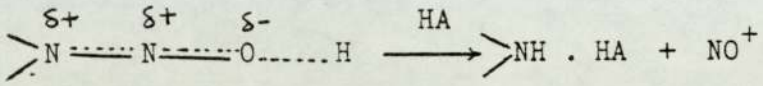


A solution of the N-nitrosamine in dry ether or ethanol with a solution of hydrochloric in ethanol give the best conditions for a high yield of rearrangement product (199).

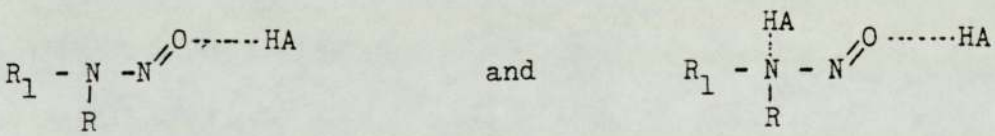
Hydrogen Bonding and Adduct Formation

Hydrogen bonding has a significant role in the denitrosation

reaction (196). It is believed that a more correct mechanism of the denitrosation of aliphatic nitrosamines involves electrophilic attack on the oxygen atom of the nitroso group. The $(NO)^+$ is eliminated as a result of the generation of partial positive charges at the nitrogen atom.



The reaction of nitrosamines with trichloroacetic acid in cyclohexane suggests that the oxygen atom of the nitroso group is involved in adduct formation (200). This reaction yields compounds with the following structures and one or two hydrogen bonds are also involved :



Hydrogen bond formation also takes place with formic acid, acetic acid, phenols, alcohols and amines.

The capacity of nitrosamines to form stable coloured complexes with bromoplatinic acid $(R_2N\text{-}NOH)_2Pt\ Br_6$ (201) has demonstrated the basic nature of the oxygen atom. Other adduct reactions have been reported (196) between dimethylnitrosamines and BF_3, PCl_5, $SbCl_5$, $AlCl_3$ and $ZnBr_2$.

The formation of adducts of the nitrosamines may prove to be useful analytically, Brooks et.al (203) attempted to produce a fluorinated

derivative of N-nitrosodimethylamine with the intention of applying gas chromatography with electron capture detection (i.e. enhance the sensitivity of detection methods).

Transnitrosation

It has already been mentioned in the previous section that the rearrangement of aromatic nitrosamines on treatment with acids, particularly HCl and HBr, gives ring-substituted isomerides and is known as the Fischer-Hepp rearrangement. Morgan and Williams (204) have suggested that transnitrosation occurs in aqueous acid in this rearrangement. Another condition for transnitrosation involves the heating of a nitroso acceptor in an organic solvent with an aromatic nitrosamine (205).

Reduction and Oxidation

The nitroso group undergoes reduction and oxidation reactions. Reduction leads to either the appropriate N,N-substituted hydrazine or the corresponding secondary amine. Many reducing agents have been employed in the reduction process of N-nitrosamines; e.g. zinc in acetic acid, sodium amalgam, and tin in hydrochloric acid. Oxidation yields secondary N-nitramines. Oxidizing agents which have been utilized include hydrogen peroxide with nitric acid, nitric acid and ammonium persulphate and trifluoroperacetic acid. For further details about reduction and oxidation of nitrosamines, the recent review by Fridman et.al (196) may be consulted.

Photochemistry

Photochemical reactions have found extensive application in the development of analytical methods. It is well-known fact now that N-nitrosamines undergo photolytic decomposition. Many workers have investigated methods for the identification and analysis of the nitroso compound utilizing this fact. Some methods are based on the photolytic cleavage of the NO groups. Other methods are based on detection of nitrous acid and secondary amine.

Degradation has been noticed following exposure of N-nitroso-dialkylamines in hexane to sunlight (206) and the products have been analyzed using gas-liquid chromatography and mass spectrometry.

Photochemical transnitrosation processes have been reported (207, 208) which take place between nitrosamines and diphenylamine in the presence of ethanol and palladium(II) chloride, Preussmann et.al (209) have developed a TLC method utilizing this reagent system as a spray reagent.

(3) Analytical Aspects

The main analytical problems of N-nitrosamines are concerned with detection methods which high sensitivity required. Concentrations of parts per million or even parts per billion of these compounds have been found in foods and this needs very careful and well-developed methods of analysis. There is no general method available for isolation of these compounds in a variety of foodstuffs.

Much attention has been given in recent years to the development

of reliable methods of analysis for the detection and determination of nitroso compounds. Significant developments in this area have been achieved and the methods employed are capable of detecting, identifying and measuring small amounts of nitroso compounds in food matrix or in biological samples in which the available sample is very limited. The methods which have been developed are applicable for the determination of N-nitrosamines distributed in the environment and also are useful for monitoring the diets of animals used in laboratory experiments.

The full analytical procedure usually involves an extraction step, followed by distillation, partitioning with solvents, a clean-up step with columns (using ion-exchange resins or celite or alumina) or thin-layer chromatography and finally separation, detection and confirmation (using GLC and MS).

Clean-up procedures may be applied to isolate nitrosamines from matrixes. The low molecular weight nitrosamines (the most highly carcinogenic), are all steam volatile. First stage of most clean-up procedures (after dissolution) is a steam or vacuum distillation. Normally, the distillation is carried out from an alkaline or neutral solution. Solvent extraction has been employed using solvents such as dichloromethane (a primary extractant), acetonitrile/heptane and ether/boiling water. A variety of chromatographic methods are also used for isolation. Most of the methods reported have utilized distillation and solvent extraction procedures in one combination or another as the primary or secondary extraction stages. Ion-exchange resins have been employed also for clean-up step.

Many physical methods of analysis have been reported for nitrosamine detection and estimation. The diagram in fig.(5.2) summarizes

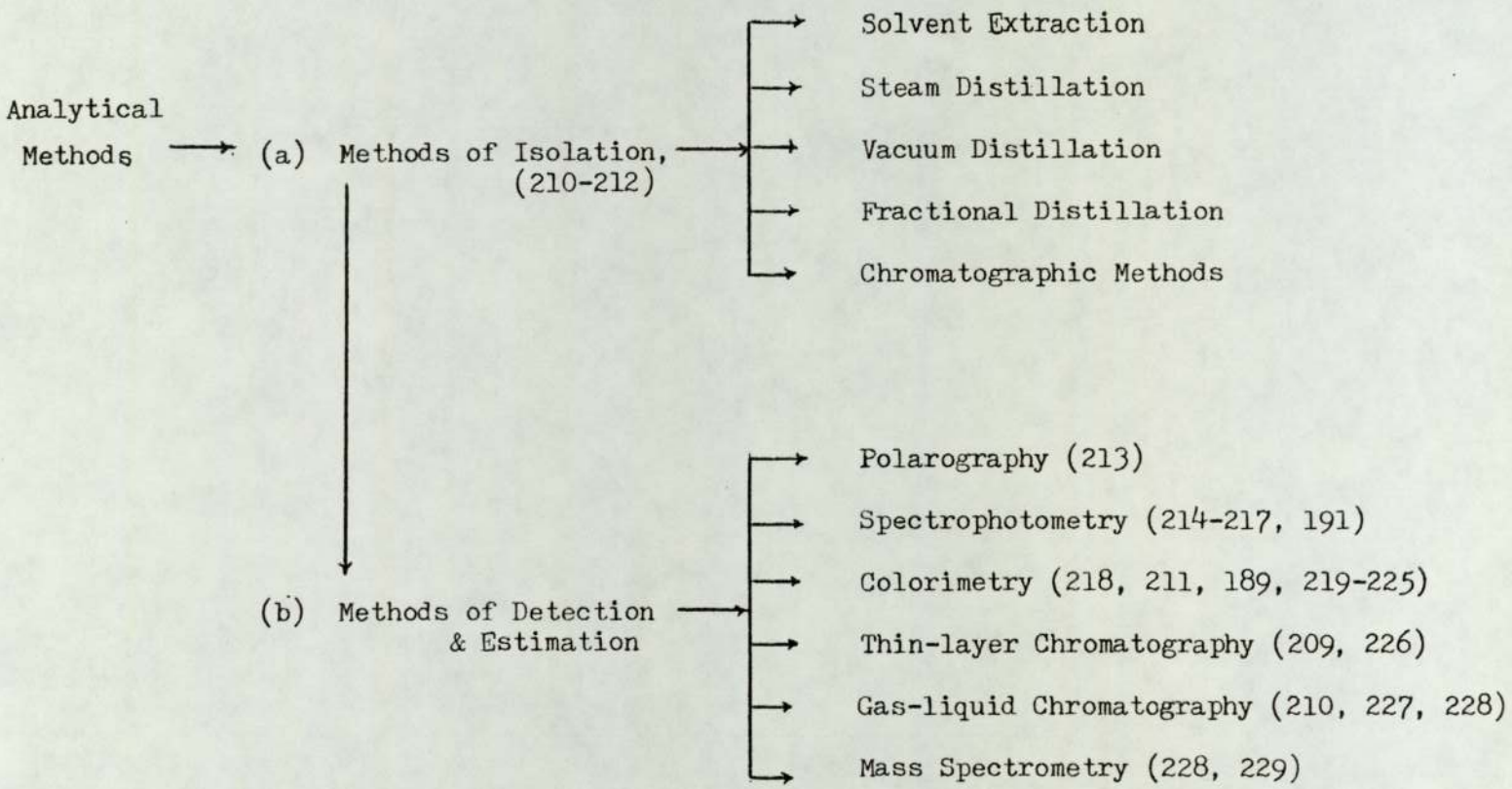


Fig. (5.2) Analytical Methods Available for the Determination of N-Nitrosamines

some methods used for the analysis of N-nitrosamines. Analysis invariably involves two stages;

(a) isolation of the N-nitrosamines by one(or more) of the methods listed followed by

(b) detection and determination using one of the techniques listed.

Some of the methods, such as infrared spectrophotometry and nuclear magnetic resonance spectrometry are specific but they suffer lack of sensitivity. It must be borne in mind that these compounds are at the nano-gramme (ng) level in some cases. On the other hand, ultraviolet spectrometry and polarography have the sensitivity but lack specificity. Many of these methods have found wide applicability in analytical problems because their lower cost compared to a mass spectrometry. Other methods such as TLC and GLC are the most frequently employed techniques in nitrosamine analysis. It appears that GLC followed by mass spectrometry is the most acceptable procedure (228, 229). Most workers now believe that mass spectrometry (or mass spectrometry and gas chromatography) is essential for confirmational work.

Spectrophotometric and Colorimetric Methods

N-nitrosamines as such do not lend themselves to the formation in solution of derivatives useful for spectrophotometric evaluation. However, many workers have utilized measurement of the intensity of uv absorption as a means of qualitative estimation. The colorimetric methods mostly may be carried out after either a reduction process (using lithium aluminium hydride, or Zn/HCl or others), or a photochemical process using uv light irradiation. The reduced products are either secondary amines

or hydrazines or nitrosylbromide and they react with many complexing agents to give coloured solution. The photochemical step leads to nitrite which may be react with another reagent to give a colour.

Alkyl nitrosamines in aqueous solution have been estimated utilizing the absorption band at 230 nm as a means of quantifying the nitroso compounds (214, 215). In order to determine the partition of nitrosamine in the n-heptane acetonitrile system; Eisenbrand et.al (216) have estimated nitrosamines in concentrations 2 to 3 μg/ml and 200 to 2500 μg/ml. Möhler and Mayrhofer (217) have used different solvents in their studies. The UV and IR absorption measurements have been employed as confirmation means for the presence of nitrosamines in herring meal processed with large amounts of sodium nitrite (191, 215).

The disadvantage of these methods their limitation by interferences from the other coextracted substances which also absorb in the UV region.

Neurath et.al (218) have described a colorimetric method, in which the nitrosamines were reduced with lithium aluminium hydride to hydrazine. This was then condensed with 5-nitro-2-hydroxybenzaldehyde to form a benzalhydrazine. The method is applicable to cigarette smoke condensates and on extracts from flour and cheese.

Ender and Ceh (215, 189) have described a method based on reduction of nitrosamine by zinc and hydrochloric acid and condensation of the product with p-dimethylaminobenzaldehyde giving a product which absorb at λ_{max} 458 nm. They have also been reported a reduction method in which the N-nitrosodimethylamine was reduced to dimethylamine and reacted with 4-nitro-4-azobenzoyl chloride to give the corresponding amide. This has an absorption maximum at 336 nm. The detection limit

was found to be 0.3 to 0.4 μg/ml.

The release of nitrite by irradiation of nitrosamines with UV light has been well-known method for the detection and estimation of nitrosamines. Daiber and Preussman (219) have described a colorimetric method based on this property. The resultant solution was made alkaline with sodium carbonate, treated with Griess reagent and the absorbance of the reaction product was measured at 525 nm.

Nitrite release reactions may also carried out using methanol as an inhibitor (220-223). Sensitivity of these methods was found to be in the order of 0.1 μg/ml for N-nitrosodimethylamine. Sander (224) has reported similar method using acetone with sensitivity of 1 μg/ml.

In another approach, a method has been devised (225) describing a reagent system based on HBr in glacial acetic acid. The resultant components were amine and nitrosylbromide. The nitrosylbromide was then reacted with sulphanilic acid; the diazo ion coupled with N-(1-naphthyl)-ethylendiamine and the absorbance was measured at 550 nm. Sensitivity was found to be 1 μg/ml.

(4) Possible Routes to Indirect AAS Mthods

The main aim of this chapter is to introduce a basis for investigating the possibility of developing new methods i.e. utilizing particular chemical reactions. There are many well-known reactions involving nitrosamines could be potentially useful in analytical applications. In the previous sections, such reaction have been discussed.

In brief, the following chemical reactions are worthwhile studying and may be adapted to either the preconcentration procedure or

final stage of the determination.

(a) Reaction of N-nitrosamines and Metal Complexes; Palladium(II) chloride Complexes in Particular

Stable coloured complexes may be formed between N-nitrosamine and bromoplatinic acid (201). Organic hexabromoplatinates such as $\left[(CH_3)_4.N\right]_2PtBr_6$ may be produced by this reaction and extracted into suitable organic solvents, followed by subsequent platinum determination.

Yellow crystalline complexes; such as dichlorodialkylnitrosamine-palladium; $(R_2N_2O)_2\ PdCl_2$, are formed when dialkylnitrosamine is added to palladium chloride. The precipitation of the complex; $\left\{\text{trans-}\left[(C_2H_5)_2N_2O\right]_2PdCl_2\right\}$ has been reported (230). The complexes were prepared by reacting two moles of nitrosamine and a concentrated aqueous solution of sodium tetrachloropalladate(II) according to the reaction below :

$$2\quad RR_1\text{-}N\text{-}NO\ +\ Na_2\left[PdCl_4\right]\ \longrightarrow\ (RR_1\text{-}N\text{-}NO)_2PdCl_2$$

The methyl complex $\left[(CH_3)_2N_2O\right]_2PdCl_2$ is reported to be moderately soluble in cold water and particulary insoluble in non-polar solvents. The butyl complex is insoluble in water and readily soluble in most organic solvents.

Dialkylnitrosamines form 1:1 or 1:2 addition compounds with many metal and non-metal halides (198). Addition compounds have been reported in which nitrosamines were added to the following : PCl_5, $SbCl_5$, $AlCl_3$, $ZnBr_2$, $CdCl_2$, $CoCl_2$, $CuCl_2$ and $PdCl_2$. The chemical reactions with some experimental conditions are listed below :

$$Me_2NNO + SbCl_5 \longrightarrow (Me_2NNO.\ SbCl_5)$$

(1.5g) (6g/10ml CH_2Cl_2) (6.4g)

(dropwise)

$$Me_2NNO + AlCl_5 \longrightarrow (Me_2NNO\text{-}AlCl_3)$$

(15ml) (4.5g) (5.4g)

$$Me_2NNO + ZnBr_2 \xrightarrow{\text{warm}} (Me_2NNO.\ ZnBr_2)$$

(5ml) (10g)

$$Me_2NNO + CdCl_2 \xrightarrow[\text{ether wash}]{\text{warm}} (Me_2NNO.\ CdCl_2)$$

(8ml) (8.1 g)

$$Me_2NNO + CoCl_2 \longrightarrow (Me_2NNO.\ CoCl_2)$$

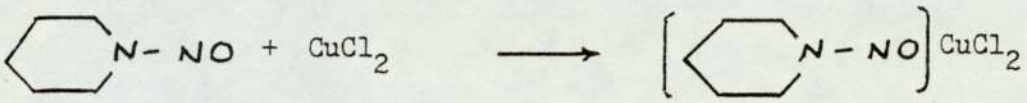
(11ml) (4.7g) (pale blue)

$$Me_2NNO + CuCl_2 \longrightarrow (Me_2NNO.\ CuCl_2)$$

(4ml) { 3.5g/35ml absolute ethanol } { 4.3g brown-red }

$$Me_2NNO + PdCl_2 \xrightarrow[100^\circ C]{\Delta} (Me_2NNO)_2PdCl_2$$

(5ml) (1g) { 1.4g golden yellow }



(2.5 ml) (2g) (3.4g)
brown-red

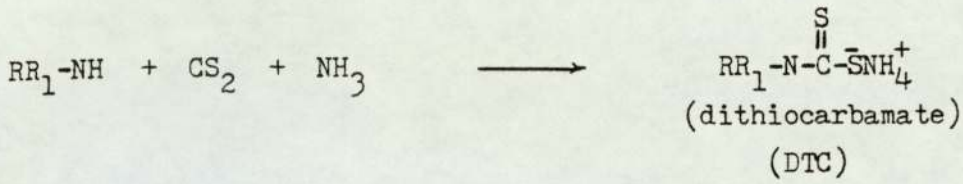
It was thought that some of the above listed chemical reactions may prove to be a useful basis for the development of an analytical method. The method may based on the reaction followed by solvent extraction process in order to extract the complexes. Either, spectrophotometric or AAS methods may be used for colour or metal measurement, respectively. Investigation may lead to the development of; (a) new indirect AAS methods and also possibly, (b) a convenient preconcentration step utilizing column chromatography.

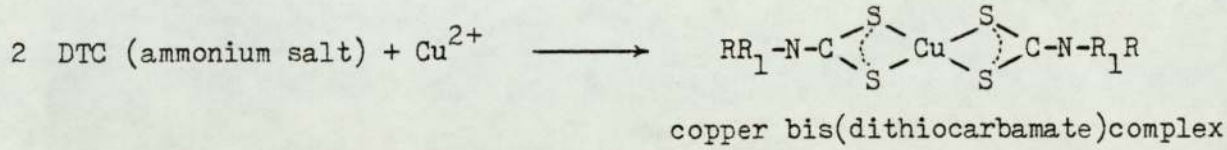
(b) Reaction Involving the Hydrolysis of Nitrosamines

The basis of this approach is to convert the nitrosamine to the equivalent secondary amine, followed by its reaction with carbon disulphide in presence of metal and ammonia to give a dithiocarbamate complex.

$$RR_1\text{-}N\text{-}N{=}O + H^+ \longrightarrow RR_1\text{-}NH\text{-}N{=}O \longrightarrow RR_1\text{-}NH + NO^+$$

(secondary amine)





(R and R_1 = H, Alk, Ar)

The reaction results in the formation of a dialkyldithiocarbamate complex which forms a stable complex with metal ions. The copper complex $\left\{ Cu(DTC)_2 \right\}$ may be then extracted into a suitable organic solvent such as chloroform or benzene (231).

1-Nitrosopyrrolidine in particular (which is formed during frying of bacon of concentration up to 40 μg/kg) could be hydrolysed to the equivalent amine 'pyrrolidine'. This product could then be reacted with carbon disulphide and ammonia to give the well-known extracting reagent; 'Ammonium Pyrrolidine Dithiocarbamate' (APDC). This reagent forms complexes with more than 30 elements (232), and most of these complexes can be extracted into organic solvents. Many applications of APDC have found use in AAS and spectrophotometry. The APDC-metal complexes are soluble in many organic solvents. MIBK and n-amylmethyl ketone are in general, the most satisfactory solvents.

The preceding facts make a determination of N-nitrosamines by AAS a feasibility. Probably, the most important step in such determinations is the hydrolysis procedure.

(c) Reactions Involving Photochemical Trans-nitrosation

The thin-layer chromatographic method reported (209) is a simple and sensitive colour reaction for N-nitroso compounds. In this method a thin spray of the reagent consisting of; 5 parts of a solution of diphenylamine, 1.5% in ethanol, and 1 part of a solution of $PdCl_2$, 0.1% in 0.2% saline, is applied to the layer. Irradiation of the moist plate with ultra-violet light ($\lambda_{max.}$ = 240 nm) for some minutes produces blue to violet spots of nitroso compounds. Detection limit was found to be 0.5 μg nitrosamine.

The mechanism of this reaction attributed to a photochemical trans-nitrosation with transfer of the nitroso group to diphenylamine and colour production of the formed p-nitrosodiphenylamine with the well-known palladium(II) chloride reaction. In this reaction (207) p-nitroso-diphenyl is reacted with palladium(II) chloride in neutral and weakly acid solution to give either a deep red coloured solution or a purplish-brown precipitate, depending on the concentration of the metal. This reaction has found to be extremely sensitive for the detection and determination of palladium. The study has been applied to the colorimetric detection and determination of palladium. Highly coloured complexes are formed between compounds containing p-nitrosophenylamino group and small amounts of palladium (208).

It was thought that, these facts may be useful contribution to the development of new methods employing AAS technique. Investigation of this sensitive reaction may include a solvent extraction step in order to extract palladium complexes which could be formed by photochemical trans-nitrosation. Also, in addition the relationship between the

concentration of nitrosamine to be analysed and the acceptor for nitroso group i.e. diphenylamine could be determined. Spectrophotometric or AAS methods could be applicable in the final stages of this analysis.

Another technique which also may be developed is the violet or blue spots of nitroso compounds produced on colourless plates. They could be isolated for palladium content determination. The AAS method may be applied in this case by direct carbon furnace measurements.

5.2 Experimental, Results and Discussion

"Preliminary Study of the N-Nitrosamine Reaction with Na_2PdCl_4 and its Possible Application to Spectrophotometry and AAS"

The Ultraviolet Spectra of N-Nitrosamines

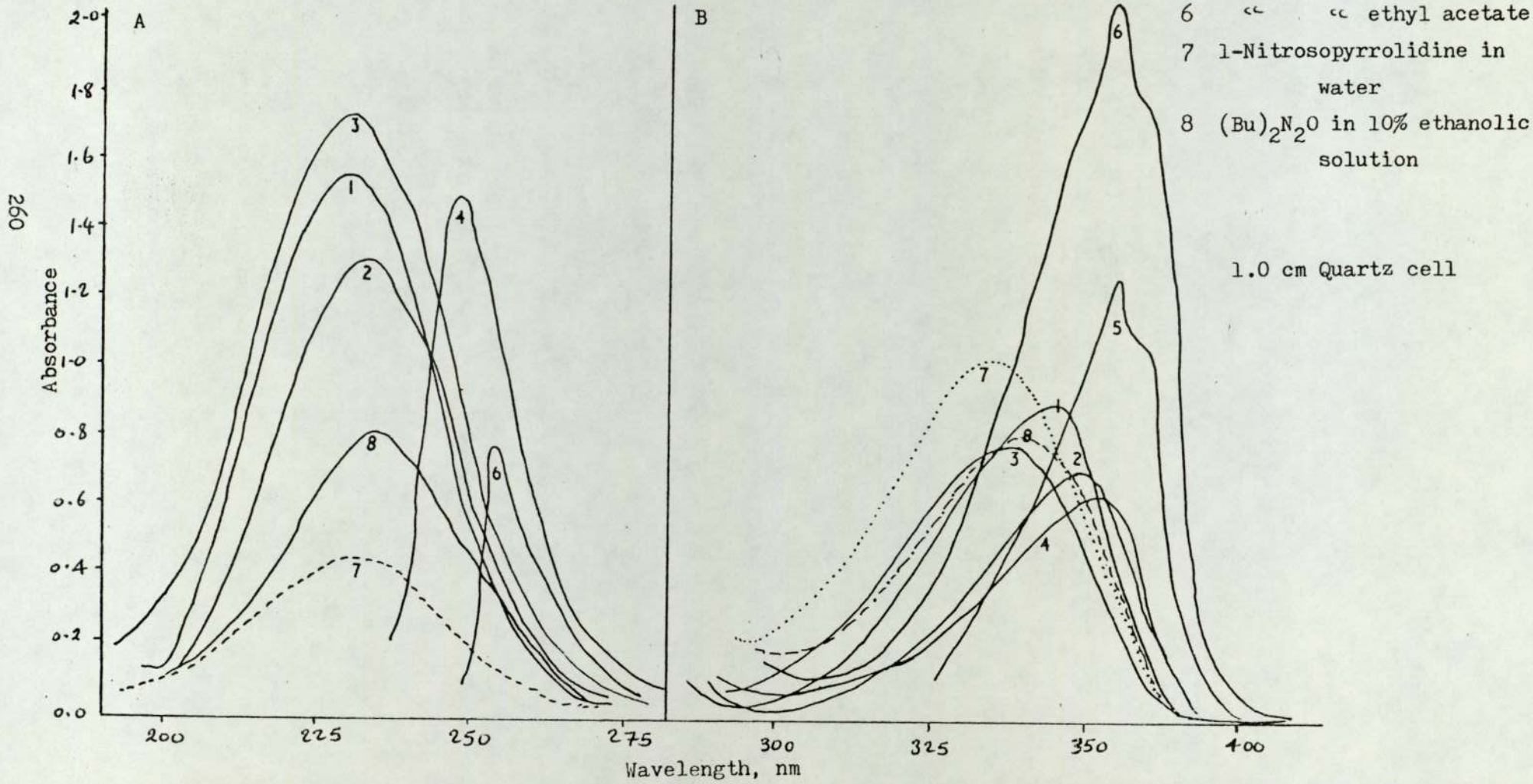
Spectroscopic characteristics of N-nitrosamine have been studied by Hazeldine and Jandar (233, 234). The ultraviolet spectrum of a nitrosamine is characterised by a low-intensity nitrosamine band at 365 nm is solvent-dependant. It has been suggested that the effects due to changes in solvent, supported the idea that the band at 235 nm is characteristic of the N-N bond and that the absorption at the higher wavelength is due to -N=O. The large shift of the main peak due to a change from nonpolar solvents to water (30 nm) is further confirmation of this conclusion.

Table(5.2)shows different $\lambda_{max.}$ for N-nitrosamines using different solvents (see fig. 5.3 A&B).

Table (5.2) Ultra-violet spectra of N-nitrosamines

No	N-nitrosamines	Solvent	$\lambda_{max.}$ (nm)
1	$(CH_3)_2.NNO$	ethanol	345 230
2	$(C_2H_5)_2.NNO$	water	338 230
3	$(C_2H_5)_2.NNO$	ethanol	350 232
4	$(C_2H_5)_2.NNO$	chloroform	354 247
5	$(C_2H_5)_2.NNO$	dichloromethane	358 238
6	$(C_2H_5)_2.NNO$	methyl-iso-butyl ketone	360 -
7	$(C_2H_5)_2.NNO$	ethylacetate	362 253
8	$(C_2H_5)_2.NNO$	carbon tetra-chloride	364 264
9	$(C_4H_9)_2.NNO$	ethanol (10%)	340 233
10	$(C_4H_9)_2.NNO$	methyl-iso-butyl ketone	360 -
11	$(C_4H_9)_2.NNO$	chloroform	356 247
12	$(C_4H_9)_2.NNO$	ethylacetate	360 254
13		water	334 230
14		methyl-iso-butyl ketone	362 -

Fig. (5.3) Spectra of N-nitrosamines in different solvents.



Preparation of Trans-$\left[(C_2H_5)_2N_2O\right]_2 PdCl_2$ Complex

The complex was prepared for this work on the basis of method reported by Schmidpeter (198) as follows :

One gram of palladium(II) chloride was added to 5 ml of diethylnitrosamine and about 50 ml of water. The mixture was heated to 100°C and then cooled. This yielded an air-stable golden yellow crystals. The complex may also be prepared using a concentrated aqueous solution of sodium tetrachloro-palladate(II) (230).

Some Characteristics of the Complex

(a) Melting Point

The melting point of trans-$\left[(C_2H_5)_2N_2O\right]_2 PdCl_2$ complex was found to be 150°C [reported 163°C, (230)]

(b) Elemental Analysis

element	calculated%	found%
C	25.2	24.8
H	5.2	5.2
Cl	18.6	18.6
N	14.7	13.9

(c) Infrared Spectra of the Complex

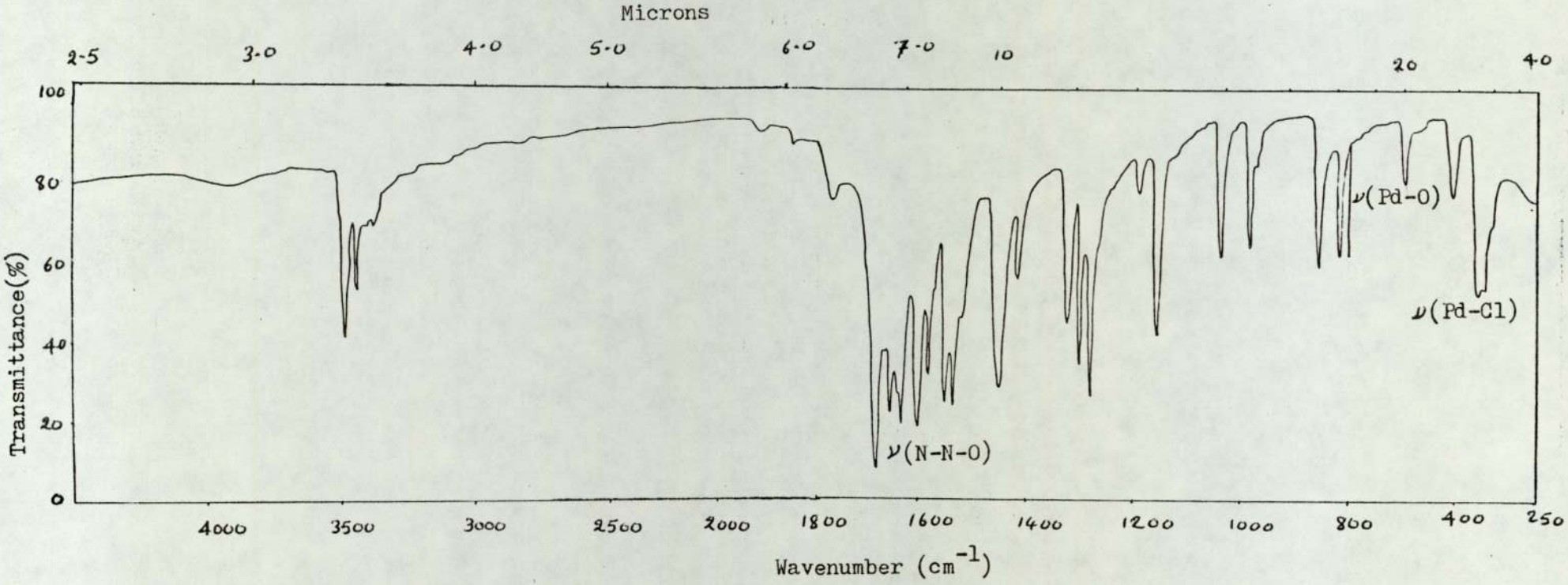
The infrared spectra (shown in fig. 5.4) of the palladium complexes contain a very strong band at 1480 cm^{-1}, which is the frequency of the asymmetric N-N-O vibration reported for gaseous monomeric dialkylnitrosamines (233).

Coordination is from the oxygen atoms to the palladium. The spectra also contain weak bands at 498 cm^{-1} in the region where metal-oxygen ν(Pd-O) stretching bands would be expected. The spectra are less complex above 20μ. A single strong band in the 340-360 cm^{-1} region is characteristic of trans-dichloropalladium(II) complexes ν(Pd-Cl) (235).

(d) Solubility of the Complex in Organic Solvents

No	Solvent	Observations
1	ethanol	soluble-yellow greenish colour-deposit
2	methanol	« « « -clear
3	isopropyl alc.	insoluble-black turbidity after time
4	isoamyl alc.	slightly soluble-yellow colour-deposit
5	acetone	very soluble-yellow colour-clear solution
6	n-amyl methyl ketone	slightly soluble-yellow colour-deposit
7	methylisobutyl ketone	very soluble-yellow colour-clear solution
8	benzene	slightly soluble-yellow colour-orange precipitate.
9	toluene	soluble over night-yellow colour
10	o-nitrotoluene	soluble-yellow colour-clear solution
11	hexane	insoluble
12	carbon tetrachloride	«
13	chloroform	very soluble-yellow colour-clear solution
14	dichloromethane	« « «
15	nitrobenzene	very fine needles formed overnight
16	n-amylacetate	slightly soluble-yellow colour-precipitate
17	ethylacetate	soluble-yellow colour
18	diethyl ether	slightly soluble-yellow colour

Fig. (5.4) IR- Spectra of (Dichlorobis-diethyl-nitrosamine palladium) complex.



KBr disc

Concentration 1.0%

Reference : Air

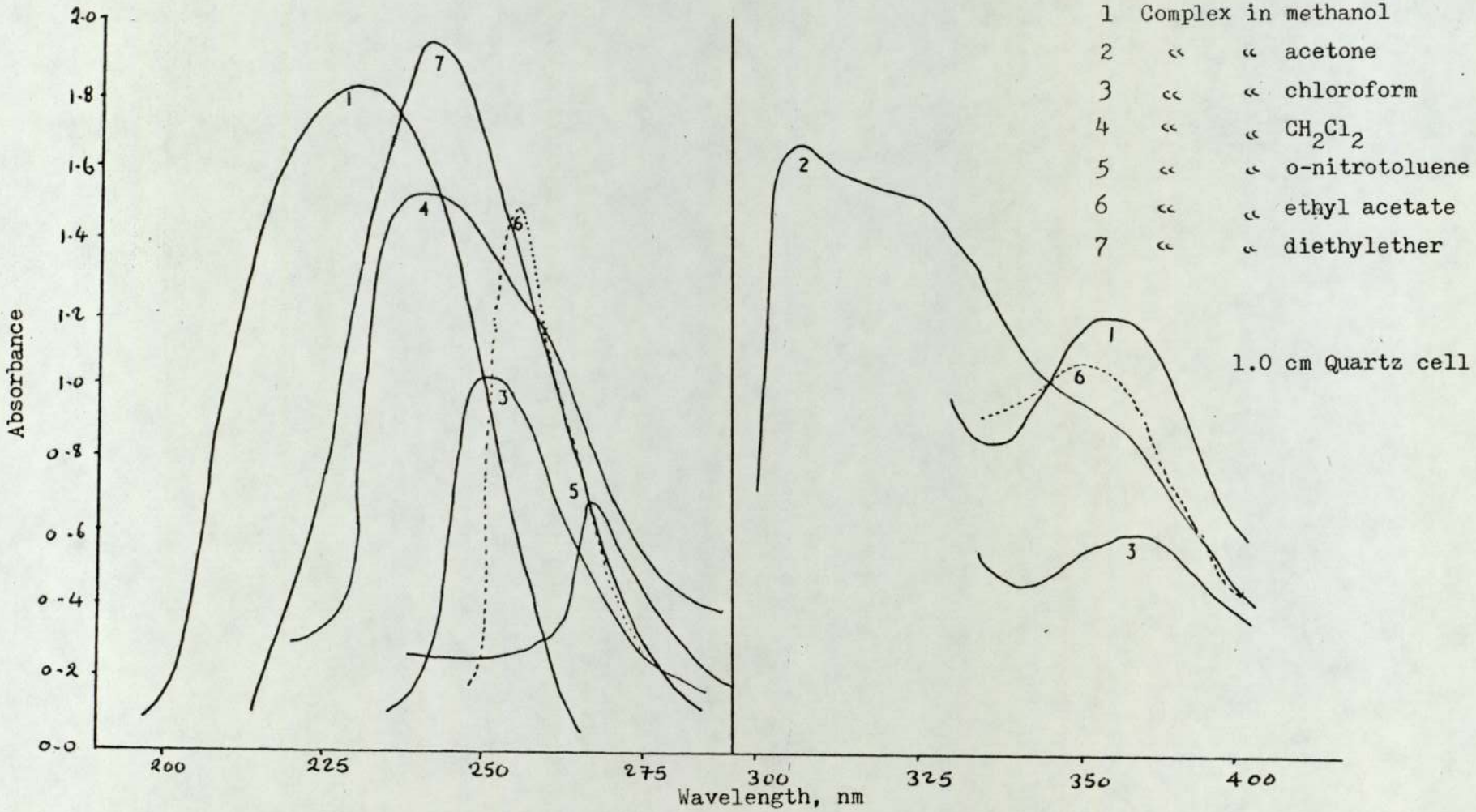
(e) The Ultra-violet and Visible Spectra of the Complex in Different Solvents

The ultra-violet and visible spectra were recorded in order to observe any new peaks which may be present and which may be potentially useful for analytical colorimetric methods. Some results are shown in the table below and some of the spectra shown in fig. (5.5).

Solvent	λ max.	Solvent	λ max.
methanol	408 228	dichloromethane	305 240
		o-nitrotoluene	430
acetone	328		
		ethyl acetate	396 254
methylisobutyl ketone	332		
		diethylether	242
toluene	283		
chloroform	414 251		

There are two peaks for Na_2PdCl_4 in water i.e., at 424 and 209 nm. It is noticeble that the absorption maxima occur at similar maxima for the nitrosamine.

Fig. (5.5) Spectra of the complex $(Et_2N_2O)_2PdCl_2$ in different organic solvents.



(f) Practical Observations on Solvent Extraction System

$(C_2H_5)_2N_2O$ / Na_2PdCl_4 / Organic Solvent

Attempts were made to investigate the practicability of extraction the palladium complex formed when nitrosamine is added to a palladium(II) solution. For this purpose the following reagents have prepared :

Sodium tetrachloropalladate(II) solution (10^{-2}M) :

0.2942 g/100 ml distilled water.

Dimethylnitrosamine solution (10^{-2}M) :

0.07408 g/100 ml H_2O or ethanol.

Diethylnitrosamine solution (10^{-2}M) :

0.10214 g/100 ml H_2O or ethanol.

Dibutylnitrosamine solution (10^{-2}M) :

0.15821 g/100 ml H_2O

1-Nitrosopyrrolidine solution (10^{-2}M) :

0.10012 g/100 ml H_2O

The experiments here were designed to study many solvent extraction systems including different pH's, temperatures and organic solvents. The following systems have been examined :

1. $(C_2H_5)_2NNO$ - Na_2PdCl_4 - MIBK
2. " " - CCl_4
3. " " -$CHCl_3$
4. " " -ethylacetate
5. $(C_2H_5)_2NNO$ - Na_2PdCl_4 - CH_2Cl_2
6. $(C_4H_9)_2NNO$ - Na_2PdCl_4 - MIBK

7. $(C_4H_9)_2NNO$ - Na_2PdCl_4 - CCl_4

8. " " - ethylacetate

9. " " - diethylether

10. " " - benzene

11. " " - toluene

12.  NNO - Na_2PdCl_4 - MIBK

13. " " - $CHCl_3$

These systems showed no evidence of a useful absorption peak when the extract was examined in the uv and visible ranges. The absorption profiles were found to be similar to those of nitrosamines. However, there is some enhancement in absorbance for the extracted species, in particular when using MIBK solvent. It was also noticeable that Na_2PdCl_4 is soluble in some organic solvents and may be extracted into MIBK to give a satisfactory blank reading. N-Nitrosamines are also extracted into MIBK.

When considering an AAS method the extraction of nitrosamines into MIBK is not the problem but the palladium(II) solution causes problems in such systems.

Figures 5.6,5.7 and 5.8 show the spectra obtained from quantitative extraction process for $(C_2H_5)_2NNO$, mixture $(C_2H_5)_2NNO$-Na_2PdCl_4 (immediate extraction) and the same mixture (extraction ofter 24 hr, solutions in contact). An enhancement of absorption of the extracted species in MIBK has been noticed in which the maximum absorption occurs at the same maximum for N-nitrosamines. This enhancement is ploted vs. the nitrosamine concentration added to the mixture. The enhancement values are shown in the following table.

Fig. (5.7) Spectra of extracted species, (nitrosamines + Na_2PdCl_2) into MIBK- immediate extraction.

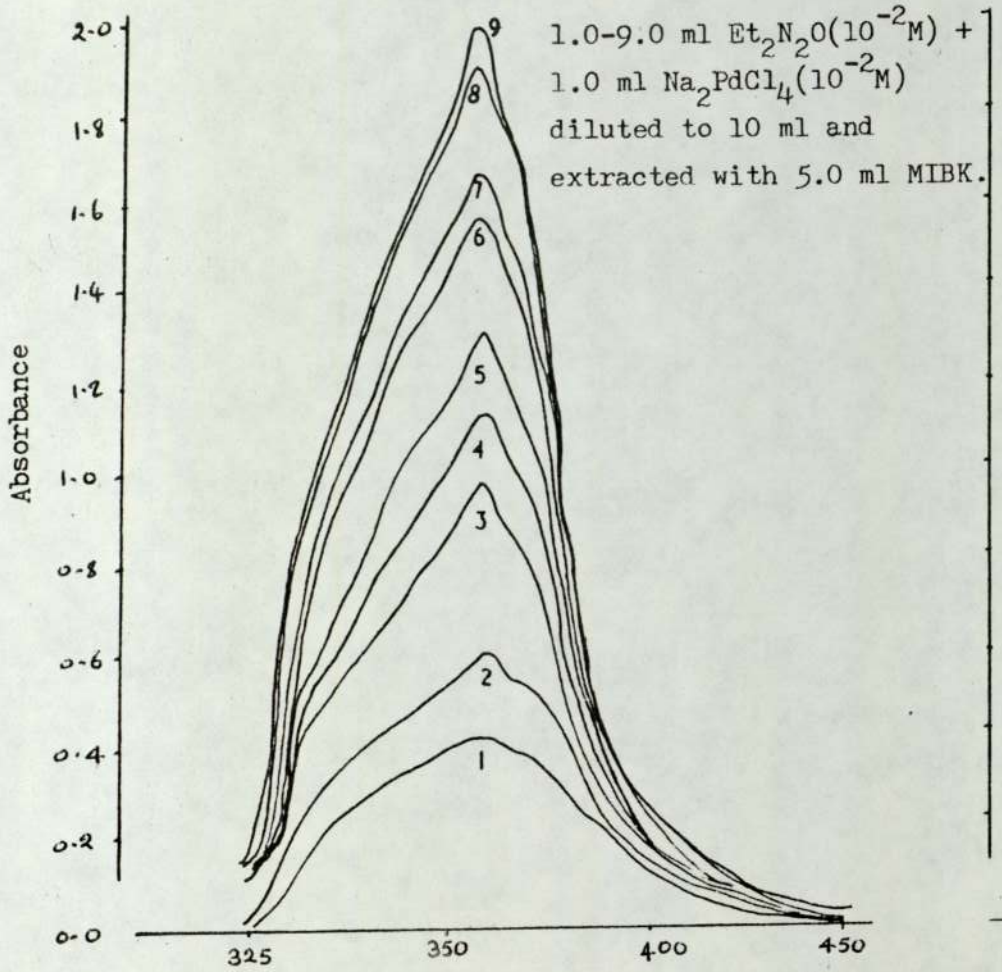


Fig. (5.6) Spectra of Et_2N_2O extracted into MIBK.

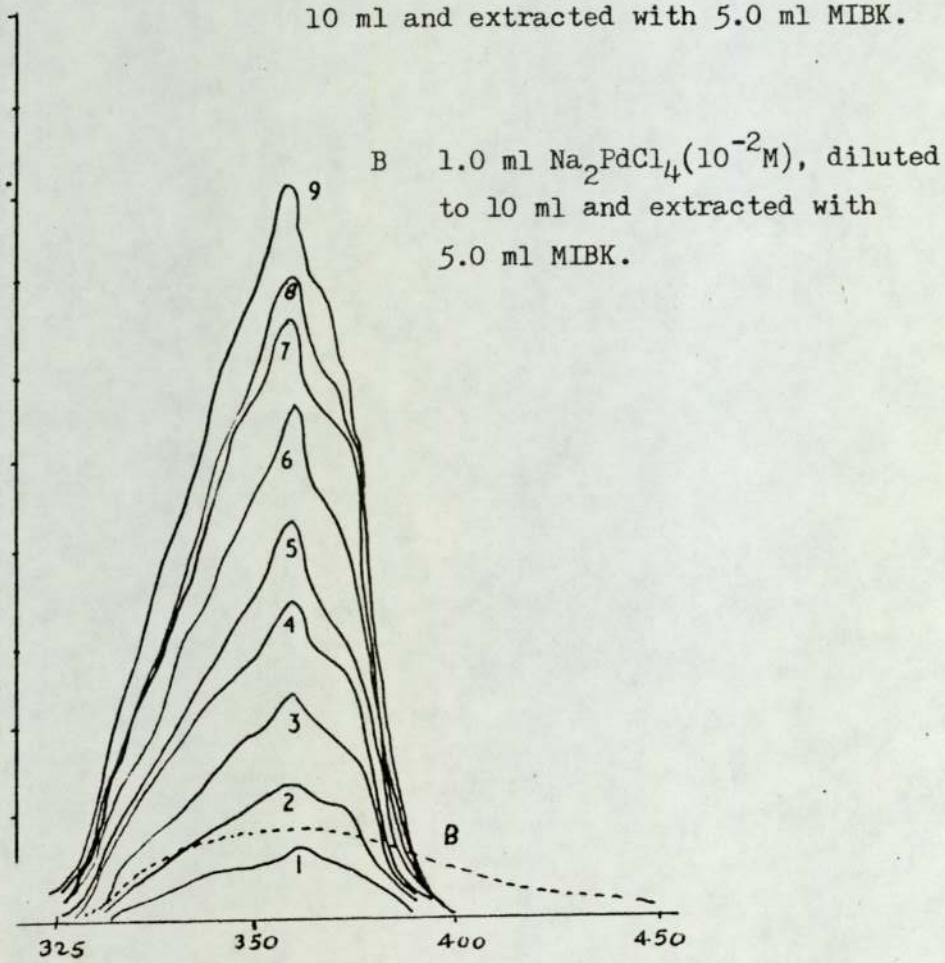
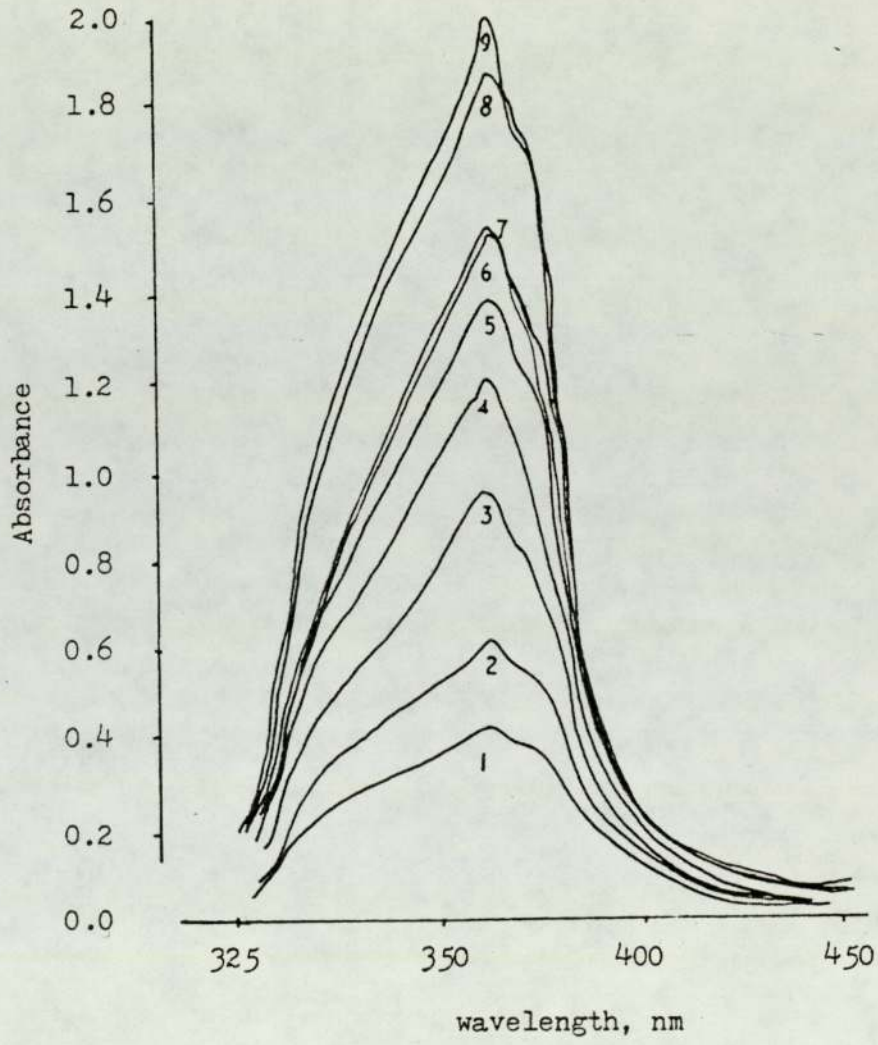


Fig. (5.8) Spectra of extracted species, (nitrosamine + Na_2PdCl_4) into MIBK - after 24 hrs.



1.0-9.0 ml Et_2N_2O (10^{-2}M) + 1.0 ml Na_2PdCl_4 (10^{-2}M) diluted to 10 ml and extracted after 24 hrs. into 5.0 ml MIBK.

$(C_2H_5)_2NNO$ ml (10^{-2}M)	Absorbance at 360 nm		
	Blank	Mixture (1)	Mixture (2)
1	0.13	0.41	0.405
2	0.28	0.60	0.60
3	0.47	0.97	0.93
4	0.69	1.14	1.18
5	0.86	1.31	1.35
6	1.12	1.58	1.49
7	1.31	1.66	1.53
8	1.41	1.90	1.85
9	1.61	1.99	1.98

mixture (1) = immediate extraction

mixture (2) = extraction after 24 h.

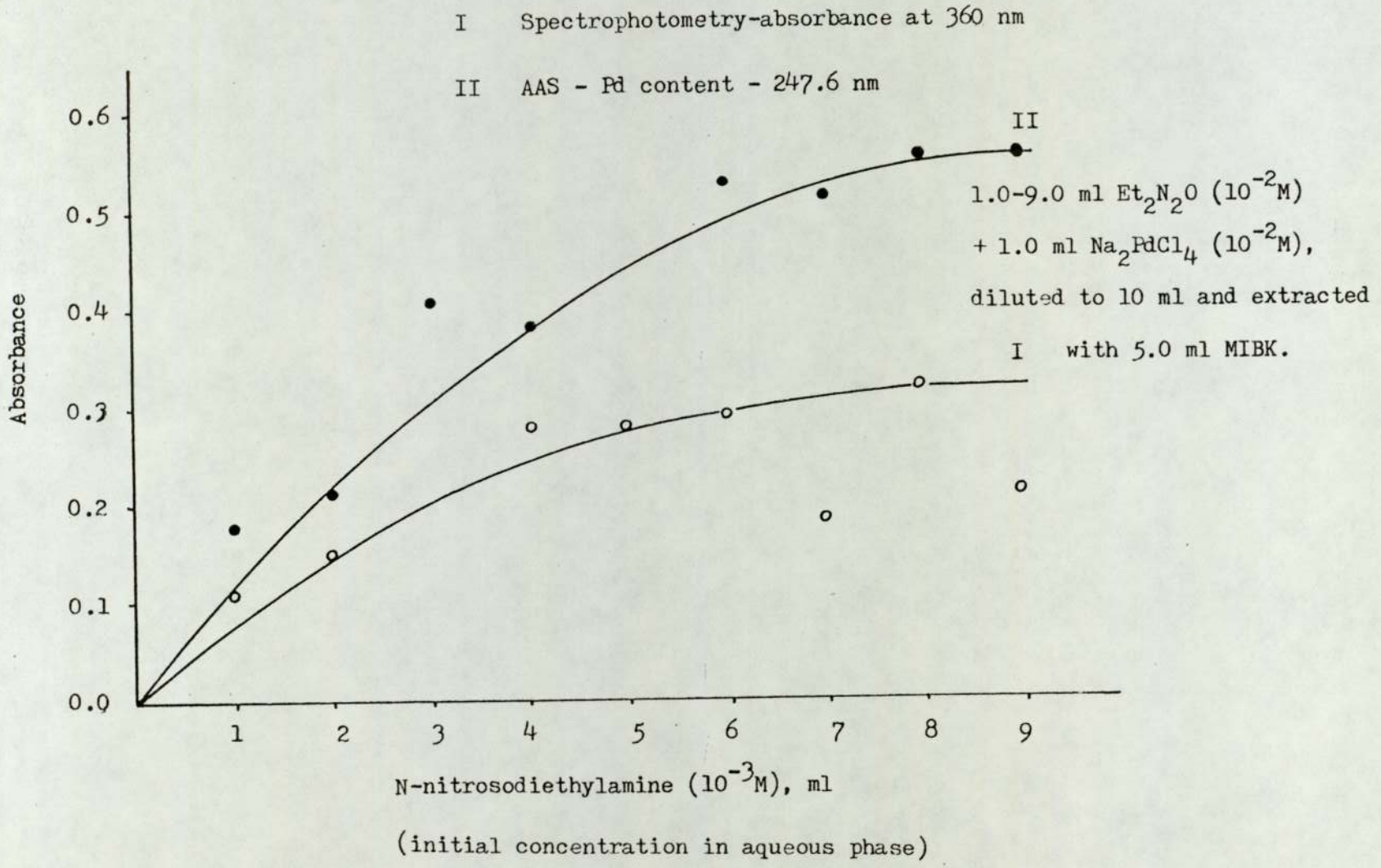
The absorbance reading at 360 nm for Na_2PdCl_4 (10^{-2}M, 1 ml) was 0.17.

The extracted species in MIBK was aspirated into the AAS-flame using Perkin-Elmer AAS 303 under the following conditions :

Acetylene	5 l/min.
Air	8 l/min.
Pd	247.6 nm.

The graphs obtained are reproduced in fig.(5.9)for both; spectrophtometric and AAS measurements. The curves show that there is possibility of extracting Pd(II) in a quantitative manner with nitrosamine. The reaction might take place in dilute solutions but very slowly. It is suggested that this reaction needs further investigation using dilute

Fig. (5.9) Calibration curves for N-nitrosamine determination



solutions. The extractability of the complexes in different solvents and at different pH's also needs further study. The results obtained in this work show no immediate analytical application for the determination of nitrosamine but there seems potential for further study.

CHAPTER SIX

CONCLUSIONS

AND

SUGGESTIONS FOR FURTHER WORK

6.1 Conclusions

In reviewing indirect methods by AAS for the period 1966- 1980 and examining the present work, certain observations and conclusions emerge which can be summarised as follows :

I. Potential and Feasibility of Indirect Methods

(1) The AAS technique has played a very important role in indirect methods of chemical analysis. The procedure can be applied widely particularly in the determination of organic compounds encountered in environmental analysis.

(2) It has been apparent that the topic of 'indirect methods' occupies either little or no space in books on AAS, the principles of the technique being stated only briefly. The reason for this most probably lies in the high specificity and selectivity in metal determinations which has somewhat overshadowed the estimation of organic species. Moreover, much of the sporadic work that has been published or reported is in the Japanese literature.

(3) Indirect methods are a recent extension for the applicability of AAS technique which have resulted in improved sensitivity for some metals and has enabled non-metals and organic species to be determined.

(4) The methods offer some advantages. They furnish a means of analysis when an alternative method may be not available. They sometimes offer a more sensitive and better selectivity of the determination coupled with the avoidance of interferences. The methods may be cheaper and more rapid than alternative procedures. They may avoid the purchase of

additional equipment.

(5) Numerous methods of indirect analysis have been reported and these show the feasibility of AAS in order to determine organic species. A thorough search of the literature on the current methods available has led to a comprehensive review for the determination of organic compounds covering the period 1966-1980.

(6) There are few reviews dealing with this topic. Published reviews have discussed very few applications to organic species and appeared before 1973. Since this time much work on indirect methods has been reported in analytical journals. These reviews have been orientated towards the determination of metals and non-metals rather than organic species. No attempts have been made to include a fully comprehensive treatise on the applicability of AAS to organic species.

(7) No systematic investigations for the determination different organic classes or functional groups have been reported. The technique however, has found practicability for certain organic classes, such as; alcohols, aldehydes, sugars, proteins, amino acids, primary and secondary amines, organic acids and the nitro-groups, etc. The methods should be applicable to many other organic compounds.

(8) Methods were developed either to meet a certain need in chemical analysis or on the other hand, some of these methods are described in order to extend the applicability of AAS.

(9) Methods are based on selecting an appropriate chemical reaction which is assumed stoichiometric. In some cases, the initial chemical reaction loses some of its selectivity for the method but the technique can still be quite valuable in determination of species in an analytical method.

(10) Some spectrophotometric methods are modified and/or adapted to

include AAS for the final stages of analysis, but all the methods involve a metal determination rather than measurement of absorption of colour of a solution.

(11) Of the indirect methods reported, solvent extraction is extensively employed. The other techniques have been used to a lesser extent but in practice solvent extraction has greater applicability.

II. Saccharin-Ferroin : Solvent Extraction with Nitrobenzene

(1) One of the indirect approachs for determination of an organic species by AAS has been employed in the present work. Saccharin, an important artificial sweetener, and common food additive, may be determined by AAS via complexation as its tris-(1,10-phenanthroline)iron(II) cation. The method is based on the spectrophotometric determination of saccharin by selective solvent extraction from aqueous solutions containing an excess of tris-(1,10-phenanthroline)iron(II) cation into nitrobenzene as an ion-association system. The best conditions have been determined after thorough investigation and the method has been adapted to give an atomic absorption spectroscopy finish using both flame and flameless technique, in which both the organic and aqueous phases have been analysed.

(2) The use of nitrobenzene within the carbon furnace has been examined. Problems associated with this work have been found and discussed. Some practical considerations have been mentioned including the advantages and disadvantages of nitrobenzene in this system. It causes matrix and background absorption interferences. Because its relatively high viscosity, the smoke presents the most serious problem associated with carbon furnace work. In spite of the toxicity of nitrobenzene, it has

still been widely used in analytical flame AAS and spectrophotometric methods. This is because its high distribution coefficient and its relatively high dielectric constant which is suitable for extraction of the ion-pair.

(3) In order to avoid the interference of nitrobenzene in the measurements of carbon furnace, certain steps have to be taken. A stripping method has been devised in order to strip the iron content back into aqueous solutions before the usual measurements using either flame or flameless techniques. A few millilitres of carbon tetrachloride have been added to the organic phase containing ion-association complex and then the mixture has shaken with distilled water. This causes dissociation of the ion-pair bringing the easily soluble cation $(Fe\ Phen_3)^{2+}$ in aqueous phase.

(4) When injecting a nitrobenzene matrix into the carbon furnace, the smoke produced during drying and charring temperature stages absorbs less at the iron 372 nm line than at the 248.3 nm line. Significant reduction of signals has been recorded.

(5) Stripping methods have proven to be practical. A set of standard saccharin solutions are extracted with $(Fe\ Phen_3)^{2+}$ into nitrobenzene and is then stripped back into aqueous solutions with aid of carbon tetrachloride. For the flame work, the calibration curves were found to be linear over a wide range of concentration; up to 800 μM saccharin. Sensitivity of the method in term of $C_{1\%}$ was found to be 11.88 μM saccharin using iron 248.3 nm line.

(6) The best conditions for the use of carbon furnace in this determination have been found and the Perkin-Elmer HGA70 carbon furnace has been evaluated. The graphite tubes were found to be used for maximum of 170 injections using aqueous matrix. They were not found

to age significantly during the use of nitrobenzene matrix, in which they have useful life times of 70 determinations. After this, sensitivity and precision start to decrease with tube age. Time factor for analysis is very important, and using nitrobenzene matrix the time factor is 190 seconds per sample. This is longer than the time taken with aqueous matrixes i.e. 45 seconds per sample. Stripping methods have been proven to be superior in precision and also reduce the time of analysis, and lengthen the useful time of the graphite tube thereby, reducing the cost of analysis. The method may be conveniently employed using flame in order to avoid aspirating nitrobenzene a solvent which is toxic and has a low nebulization rate.

(7) Using the carbon furnace, the calibration curve was found to be linear up to 60μM saccharin. Sensitivity of the method ($C_{1\%}$) was found to be 0.748μM saccharin using iron 372 nm line. The average range (R) and relative standard deviation (S) in the measurements were found as follows :

$$\left.\begin{aligned} R &= 0.0175 \\ S &= 0.0057 \end{aligned}\right\} \quad \text{for saccharin} < 60\mu\text{M}.$$

$$\left.\begin{aligned} R &= 0.0383 \\ S &= 0.0122 \end{aligned}\right\} \quad \text{for saccharin} > 60\mu\text{M}.$$

III. Saccharin-Ferroin : Adsorption-Desorption on Silica Gel

(1) A new technique involving a spectrophotometric finish for saccharin determination has been developed. The method is based on the quantitative formation of ion-association complex $\left\{ (\text{Fe Phen}_3)^{2+}; (\text{saccharin})_2^- \right\}$ when

an ethanolic, or methanolic or acetone solution of saccharin is shaken with ferroin-impregnated silica gel. The absorbance of the characteristic orange colour of the desorbed ferroin ion-association complex is measured at 510 nm.

(2) 'Ferroin' has employed as a 'modifier' for the surface of silica gel to provide a new modified-surface matrix. The matrix has prepared and conditioned in standard manner.

(3) The severe health hazardous problems associated with nitrobenzene as extractant could be replaced by other solvents such as ethanol, methanol, and acetone for the ion-pair; $\{ (Fe\ Phen_3)^{2+} ; (saccharin)_2^- \}$ and replacing the solvent extraction process by a desorption technique.

(4) The advantages of the adsorption-desorption method may be summarised as follows :

(i) the method is simple, cheap, easy to carry out and less time consuming than other methods. It is, therefore, ideal for routine work,

(ii) the desorption process is rapid and results in a solution which does not contain excess of ligand. Blanks are low in absorbance readings,

(iii) the method is accurate; the results were found to be reproducible and the relative standard deviation percent for the overall process was found as :

1.852, 1.264, 2.311 and 1.773% when using distilled water, 60% ethanolic, 80% methanolic or 70% acetone solutions respectively,

(iv) the ferroin-impregnated silica gel matrix may be prepared in large batches and is stable and homogeneous. Reproducibilty is good and the sorption capacity from batch to batch may be reproduced,

(v) the ferroin-impregnated silica gel matrix may be used several times and may be reactivated until exhausted,

(vi) other reagents are kept to a minimum in the system,

(vii) there is no need for the use of drying agents, no time consuming solvent extraction procedures.

(5) The calibration curves for the colorimetric method were found to be linear over the ranges of saccharin concentrations :

1.25×10^{-4}M - 5.0×10^{-4}M	distilled water
7.5×10^{-5}M - 5.0×10^{-4}M	60% ethanolic soln.
7.5×10^{-5}M - 5.0×10^{-4}M	80% methanolic soln.
5.0×10^{-5}M - 5.0×10^{-4}M	70% acetone soln.

(6) The method suffers from some interferences such as sodium cyclamate, sorbic acid, benzoic acid, citric acid and sodium chloride; but this problem may be overcome by removal of these interfering matrials prior to the determination. Large amounts of glucose, sucrose and dulcin do not interfere with the determination.

(7) A procedure for the routine analysis of saccharin tablets is proposed. The presence of bicarbonates in tablets could be eliminated by adding few drops of 2% (v/v) sulphuric acid (0.8 ml) to each tablet.

(8) Furthermore, the method is applicable to an atomic absorption finish since the ethanolic, methanolic or acetone solution obtained in the desorption process is a satisfactory matrix for the determination of iron. This new method demonstrates a very simple, rapid and accurate indirect AAS approach for determination of organic species and this has extended the applicability of AAS.

(9) Sensitivity for the indirect AAS method, in term of ($C_{1\%}$) was found to be as :

distilled water	20.46 μM saccharin
60% ethanolic solution	6.60 μM
70% acetone solution	6.06 μM
80% methanolic solution	4.62 μM

(10) The linearity of the calibration curves for AAS technique was found to be over the ranges of saccharin concentrations :

distilled water	$1.25X10^{-4}M$-$5.0X10^{-4}M$
60% ethanolic solution	$7.5\ X10^{-5}M$-$5.0X10^{-4}M$
70% acetone solution	$2.5\ X10^{-5}M$-$5.0X10^{-4}M$
80% methanolic solution	$7.5\ X10^{-5}M$-$4.0X10^{-4}M$

IV. N-Nitrosamines; Potentially Useful Chemical Reactions

(1) Some possible routes to an indirect AAS method for the determination of N-nitrosamines have been investigated in the present work. The chemical reactions may prove to be analytically useful. Utilisation of such reactions needs further careful investigation.

(2) One of the chemical reactions has been examined in which a palladium complex is formed between nitrosamine and palladium chloride. The complex $(Et_2N_2O)_2PdCl_2$ has been prepared and identified using IR spectra. Some practical observations have been made including; melting point, solubility of complex in different organic solvents, and the spectra of uv and visible regions.

(3) Attempts have been made in order to investigate the practicability

of extraction the palladium complex which may be formed when nitrosamine is added to palladium(II) solutions. Many solvent extraction systems have been investigated and they showed no evidence of a useful absorption peak when the extract has been examined in uv and visible ranges. The peaks have found to be similar to those of nitrosamines. However, some enhancement in absorbance for the extracted species has been noticed, in particular, when using MIBK solvent. This indicates that the reaction might have taken place in this dilute solution medium but the reaction seems to be very slow. It is suggested, therefore, that this reaction needs further investigation using dilute solutions. The extractability of the complex in different solvents and pH's also need further study. The results obtained, however, show no immediate analytical application for determination of nitrosamine under the conditions studied here.

6.2 Suggestions for Further Work

The present work has initiated and introduced some interesting points and they are worth further study in future work. They may be summarised as follows :

Adsorption-Desorption Ferroin Studies

(1) The capacity of silica gel to ferroin increases rapidly with decreasing particle size. These findings were by Vydra and Markova (181), and it is suggested that there is a need to investigate the relationship between the particle size of silica gel and desorption process in recommended saccharin determination method. It may be possible to improve

the detection limit of the method after such a study.

(2) The use of glycerol in the desorption process may prove to enhance the sensitivity of the method. This is due to; (a) its solubility in water, (b) the solubility of saccharin in this solvent (1 part of saccharin is soluble in 50 glycerol), and (c) its high dielectric constant (42.5 at 25°C) compared with methanol 32.63, ethanol 24.30, acetone 20.7, and even nitrobenzene 34.82. The ion-pair $\{ (Fe\ Phen_3)^{2+}; (saccharin)_2^- \}$ is expected to be more soluble in glycerol than the other solvents used.

(3) Investigations could be made on adapting the colorimetric method developed for saccharin determination to soft drinks by direct addition of the sample to the ferroin-impregnated silica gel.

Saccharin Determination by Indirect AAS Method

(1) Via Copper Complexation

During the course of the study on the possible routes to the determination of saccharin by indirect AAS methods e.g. via metal complexation reactions; a compound of copper was isolated. This fine yellow precipitate was formed when copper(II) solution and ascorbic acid was added to saccharin solution. The precipitate is soluble in hot acids and immediately soluble in ammonia solution giving a clear blue colour. An immediate gelatinous yellow precipitate is formed with sodium hydroxide solution. The compound is very soluble in pyridine giving yellow-greenish clear solution. This copper compound has also been found insoluble in many solvents such as; cold and hot water, chloroform, MIBK, ethylacetate, benzene, carbon tetrachloride, nitrobenzene, iso-propyl alcohol, hexane, dichloromethane, methanol, ethanol, acetone, diethylether,

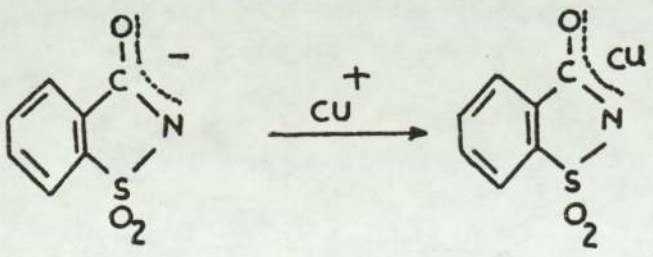
o-nitrotoluene, AMK and amyl acetate.

Elemental analysis was carried out, the results showed :

	% Calculated for $Cu(C_6H_4COSO_2N)$	% Found
C	34.22	35.0
H	1.64	1.8
N	5.70	6.4

Reviewing this point in the available literature, little has been reported about the chemical reactions involving saccharin and metals. Klasens and Terpstra (236) have reported that, light blue crystals of $(C_6H_4COSO_2N)_2Cu.(H_2O)_6$ may be formed upon addition of concentrated copper sulphate solution to an equally concentrated solution of sodium saccharinate. They have also studied the crystallography of cupric saccharinate formed. Michrochemical reactions of saccharin with Ni, Co, Mn and Cu (ammonia) have been reported (237, 238, 240). The salts of saccharin with these metals have been identified by microscopic examination. The composition of $\left[C_6H_4(CO)\ SO_2N\right]_2Mn.5H_2O$; manganous derivative has been confirmed (239).

The compound which has been precipitated when saccharin solution is added to copper(II) solution in the presence of ascorbic acid is believed to be copper(I)-saccharinate according to the reaction below :



The reaction is worth investigation in order to characterise and identify the compound and also in order to study its possible utiliztion as possible route to an indirect AAS method for saccharin determination.

(2) Via Silver Compound

Saccharin has been reported to be quantitatively precipitated as its silver salt from solutions at pH values below 6 (241, 242). This fact has been utilized in order to develop titrimetric methods for saccharin determination. This reaction seems to have potential as the basis for an indirect AAS method which wolud involve a precipitation step before determination of the silver content either in the precipitate or in the filtrate (unreacted silver) solution.

N-Nitrosamines Determination

The chemical reactions which have been discussed in chapter five concern the development of indirect AAS methods for the determination of N-nitrosamines. These reactions are worthy of more attention and investigation. Further study may lead to new and useful analytical methods of analysis.

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