A THESIS

# QUANTITATIVE AQUEOUS AMMONIUM ION ANALYSIS

by

# TRANSMISSION INFRARED SPECTROSCOPY

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# ABSTRACT

## QUANTITATIVE AQUEOUS AMMONIUM ION ANALYSIS by TRANSMISSION INFRARED SPECTROSCOPY

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The main objective of this research was to study the behaviour of the ammonium ion in aqueous solutions and dilute Kjeldahl distillates using the double beam infrared (IR) spectrophotometer and to investigate the possibility of transferring the obtained methodology into commercially available infrared filter instruments. The spectral characteristics of ammonium sulphate in water were determined and the analytical band of 6.89 μm was chosen for further quantitation of the ammonium ion. The effect of the pH on the ammonium ion quantitation was examined and it was concluded that accurate results were obtained if the pH was kept within the 3-8 range. Spectra of ammonium sulphate in both dilute sulphuric acid and dilute Kjeldahl digest were obtained. Based on the data gathered on the Spectroprocessor IV instrument, a 6.89 μm sample filter and a 5.56 μm reference filter were installed in the commercial filter IR spectrophotometer, Multispec M. The infrared method using the Multispec M was compared to the traditional micro-Kjeldahl procedure for the determination of ammonia content in Kjeldahl distillates and was found to be more accurate. The conclusion of this study was that the measurment of the ammonium ion in Kjeldahl distillates may be feasible if a low cost, simple and dedicated filter IR instrument were available.

# RÉSUMÉ

## L' Analyse Quantitative des Ions d''Ammonium en Solution Aqueuse par Spectroscopie de Transmission à l' Infrarouge.

Cette recherche avait pour but principal l'étude du comportment des ions ammonium en solution aqueuses et dans les distillats Kjeldahl dilués en utilisant le spectrophotomètre à double faisceau infrarouge (I R) et adapter la méthode développée aux instruments commerciaux à filtre I R Les caractéristiques spectrales d'une solution aqueuse de sulphate d' ammonium ont été déterminées et la bande analytique de 6 89 µm a été choisie pour les études quantitatives sur l'ion ammonium. Les résultats quantitatif du dosage de l'ion ammonium étaient les plus précis lorsque le pH se situait entre 3 et 8. Se basent sur les données recueillies avec le Spectoprocessor IV, un filtre de 6.89 µm pour l' échantillon et un filtre de 5 56 µm pour la référence ont été installés sur un spectrophotomètre IR commercial, le Multispec M La détermination du contenu en ammonium distillats Kjeldahl etait plus precis avec la methode infra - rouge utilisant le Multispec M que lorsque la méthode traditionnelle du micro-Kjeldahl était utilisée. En conclusion, la détermination quantitative de l'ion ammonium des distillats Kjeldahl peut être faité précisément si un instrument IR a filtres spéciaux, simple et plus coûteaux était disponible sur le marché.

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# Chapter I
# INTRODUCTION

The Kjeldahl method is a major reference method for protein determination in food systems, however it is generally considered to be a tedious and troublesome analysis to perform because of its lengthy digestion, distillation and titration steps. Although a variety of methodologies have been developed to try to eliminate at least the distillation and titration steps (Fleck and Munro, 1965), few of these methods have gained widespread use in the industrial and goverment laboratories.

Recent years witnessed substantial development of infrared (IR) applications in the field of quantitative food analysis (Gould, 1959; Bjarno, 1981; Mills et al., 1982; Mills et al., 1983, and Mills et al. 1984). Nevertheless its main application is still in milk analysis (Biggs, 1978 and Biggs et al., 1984) in which quantitative determination of fat, protein, lactose and total solids can be done simultaneously at the rate of 250-300 samples per hour using the infrared filter instrument, Multispec M (van de Voort, 1980) The newer version, Multispec M instrument, having capabilities of analyzing up to eight components simultaneously (Shields, 1985), will be soon on the market.

The success of quantitative IR milk analysis for fat, protein and carbohydrates has indicated the possibility of other applications. Since there is a great need for a rapid and accurate protein methodology which would replace the traditional Kjeldahl procedure, the IR spectroscopy as a primary method for protein determination in foods was considered.

Hypothetically, ammonium sulphate, the end product of Kjeldahl digestion, could be analyzed directly in a distilate eliminating the need for titration. If a

method for the direct analysis of ammonium sulphate in digests were available, both the distillation and the titration steps could be eliminated

Based on these facts and the advice of experts in the field, this research deals mostly with studying the possibility of using IR transmission spectroscopy to quantitate ammonium ion directly in simple solutions such as distillates. Furthermore, an attempt was made to elucidate the potential problems involved if this method is to be extended to more complex solutions as Kjeldahl digests.

In terms of sequence of events, the first objective was to study the behaviour of ammonium ion in aqueous solution and to confirm the theoretical considerations found in the literature. The Spectroprocessor IV, a specially designed scanning IR spectrophotometer capable of working with aqueous samples, was used for this work A substantial effort was put into studying the ammonium ion spectra in distilled water and the effect the pH and temperature changes had on them. Subsequently, the spectra of ammonium sulphate in dilute acid and dilute Kjeldahl digest were examined, followed by the assessment of their suitability for the quantitative work.

The second stage of the research involved the transfer of the data obtained on the Spectroprocessor IV into the commercial, single cell, filter IR instrument and the development of a methodology suitable for the rapid analysis of proteins in Kjeldahl distillate.

In summary, the two main objectives of this masters research were the following: a) to study the fundamental problems associated with IR analysis of ammonium ion in aqueous solutions and b) to extend the methodology into a commercial IR filter instrument such as the Multispec M.

It is hoped that the information derived from this research will add knowledge to the application of IR analysis in food science and help other investigators interested in this field.

# Chapter II
# LITERATURE REVIEW

## 2.1 IR Spectroscopy in Aqueous System

The IR spectroscopy dates as far back as 1800 with Sir William Herschel's experiment. Using the thermometer as a detector, he observed a large heating effect in the region proceeding the red of the visible spectrum. He also recorded the first infrared spectrum by measuring the intensity of radiation as a function of distance beyond red. The term 'infrared' which originates from his observations was later defined more precisely as a region of the electromagnetic spectrum which lies between the visible and microwave region and covers the wavelengths between 0.7 and 100 microns

More than 100 years latter, William W. Coblentz (1905) devoted himself to systematic studies of infrared absorption. He published a collection of IR spectra in the region between 2.5 and 15 μm and was able to show the correlation of molecular structure to infrared spectra.

The classical monograph of Coblentz escaped the attention of organic chemists and until the mid-thirties IR analysis was mainly of interest to theoretical physicists who used the spectra to obtain fundamental data on the mechanics of simple molecules. They successfully classified the vibrations associated with covalently linked atoms into stretching and bending vibrations These were further subdivided into symmetrical and asymmetrical stretching, scissoring, twisting, wagging and rocking (Rao,1963). The detailed vibrational analysis of the infrared spectrum of a variety of symmetrical molecules clearly indicated that some of the vibrational frequencies were associated with specific groups of atoms, due to the fact that bond forces are constant from molecule to molecule. The significance of these findings was finally appreciated by chemists who

started using IR spectroscopy for functional group analysis. Infrared analysis was also successfully utilized for the determination of isomerism, inter-molecular interactions, solute-solvent interactions, hydrogen bonding and as a powerful method for quantitative analysis of compounds.

The first extensive survey of infrared absorption spectra was done by Lacomte and his co-workers (1948). This was followed by the Colthup chart (Colthup,1950) containing characteristic frequencies for the nitrate, sulphate, carbonate, phosphate and ammonium ion. An excellent paper by Hunt and his co-workers (1950) contained the spectra of 64 naturally occuring minerals and related inorganic compounds. At that time there was still no extensive compilation of infrared spectra of inorganic salts obtained with a modern spectrometer. A few years latter, Miller and Wilkins (1952) investigated and presented infrared spectra of 159 pure inorganic compounds obtained with a Baird Model A and a Perkin-Elmer 12B spectrometer. These characteristic frequencies were shown to be useful in quantitative analysis of inorganic unknowns. At that time the authors also pointed out that quantitative analysis was difficult, because of the necessity of working with powders.

Until the late forties, water was excluded as a solvent because of its strong absorption in the infrared range. The absorption bands due to the components analyzed were usually obscured by water with the instruments available at that time. The first major development in the field of aqueous IR analysis took place when Gore and co-workers (1949) had published the infrared spectra of several amino acids These workers used water insoluble, infrared transparent materials, such as special glass, fluoride and silver chloride, for fabrication of cells. They also developed a new IR spectrophotometer, the 12A Perkin-Elmer, which was more suitable for work with with aqueous systems. This instrument maximized the energy available for analysis by chopping the

radiation at fixed frequency thereby allowing only the required signal to be picked up by the detector.

In 1953, a major advance occured in the field of biological chemistry when spectra of solutions of plasma were obtained on a double-beam spectrophotometer (Lenormant and Blout, 1953) The use of a double-beam spectrophotometer made it possible to compensate for solvent absorption by placing the appropriate solvent in the reference beam The same workers (Lenormant and Blout, 1953 ) had published many IR spectra of biological polymers in water and deuterium oxide solutions. Since heavy water is transparent in some IR regions where water absorbs, using both liquids made it possible to obtain spectra over the entire IR region. A double-beam spectrophotometer and polished plates of thallium bromide-iodide with short path lengths of 0.010-0.025 mm were used for optimal results. Slit openings of 0.072 mm at 5 μm, 0.125 mm at 7 μm and 0.350 mm at 12 5 μm were required for their work with the Perkin-Elmer Model 21 double-beam spectrophotometer Their research indicated that with suitable instrumentation good IR spectra of compounds in aqueous solutions in the region from approximately 6 5 to 10 μm could be obtained.

Neverless, further studies were needed to maximize the available energy for quantitative work. Phyler and Acquista (1954) were first to obtain quantitative data on compounds in aqueous solutions. They also published spectra of pure water, showing the region from approximately from 6.5 to 10 μm to have sufficient transmittance to be applicable for quantitative analysis.

At that time, major experimental difficulties arose from the need to find suitable cell windows which were not attacked by solvent. Potts and Wright (1956) constructed one of the earliest permanent barium fluoride cells with a

path length of 0.027 mm. Barium fluoride seemed to be ideally suited for this use as it was commercially available, hard, easily polished, essentially insoluble in water and IR transparent up to 12 5 μm. Using these cells in a double-beam spectrophotometer built in their laboratory (Dow Chemicals Co.), the authors obtained quantitative spectra of metallic salts of organic acids, glycols and other compounds which could be analyzed accurately only in aqueous solution To compensate for the energy lost by water absorption, both entrance and exit slit widths were increased by a factor of $T^{-1/2}$ where T is the percentage transmittance of water in the region from 6.5 to 10 μm. Further research was done by Kaye (1955), who presented the details of infrared transmission, water solubility and refractive index of a number of useful window materials. Subsequently, Sternglantz (1956) reproduced the transmission curves of a number of suitable window materials useful for taking IR spectra in aqueous solutions.

At this time, a major advance in IR spectroscopy occured with the inven - tion of the diffraction grating. The first commercially available reflection grating instrument, Grubb Parsons GS2 grating spectrometer, was designed by Shields under the guidance of Martin (1956). The main advantage of this instrument was its grating efficiency and its ability to function over the entire region of the spectrum.

From then on, having developed suitable instrumentation and cell mate - rials, the researchers conducted many studies which indicated that IR spectro - scopy in aqueous solutions may be a technique of choice for studies of inorga - nic and biological substances. Falk and Giguere (1957) detected $H_3O^+$ ion in concentrated aqueous solutions of strong acids such as HCl, HBr, $H_2SO_4$ and $H_3PO_4$ using silver chloride cells. The same authors (Falk and Giguere,1958) also studied aqueous solutions of $SO_2$ between silver chloride windows and

concluded that $SO_2$ exists mostly in the molecular state with small amounts of $HSO_3^-$ and $HS_2O_5^-$ being formed. Antikainen (1958) has presented IR spectra of some oxyions of sulphur in aqueous solution and has showed their characteristic absorbance maxima using polyethylene-NaCl cells. These type of cells were replaced with teflon-NaCl windows by Fogelberg and Kaila (1957) who studied aqueous acidic and basic solutions IR spectra of amino acids in aqueous solutions and related substances have been recorded by Parker and Kirchenbaum (1961) They reported that these spectra have generally fewer but broader bands than similar spectra in a solid state.

Goulden (1959) further contributed to this field of research He used barium fluoride cells, the most suitable material in the 6 5-10 μm region, to obtain spectra of inorganic and organic compounds such as carbohydrates, amino acids and other biological compounds. Goulden concluded that, despite experimental difficultes, IR spectra could be potentially very useful in the analysis of aqueous solutions of a variety of compounds, particularly inorganic salts He also presented spectra of lactates in aqueous solution (Goulden,1960) whose application to milk analysis will be discussed later.

Some important IR spectroscopic studies were done by Thompson (1965). He described a technique for preparing thin cells of known thickness in the range from 0.4 to 1 7 μm. These cells are most suitable for taking the spectra of liquids and solutions since they minimize the effect of strong water absorption in the mid-IR region. Using the double-beam instrument and calcium fluoride cells of 0.8 μm thickness, he accurately determined the molar extinction coefficient of some fundamental IR absorption bands of water, deuterium oxide and other solutions The same workers (Thompson, 1966) studied the effect of concentration on the IR active vibrational frequencies of the ammonium ion in the 2.5-10 μm region. He noted shifts in frequencies by comparing the values

for several ammonium salts in their crystaline state, and in saturated and dilute solutions Shifts in ammonium ion frequencies were attributed to hydrogen bonding and ionic interactions. This was confirmed by further experiments which showed that, in saturated solutions, ammonium ion frequencies differ as a consequence of strong ionic interactions and they approach a constant set of values for dilute solutions where only hydrogen bonding is in effect.

Work in the field of aqueous IR spectroscopy was carried further by Goulden and Manning (1967). A frequency correlation chart was prepared which allowed for identification of the most common polyatomic inorganic ions. Using pH resistant calcium fluoride cells of 50 μm, the authors were able to study the pH dependent changes in the ionic structure of numerous inorganic ions and the effects of complex ion formation on IR spectra. All the spectra recorded were obtained from instruments fitted with a sodium chloride prism. The spectra were not of high resolution but allowed for substantial increase in the spectrometer's slit-width which compensated for strong water absorption in the mid-IR region. Goulden and Manning (1967) also studied the effects of a change in cations and of the solute concentration on the absorptivity of the sulphate band. They demonstrated that the sulphate band is affected by both the solute concentration and the nature of the cation. They also noted that, at high concentrations, ionic interactions can sometimes cause the absorbance-concentration relationship to become non-linear and may even lead to the appearance of additional absorption bands as the symmetry of the free ion is reduced.

The use of IR spectroscopy in food analysis has been limited mainly due to complexities of sample preparation and to the presence of water in most food systems. Recent developments in instrumentation have solved some of the practical difficulties of aqueous solution spectroscopy and there has been a

steady increase in use of IR spectroscopy in the quantitative analysis of food systems.

Goulden (1959) was the first to demonstrate the application of quantitative aqueous IR analysis to the determination of lactose in milk with an accuracy of approximately 1.5%. He further indicated (Goulden,1961) that under suitable experimental conditions the infrared spectra of homogenized milk samples show absorption peaks near 5.8, 6 5 and 9 6 µm, which correspond to fat (carbonyl ester or C=O linkage), protein (peptide or CONH linkage) and lactose (hydroxyl or OH group) absorbance bands respectively The author noticed that except for the effects of the fat band on the 6 5 µm protein band, the absorbance measured at 5 8 and 9.6 µm wavelength is proportional to the concentration of fat and lactose. The concentration of protein in the sample could be obtained by applying a correction factor. The author also demonstrated that quantitative absorption measurments of milk samples are only accurate if the effects of light scattering are minimized by the proper homogenization of fat globules, their diameters having to be smaller than the wavelength at which absorbance is measured.

A concept developed by Goulden was successfully employed in the design of a commercial infrared milk analyzer (IRMA) produced by Grubb-Parson in England in 1964 which could determine fat, protein, lactose and solid-not-fat (SNF) in less than 1 minute. This split beam, dual cell IR analyzer had some limitations such as a relatively long light path, a complex optical system and an unstable infrared source. Neverless, in spite of of these limitations, it was superior to any other instrument available at that time.

The IR method for milk analysis was extensively studied by Biggs (1972) who demonstrated that the IRMA method was as accurate and precise as, but less expensive and faster, than the accepted standard methods (Babcock for fat,

Kjeldahl for protein and polarimetry for lactose). After an extensive collaborative studies the IRMA method was adopted as an official method for milk analysis (AOAC 1975, Method 16.074).

Subsequently Biggs (1979) recommended performance specifications for the Infrared Milk Analyzer. The proposed specifications dealt with a number of factors which could reduce the accuracy of the instrument's performance such as poor homogenizing efficiency, the effect of moisture and the quality of milks used for calibration. Since infrared milk analyzers are calibrated to repro - duce the analytical results achieved with the accepted standard reference methods, the author recommended that the instruments using the reference methods be well calibrated to perform within specifications for precision and accuracy.

In 1975, Foss Electric introduced the first single cell, dual wavelength infrared milk analyzer, the Milkoscan 203 and 300. In these instruments the diffraction grating was eliminated, and replaced by filter pairs specific for the fat, protein and lactose wavelengths. By chopping the beam and passing it through the sample and reference filter, the levels of energy from each filter reaching the detector could be compared. The percent of each component in the sample could be then calculated because it is proportional to the log difference in signal. The obvious advantage of this system is the speed of analysis and higher levels of energy available due to the wide band filters. van de Voort (1980) evaluated the Milkoscan 104, an improved model of IRMA, to establish if it meets the AOAC specifications. This assesment was done using a variety of milks and the results indicated that the Milkoscan 104 was capable of meeting the AOAC specifications for fat, protein and lactose analysis.

A study of IR spectroscopic application to food analysis was done by Mills and van de Voort (1982). These workers used the C-H stretch (3.4 μm) filter to successfully measure the IR absorption of fat in aqueous solutions and suggested that the use of the C-H filter, the C=O filter or a combination of both could predict the sample fat content of consistent molecular weight and degree of saturation. For fats which varied in their degree of unsaturation, the most accurate results could be obtained when the C-H filter measurments were used together with the iodine value

Bjorno (1981) at Foss Electrics tried to apply the established IR analysis technique to samples other than milk. The meat samples analyzed were converted to a milk-like emulsion, homogenized and transfered to the cell for IR measurments. The absorbance bands investigated were the C=O stretch (5.73 μm) for fat, the N-H stretch (6.5 μm) for protein and the OH group (9.6 μm) for carbohydrates. Bjorno's results indicated that the IR spectroscopy could be used successfully for meat products. The collaborative studies (Bjorno,1982) confirmed the above results. They indicated that there was no significant difference between the infrared method and the reference method at a 95% confidence level for the determination of protein, fat and water in meat products.

## 2.2 Kjeldahl method and its modifications.

The analysis of nitrogen is widely used for the determination of the protein content of food and other biological materials For more than 100 years this determination was done by two basic procedures; the Dumas method (1831) and the Kjeldahl method (1883)

The Dumas method is based on the principle of complete combustion of the material to yield gaseous nitrogen which is then determined by volume. As dry samples are required for the Dumas method, it is less commonly used.

In the Kjeldahl method, the sample is treated with hot sulphuric acid which converts the organic nitrogen to ammonium sulphate. The product of the digestion may then be measured by various methods. As the Kjeldahl method measures not only the nitrogen from proteins but also any nitrogen present in other classes of compounds, the total nitrogen times the Kjeldahl factor gives only an estimate of the protein present. The Kjeldahl factor, which converts the nitrogen found into the protein content, has a value of 6.25, unless it has been determined that some other factor is more accurate for a particular material.

The extensive application of the Kjeldahl method to organic sample analysis has led to numerous publications and also to comprehensive literature reviews by Kirk (1947), Bradstreet (1954), Steyermark (1961), Ingram (1962), and, more recently, by Fleck (1965). To present the available material, the method has been divided into its component parts: the digestion of the sample and the estimation of its protein content.

### Digestion process

Quantitative conversion of organic nitrogen to ammonia in the presence of sulphuric acid is a critical step in the Kjeldahl method. The objective is to

oxidize the carbon of the organic matter without oxidizing the liberated ammonia to gaseous nitrogen. There are several factors that affect the digestion process: the catalyst, the salts in the digestion mixture, the temperature, the time of digestion and the oxidizing agents. There is no one single universal digestion method (Kirk,1947) and the conditions chosen depend mostly on the nature of the sample being analyzed.

Catalysts

Numerous elements and their salts have been used as catalysts in the Kjeldahl digestion. Wilfarth (1885) first proposed the use of mercuric oxide in 1885 and since then 40 elements have been tested for catalytic activity Although Osborne and Wilke (1935) tested 39 elements and found the order of efficiency of the best six to be Hg, Se, Te, Mo, Fe, Cu; most of the studies have been on Hg, Se, Cu or a mixture of these three

Baker and Shuttleworth (1939) compared minimum digestion times and found that catalytic efficiency decreased in the following order mercury, selenium, copper and manganese. Other studies such as those of Osborne and Wilke (1935), Hiller (1948) and Phelps (1951), confirmed that mercury is the best single catalyst. A thorough AOAC study (1955) has shown that, for easily digested materials such as proteins the results are higher with mercury as a catalyst than with copper. The only disadvantage in using mercury as catalyst is the necessity to precipitate the mercury before distillation since it forms com - plexes with ammonia (Bradstreet ,1954).

Selenium has been thoroughly investigated as a catalyst since Lauro (1931) first reported it. Patel and Sreenivasan (1948) considered selenium a controversial catalyst because of the nitrogen loss that may occur if the tempe- rature of the digest is too high or the digestion time is too long. Selenium, either

as a metal or a selenite salt, is seldom used alone. It is usually used with mercury for the purpose of increasing the digestion rate over that of mercury used alone Willits et al. (1951) showed that the higher the selenium content, the greater the nitrogen loss and that the presence of mercury increased the loss caused by the selenium alone. Patel and Sreenivasan (1948) on the other hand, found that the presence of mercury reduced the loss due to selenium They suggested that the reason for this could be the formation of a mercury - ammonia complex which protects the nitrogen against further oxidation.

There is a conclusive evidence that copper is inferior to mercury as catalyst (Baker, 1953) and (Bradstreet, 1954) and therefore it is seldom used.

Salts in the digestion mixture:

Gunning (1889) was the first one to report the use of potassium sulphate to raise the temperature of the digest, thus shortening the digestion time required. The effect of varying the salt / sulphuric acid ratio was reported by Phelps (1920) . His work showed that, with mercuric oxide as a catalyst and a 2:3 salt / acid ratio, pyridine salts, could be analyzed with a 2.5 hour digestion. Lepper (1930) reported that rapid digestion was obtained with potassium sulphate/ acid ratio of 3:4. Ogg and Willits (1950) measured the boiling temperature of potassium sulphate - sulphuric acid solutions with ratios ranging from 1:4 to 7:8 and found that the boiling point increased from 332$^{o}$C to 358$^{o}$C. They also observed that a temperature increase of 10$^{o}$C doubled the reaction rates.

Lake et al. (1951), studying the effects of potassium sulphate on temperature, concluded that at temperatures exceeding 410$^{o}$C nitrogen loss may

occur. To avoid the possible losses, the workers suggested adjusting the amount of potassium sulphate added to obtain a temperature of 370°C.

Extensive work was done by Bradstreet (1954) on the effect of the salt / acid ratio on nitrogen recovery. Using the term "acid index", defined as the milliliters of acid divided by the grams of potassium sulphate, he concluded that the limiting value of the acid index at the end of digestion should be 0.88. This value was established by considering the amount of acid lost by boiling plus that lost by reaction with different sample materials. Bradstreet suggested that the acid index may be useful to determine the minimum volume of an acid necessary to oxidize the sample and, as a consequence, prevent nitrogen losses that may occur due to a too high temperatures.

Other workers also suggested that the best means of controlling the temperature of digestion is by varying the amount of potassium sulphate in the digestion mixture. McKenzie and Wallace (1954) regarded a ratio of 1g potassium sulphate to 1ml sulphuric acid as optimal and a resulting temperature of 360°C as satisfactory for an open tube micro-Kjeldahl digestion.

Salts other than potassium sulphate have been used to increase the boiling temperature of the digest. Perrin (1953) completely replaced potassium sulphate with potassium phosphate and obtained a marked increase in the digestion rate. He also noted glassware etching during this procedure and occasional low results possibly due to an excessive increase in the digestion temperature. McKenzie and Wallace (1954) reported that sodium sulphate causes solidification of the digestion mixture and does not raise the digestion temperature as effectively as a similar weight of potassium sulphate. Potassium sulphate has none of the disadvantages listed above and is the usual salt added to control the digestion temperature.

Time of digestion

The digestion time needed for complete conversion of organic nitrogen to ammonia is inversely proportional to the temperature of the digest. McKenzie and Wallace (1954) found that samples of tryptophan cleared after 5 min of vigorous boiling and conversion to ammonia was complete after 20 min. On the macro scale, Lake (1951) found that a 1 hour digestion of the mixture gave satisfactory conversion of organic nitrogen to ammonia. Steymark (1961) recommended longer periods of digestion. His procedure required 4 hours of boiling; however the digestion mixture provided a boiling temperature of only 340$^{o}$C A short digestion time is generally recommended. Loss of sulphuric acid due to prolonged boiling changes the ratio of salt to acid and increases the temperature in an unpredictable manner (McKenzie, 1954) as solidification of the digest may occur (Bradstreet, 1954).

Oxidizing agents

Oxidizing agents are used to speed up the digestion. The most commonly used agents have been hydrogen peroxide, potassium permanganate and perchloric acid, with hydrogen peroxide being the oxidant of choice. Kirk (1950) and Koch and McMeckin (1924) recommended using hydrogen peroxide but did not report the loss of nitrogen during the digestion. However, Miller and Miller (1927) pointed out the importance of the addition of hydrogen peroxide only after the sample has been partially digested. McKenzie and Wallace (1954) noted that the practice of cooling the digest before the addition of the oxidizing agent may prevent nitrogen loss. They also noted that when a high salt / acid ratio of 1:1 was used, the digestion process did not benefit from the addition of hydrogen peroxide.

Reducing agents

Certain compounds such as nitrates, azo-compounds and nitroso-compounds are not successfully analyzed by standard Kjeldahl methods without a preliminary reduction. Fridrich et al. (1933) used hydriodic acid and red phosphorous as reducing agents. The acid and iodine formed were distilled off and the normal Kjeldahl digestion was carried out However, the collabo - rative studies showed that the results were erratic. Dickinson (1958) proposed a macro-method and Steyermark (1958) a micro-method using zinc and formic or acetic acid, followed by iron and hydrochloric acid to reduce the N-O and N-N linkages.

The carbon reduction method, in which the sample is heated with carbon, is also recommended. Bradstreet (1957) studied extensively this method of reduction and recommended the use of sucrose as a reducing agent. Another reducing agent which has been used with some success was salicylic acid (Steyermark, 1958).

Other digestion procedures

Most digestions are carried out in an open tubes or Kjeldahl flasks. However, closed tube digestions have also been reported. Levi and Gimignani (1929) presented a close-tube macro-method in which temperature was only raised to 330°C and the digestion time was as long as in the open flask proce - dure. Grunbaum (1955) observed that maintaining the temperature at approxi - mately 400-440°C achieves a quantitative conversion of nitrogen to ammonium sulphate without a catalyst. White and Lang (1953) developed a micro method for the determination of heterocyclic nitrogen in sealed glass tubes at 470°C with concentrated sulphuric acid and a mercuric oxide catalyst. This method

offered good accuracy and precision because there was no nitrogen loss due to the thermal decomposition of the ammonium sulphate.

Block and microwave oven digestion.

Recently a new method has been developed using heated aluminum blocks for sample digestion. Nelson and Sommers (1972) introduced with good results digestion blocks as a mean of digesting samples. Shulman et al. (1973) used a commercially available block for the digestion of nitrogen in soil samples and shortened the digestion time to 2.5 hours. Hambleton and Noel (1975) evaluated the Technicon BD-20 block digestor in the determination of crude protein in animal feed. When compared, the results obtained with the BD-20, proved to be as accurate as those obtained by the official final action Kjel - dahl method.

Isaac and Johnson (1976) confirmed block digestors as one of the best analytical tools for Kjeldahl digestions. They found the block digestor technique compatible with the automated continous flow methodology of an Autoanalyzer. Wall and Gehrke (1977) developed a semi-automated method for the determination of nitrogen using the Technion block digestor for sample digestions followed by an automated spectrophotometric analysis of the ammonia in the digests. After comparying this method to the official AOAC manual method (1976), they concluded that the Technicon block digestion method showed an accuracy and precision equal or superior to that of the official AOAC method.

The newest development in the digestion methodology is the utilization of a microwave oven for sample digestion. Abu-Samra et al. (1975) overcame some of the difficulties of the established digestion procedure by using a commercially available microwave oven for sample digestion. In this method,

acid mixtures are heated internally by the oscillating electromagnetic field, which results in a very rapid, safe and efficient digestion. The Abu-Samra et al. method was further modified by Barret and Davidowski (1978) to eliminate problems associated with the build-up of acid fumes in the oven cavity, their removal and subsequent treatment The workers used Pyrex chromatography glass for the oven cavity which was coupled to a simple trap and a KOH bubbler system for removal of the corrosive fumes. The workers also noted that since sufficiently high temperatures were generated in such a closed system, the need for potassium sulphate was eliminated. Nadkarni (1984) investigated the use of microwave oven for the acid dissolution of biological and geological materials. He concluded that this technique offers good reproducibility and significantly decreases analysis time.

**Estimation of ammonia**

To estimate the ammonia present in a sample, the digest containing ammonium sulphate is steam distilled into a standardized acid after the addition of alkali. If mercury has been used as a catalyst, the mercury-ammonium complex formed has to be precipitated since it interferes with the complete recovery of ammonia (Hiller et al., 1948). McKenzie and Wallace (1954) suggested that both zinc dust and thiosulphate can be used effectively to precipitate mercury. They noted that thiosulphate tends to produce acid fumes during distillation, but this problem can be avoided by mixing the alkali and the thiosulphate before addition to the digest. A sufficient amount of alkali added to the digest is essential to permit the ammonia to be easily and quantitatively distilled (Ogg, 1965). The same worker demonstrated, however, that too large an excess of alkali may lead to low recovery of ammonia when the digest solution contains precipitated mercuric sulfide.

Different methods of steam distillation can be carried out as required on sample quantities ranging from macroscale (Steymark, 1961) to the ultra microscale (Tourtellotte et al.,1958). In the original Kjeldahl procedure (Kjeldahl, 1887) ammonia was distilled into standardized sulphuric acid. After distillation, the excess of standard acid was determined iodometrically. Other workers, (Hiller et al , 1948) and a Joint Committe of the AOAC and AOCS (1955) used standard acid to absorb the ammonia carried over during distillation and reported excellent accuracy using this method

A more common practice is to use boric acid for the absorption of ammonia, which has the advantage that only one standard solution is necessary Boric acid was used by numerous workers (Ma and Zuazaga, 1942; McKenzie and Wallace, 1954) but more for micro rather than macro analysis. A statistical study based on the collaborative test (Joint Committe of AOCS and AOAC, 1955) showed that there was a significant difference between the results obtained with boric acid and those with standard hydrochloric or sulphuric acid, the latter giving higher values Wingo et al. (1950) studied the problem of incomplete ammonia recovery with the boric acid method and suggested that this could arise from the heating of the boric acid solution by hot distillate, since no losses occurred if the temperature of the receiver remained below 50°C. Stetten Jr. (1951) noted an increase in the pH of a boric acid solution as it was being diluted. He suggesed the adjustment of distillate to the same volume before each titration to assure reliable results.

Methyl red is the most common acid-base indicator used for Kjeldahl titration. However, the mixed indicators are prefered by many workers Ma and Zauzaga (1942) recommended using methyl red-bromocresol green indicator They pointed out that the colour change occurs over a much narrower pH range than with a single indicator; thus leading to a sharper, more reproducible

end point. Sheer (1955) described a two step indicator such as a mixture of bromocresol green, coccine and p-nitrophenol, which progressively changes from green to blue in boric acid; signalling the approach of the grey end point

An alternate method in which potassium hydrogen iodate serves as an ammonia absorbent has been proposed by Ballentine and Gregg (1947) and Niederl et al. (1938). Using an iodometric titration with its sharp end point they obtained a standard deviation of better than 0.01 for samples containing about 1% nitrogen. However, iodometric methods have been found unsatisfactory by some workers (McKenzie and Wallace, 1954) and, in practice, their use is limited to samples containing a few miligrams of nitrogen.

**Colorimetric methods**

As colorimetric procedures are generally more sensitive than titrimetric methods, they are often employed for analyses of samples containing below 0.3mg of nitrogen.

The most common colorimetric method is the Nessler procedure (Follin and Denis, 1916). It relies on the measurement of a yellow-brown color formed when the Nessler reagent (a mixture of potassium iodide and mercuric iodide) is added to a solution containing ammonia. Thompson and Morrison (1951) have done an in-depth study on the Nessler procedure and found that numerous factors such as amount of salt, pH of the solution, temperature and time of colour development, greatly affected the reaction These workers noticed that, if the solution was alkaline during the color development, turbidity might result; whereas, if the solution was too acidic, the color development might be insuficient and the reactants might precipitate. They recommended 20°C as an optimal temperature, and 20 to 30 minute as an optimal time for colour development.

Polley (1954), working with biological materials modified the Nessler reagent to improve its stability. He also noticed that, where mercury had been used as the catalyst, erratic results were obtained unless the mercury-ammonium complex was first decomposed with zinc dust.

Fleck and Munro (1965) obtained reproducible calibration curves with ammonium sulphate but, when the procedure was applied to protein analysis, the results were erratic. They reported that the Nessler procedure, when applied to the digest, was found to be unreliable by many workers due to the numerous factors affecting the results. Applying this procedure directly to the distillate eliminated many sources of error but also added another step to the method and required special apparatus.

A colorimetric method based on the indophenol reaction was developed by Thomas (1912) and latter adapted for the quantitative estimation of nitrogen by Russell (1944). The ammonia produced during the digestion is reacted at pH 10.8 with sodium phenoxide and then with sodium hypochlorite. The reaction produces a blue coloured indophenol compound which is measured colorimetrically at 630 nm

Lubochinky and Zalta (1954) reported that sodium nitroprusside accelerated the rate of colour development and increased the sensitivity of the reaction. Bolleter et al. (1961) reported obtaining reproducible results with the indophenol method when the ammonia was buffered with boric acid. He noted that the method was rapid since only 3 minutes of heating at 100°C was needed for colour development. He also noted that copper, zinc, iron salts and bromide ions were the only interferences.

Mann (1963) developed a reliable ultra-micro method for the determination of 1 to 15 µg of nitrogen. In this method, sample digestion is followed by

zink precipitation of mercury oxide catalyst and careful neutralization of the digest. The ammonia is then directly estimated as indophenol.

Another, more recent colorimetric method is based on a reaction of protein digest or distillate with ninhydrin reagent to form Ruhemann's purple which has absorbance maximum at 570 nm Jacobs (1962) reported that when ninhydrin is applied directly to the digest, a citrate buffer dilution is recommended to ensure a linear relationship between the level of ammonia present and the colour developed. The method was found to be sensitive and amounts of nitrogen as low as 1 to 4 µg could be determined

**Ammonia electrode method**

Recently, the use of an ammonia gas-sensing electrode (ammonia probe) for ammonia determination was reported. Orion research Inc (1979) has developed an ammonia electrode that permits determination of ammonia in aqueous solutions over a concentration range $10^{-6}$ to $10^{0}$ M The electrode contains a hydrophobic gas permeable membrane through which all free ammonia can diffuse into an internal electrolyte until the partial pressures on both sides of the membrane are constant. In the equilibrium condition, there is a direct Nernstian relationship between the concentration of ammonia in the sample and the potential of the glass electrode which senses the pH change of the internal electrolyte. To assure that all the ammonium ions in solution will be converted into free ammonia the pH of the sample has to be adjusted to 11 before the analysis.

Several workers used the gas-sensing electrode as a replacement for the distillation and titration steps to estimate the ammonia content of Kjeldahl digests. Todd (1973), analyzing the digests of a variety of meat products, found close agreement between the results obtained by the ammonia-sensitive

electrode method and the distillation-titration technique. He noticed the departure from linear behaviour at low concentrations of ammonia due to complex formation with copper ions. This problem was corrected by Todd by addition of disodium EDTA to the digest.

Buckee (1974) has described a procedure for estimating the ammonia content in Kjeldahl digest solutions for barley, malt and beer. The semi-micro digestion was followed by an automatic estimation of ammonia content by an electrode in a continous flow system at a rate of 60 samples per hour. The above system was found fast and efficient and the results agreed closely with the results obtained by the distillation-titration method.

Bremner and Tabatabai (1972) used an ammonia electrode for determination of ammonia in soils. They confirmed the close agreement between the electrode and the distillation-titration method and recommended that the measurement of ammonia in a digest should be performed immediately after the addition of alkali because of the loss of the ammonium ion as ammonia gas upon prolong standing. They also examined the effects of temperature and depth of immersion of an electrode on the results and concluded that it was imperative to keep the temperature constant, but the depth of immersion of a electrode did not affect the results.

Lopez and Rechnitz (1982) studied the interference of volatile amines on the ammonia electrode response. They evaluated the potentiometric response to a series of amines and other nitrogen containing compounds with a wide range of volatilities and basicities and came up with a series of coefficients which could predict the extent of amine interference.

# Chapter III

# PROPERTIES OF AMMONIUM IN AQUEOUS SOLUTION

## 3.1 Chemical Properties

Ammonia is a pungent, colorless gas extremely soluble in water. Solu - tions of ammonia are best described as $(NH_3)aq$ with the equilibrium written as:

$$(NH_3)_{aq} + H_2O = NH_4^+ + OH^-$$

and having the equilibrium constant $K = 1.81 \times 10^{-5}$. The solution of ammonia and ammonium ion in water can also be treated as a case of salt of a weak base and a strong acid. Their equilibrium in water is as follows:

$$NH_4^+ + H_2O = NH_3 + H^+$$

The apparent dissociation constant is:

$$K'a = [NH_3][H^+] / [NH_4^+],$$

which in the negative form is:

$$- \log K'_a = - \log [H^+] - \log [NH_3] / [NH_4^+],$$

which the "p" form is:

$$pK_a' = pH - \log [NH_3] / [NH_4^+]$$

which rearanges to:

$$pH = pK'_a + \log [NH_3] / [NH_4^+].$$

This last equation, called the Henderson-Hasselbach equation, describes the relationship between the pH, the $pK'_a$ and the log of ammonia to ammonium ion ratio for a given pH (Williams, 1967). Since the $pK'_a$ for ammo - nium ion is 9.4, the final form of the Henderson-Hasselbach equation is:

$$pH = 9.4 + \log [NH_3] / [NH_4^+]$$

From the above equation, the ratios of concentration of ammonia to ammonium ion for various pHs can be calculated (Table 1).

Table 1. pH versus log $[NH_3] / [NH_4^+]$ for $(NH_4)_2SO_4$ solution.

| pH | $\log[NH_3] / [NH_4^+]$ |
|---|---|
| 3 | $3.98 \times 10^{-7}$ |
| 4 | $3.98 \times 10^{-6}$ |
| 5 | $3.98 \times 10^{-5}$ |
| 8 | $3.98 \times 10^{-2}$ |
| 9.4 | 1 |
| 10 | 3.98 |

The results in Table 1 demonstrate that the concentration of ammonia in solution is minimal below pH 8 and increases dramatically above this value as the equilibrium of ammonia-ammonium ion is shifted.

The dissociation constant for ammonia- ammonium ion system is also dependent on the temperature of the solution (Bates and Pinching,1950). There is a linear decrease in the $pK'_a$ value with an increase in temperature, which consequently affects the pH of the ammonia-ammonium ion solution. (Table 2).

Table 2. Dissociation constants versus temperature for $(NH_4)_2SO_4$ solution[a]

| Temperature | $pK'_a$ |
|---|---|
| 0 | 10.081 |
| 5 | 9.903 |
| 10 | 9.730 |
| 15 | 9.564 |
| 20 | 9.401 |
| 25 | 9.246 |
| 30 | 9.093 |
| 35 | 8.947 |
| 40 | 8.805 |
| 45 | 8.671 |
| 50 | 8.540 |

[a]From Bates and Pinching (1950).

### 3.1 Infrared properties

The unperturbed $NH_4^+$ ion would be expected to have tetrahedral symmetry $T_d$ resulting in four fundamental vibrations, two of which are infrared active having theoretical frequency values of 3350$cm^{-1}$ (2.98 μm) and 1400 $cm^{-1}$ (7.14 μm) respectively (Nakamoto, 1978). However, in the majority of crystalline ammonium salts and in their solutions, the ammonium ion frequencies are removed from these values because of the formation of hydrogen bonds, and the influence of the electric field surrounding the ion (Thompson, 1966). Table 3 shows certain site symmetries to which the ammonium ion may be subjected and the corresponding symmetry groups. As the site symmetry of the ammonium is lowered, degeneracies are lost and the number of fundamental vibrational frequencies to be observed increases.

Table 3. Fundamental vibrational frequencies and symmetry groups for ammonium ion[a].

| mode | $cm^{-1}/\mu m$ | $T_d$ | $C_{3v}$ | $C_{2v}$* | $C_s$* |
|---|---|---|---|---|---|
| v1 | 3250/3.08 | $A_1$(R) | $A_1$(R)(IR) | $A_1$ | A' |
| v2 | 1700/5.88 | E(R) | E(R)(IR) | $A_1+B_1$ | A'A" |
| v3 | 3350/2.98 | $F_2$(R)(IR) | $E+A_1$(R)(IR) | $A_1+B_1+A_1$ | A'+A"+A' |
| v4 | 1400/7.14 | $F_2$(R)(IR) | $E+A_1$(R)(IR) | $A_1+B_1+A_1$ | A'+A"+A' |

[a]From Thompson (1966).

R = Raman active.
IR = infrared active.
* All classes are infrared and Raman active.

Two additional bands occur in the case of a hydrogen bonded ammonium ion. Waddington (1958) described one near 3040 $cm^{-1}$ arising from the interaction of $v_2 + v_4$ in Fermi resonance with $v_3$ which has been lowered in frequency by hydrogen bonding, and a second band near 2850 $cm^{-1}$ arising from $2v_4$ strengthened by Fermi resonance with $v_3$. The confirmation for this assigment came from the observation (Russell, 1965) that only one $NH_4^+$ stretching band appeared in the infrared spectrum of crystalline ammonium perchlorate and of anhydrous ammonium substituted montmorillonite and saponite clays.

The infrared properties of various ammonium salts in crystalline state, in saturated and dilute solutions were studied by Thompson (1966) and are presented in Table 4.

Table 4. Frequencies observed for crystalline ammonium salts and solutions[a].

| Compound | Vibrational mode | Crystalline solid $cm^{-1}/\mu m$ | Saturated soln. $cm^{-1}/\mu m$ | Dilute soln. $cm^{-1}/\mu m$ |
|---|---|---|---|---|
| $NH_4ClO_4$ | v3 | 3290/3.03 | 3200/3.12 | 3200/3.12 |
| | v3/(v2 +v4) | -/- | 3050/3.27 | 3050/3.12 |
| | 2v4 | 2850/3.50 | -/- | -/- |
| | v4 | 1415/7.06 | 1450/6.89 | 1450/6.89 |
| $NH_4F$ | v3 | 3100/3.22 | 3180/3.14 | 3200/3.12 |
| | v3/(v2 + v4) | -/- | -/- | -/- |
| | 2v4 | 3024/3.30 | 3070/3.25 | -/- |
| | v4 | 1484/6.73 | 1465/6.82 | 1455/6.87 |
| $NH_4Cl$ | v3 | 3138/3.18 | 3180/3.14 | 3200/3.12 |
| | v3/(v2 +v4) | 3044/3.28 | 3050/3.27 | 3050/3.27 |
| | 2v4 | 2870/3.48 | 2850/3.50 | -/- |
| | v4 | 1403/7.12 | 1440/6.94 | 1450/6.89 |
| $NH_4Br$ | v3 | 3137/3.18 | 3180/3.14 | 3200/3.12 |
| | v3/(v2 + v4) | 3031/3.30 | 3050/3.27 | 3050/3.27 |
| | 2v4 | 2833/3.53 | 2850/3.50 | -/- |
| | v4 | 1401/7.13 | 1440/6.94 | 1450/6.89 |
| $NH_4I$ | v3 | 3130/3.19 | 3180/3.14 | 3195/3.12 |
| | v3/(v2 + v4) | -/- | 3050/3.27 | 3090/3.23 |
| | 2v4 | -/- | -/- | -/- |
| | v4 | 1385/7.22 | 1430/6.99 | 1440/6.91 |
| $NH_4NO_3$ | v3 | 3230/3.09 | 3200/3.12 | 3200/3.12 |
| | v3/(v2 + v4) | 3190/3.09 | 3070/3.25 | 3050/3.27 |
| | 2v4 | 2860/3.49 | -/- | -/- |
| | v4 | 1420/7.04 | 1435/6.96 | 1450/6.89 |

[a]From Thompson (1966).

The absorption band wavelengths obtained by Thompson, indicate that the spectra of crystalline solids cannot be directly compared because of their different ion symmetry. In saturated aqueous solutions, the sets of ammonium ion frequencies differ significantly from one another and their values lie mid-way between the values for dilute solutions and crystalline solids. In dilute solutions however, where only hydrogen bonding exists and there are no perturbing effects on an ion, the frequency of the ammonium ion reaches a constant set of values.

These constant absorption wavelengths for the ammonium ion demonstrate that, with the formation of hydrogen bonds, the number of fundamental vibrational frequencies increase resulting in two new bands at 3040 (3.28 μm) and 2850 (3.35 μm) $cm^{-1}$. The data also indicates that the resolution of spectra decreases as one moves from a crystalline solid to a dilute solution. As a result, the bands are broadened as they merge into each other. The above phenomenon was confirmed by Russell (1965) who noticed that, when anhydrous ammonium complexes were equilibrated to 40% relative humidity, new absorption bands at 3270 (3.05 μm), 3035 (3.29 μm) and 2855 (3.50 μm) $cm^{-1}$ appeared.

Since the structure of many inorganic ions is dependent upon pH, it is important to note the pH of the solution being recorded. In the case of ammonium ion solutions, only one absorption band at 1455$cm^{-1}$ (6.87 μm) due to $N^{+}$- H bending appears at pH 4. At pH 9.2 an additional band ammonia appears at 1115 $cm^{-1}$ (9.05 μm) due to the increased ammonia concentration at a higher pH (Goulden and Manning, 1967).

# Chapter IV

# PRINCIPLES of INFRARED QUANTITATION

## 4.1 Theoretical Considerations

The aim of quantitative analysis is to determine the concentration of a component of interest. Since the height of an absorption band is proportional to the concentration of the functional group causing the band, the amount of a component present in a sample can be determined by comparing the height of the band produced with its heigth in the spectrum of a known concentration of this component.

Beer's law describes the relationship between the absorbance and the concentration of a component. It is defined by the following equation:

$$A = e\,l\,c$$

where A is the absorbance, e is the absorptivity, l is the path length and c is the concentration of the component. Beer's law holds true only for low concentrations and assumes monochromatic and parallel light without scattering induced by particle size (Perry, 1956).

A spectrophotometer measures the energy absorbed by a sample by comparying the incident radiation of the source ($I_0$) to that which passes through the sample. To overcome the variability involved in obtaining absolute values for each wavelength, the relative intensity ($I / I_0$) which is defined as transmittance, T, is measured. The absorbance of a substance is directly proportional to a negative logarithm of the transmittance as follows:

$$A = - \log T = - \log(I / I_0)$$

or

$$A = \log(1/T) = \log(I_0 / I).$$

This relationship holds true for a single wavelength instrument. Whereas, in a dual wavelength instrument, the comb is driven by the signal difference at the detector, and the transmission is defined as follows:

$$T = I / I_0 = (I_s - I_r) / (I_{os} - I_{or})$$

where s and r represent the sample and reference wavelength respectively.

There are several significant factors which affect quantitative IR analysis. They were extensively studied by Perry and the magnitude of the resulting errors was evaluated (Perry, 1956, 1970). These factors include slit width, determination of 0 and 100% transmission, tracking error and cell inequality.

To maximize the energy transmitted, the slit should be operated at its widest possible setting (Perry, 1956, 1970). This introduces a problem of decreased resolution and reduction in peak intensity. However, in quantitative analysis, reproducibility is more important than absolute absorbance and Perry recommended increasing the slit width and controling the resultant noise level by gain adjustement.

Another factor to be considered is the determination 0 and 100% trans - mission. Uncertainities in determining the absolute values of these two para - meters can possibly lead to inaccuracy in absorption measurments. It has there - fore been generally accepted (Robinson, 1951) that, to minimize any possible error the transmission readings should be confined to the 20 - 80% range.

The concept of tracking error refers to the ability of a servo system to respond to the instructions from the detector. Since the servo system has a delayed response, the transmisson recorded is only aproximate. Tracking error can be minimized by proper settings of instrument controls and slow scanning speeds (Perry, 1956).

Another factor which could significantly affect quantitative IR analysis is the inequality of the cells utilized in the instrument. The cells can be well

matched since a cell's thickness can be determined with great accuracy from its fringe pattern. However, there always is a possibility of error being introduced if the cell windows are not ideally flat or parallel

Also band resulting from inexact solvent compensation in IR aqueous analysis may interfere with the results. The problem becomes more serious in concentrated solutions and in the regions of strong solvent absorption These sources of error should be addressed to produce accurate results and will be discussed in detail in subsequent chapters.

**4.2 Aqueous IR analysis**

Aqueous solution IR spectroscopy is particularly suitable for substances that are insoluble in organic solvents and give solid spectra which are difficult to interpret. These substances include most inorganic and ionic compounds, biological systems in which water is a natural medium, and most food systems Aqueous solution spectra show single absorption bands which can be assigned to the ionic species present and are not complicated by bands due to ionic interactions as in the solid state spectra. Since the bands are well defined, they are also suitable for quantitative work.

Water shows very strong absorption bands throughout the entire medium infrared region. The most prominent bands are in the regions 1700-1600 $cm^{-1}$ and 3500 -3000 $cm^{-1}$ due to O-H streching vibrations. Because of such strong water absorption in these regions only about 10% energy is available for analysis (Rao, 1963). Consequently the bands due to the solute are usually obscured unless both solvent compensation and suitable instrumentation are employed.

Solvent compensation is usually achieved by placing a cell containing water in the reference beam of a double-beam spectrophotometer having equal

cell pathways For quantitative analysis, effects due to non-absorbing ions must be considered. Accurate solvent compensation can be achieved by placing an alkali halide solution, instead of pure water, in the reference cell (Thompson, 1966). This also solves the problem of an apparent increase in solution trans - parency that arises in concentrated solutions when strongly absorbing water molecules are displaced from the beam

When a double beam spectrophotometer is used and solvent compensa - tion is employed, a small fraction of energy reaches the detector. To restore the instrument response, the slit width should be increased by a factor of $T^{-1/2}$ where T is the transmission of the cell containing water (Manning, 1971). With high resolving power grating instruments, it is not possible to open the slit wide enough without the image becoming larger than the detector surface. Therefore, to obtain satisfactory spectra, a reduced scan speed and an increased gain have to be employed also (Perry, 1956). Opening the slit to compensate for energy lost due to water absorbance results in a lower resolution. This could cause a large deviation from the Beer's law if the band being measured is very narrow. In practice, however, it was found that absorption bands are broad in aqueous solutions and Beer's law can be used for quantitation (Gqulden, 1959).

Temperature also affects the absorption bands. Therefore, it is important that the cells of an instrument are well thermostated and that the solutions being pumped are at the same temperature as temperature of the instrument cells (Manning, 1971).

The optimal path length to be used with aqueous solutions is 50 µm or less (Manning, 1972). With such cells, the best results are obtained with com - pounds having solubilities greater than 1% in water (Goulden, 1959). A number of optical cell window materials are available for cells used in aqueous IR ana-

lysis. Although glass and silica are much less expensive than other materials, their use is limited to the short wavelength regions as the cut-off wavelengths for glass and silica are 2.5 and 3.7 µm respectively (Manning, 1972). Barium fluoride is slightly soluble in acid but can transmit up to 12.5 µm. However, calcium fluoride is probably the most useful material. It is hardly soluble in water, inert to acids and alkalies and has good transmittance characteristics up to 9.6 µm. Intran-2 has also been extensively used but its opacity prevents the detection of small air bubbles that may be trapped within the cell. Other materials such as silver chloride, thallium bromo-iodide, polyethylene and sulphur crystals have also found an application as window materials for IR cells (Manning, 1972).

# Chapter V
# INSTRUMENTATION

## 5.1 The Spectroprocessor IV.

Most of the research was done on the Spectroprocessor IV spectrophotometer. It is an instrument specially designed for our research purpose by Mr Jack Shields of Shields Instruments Ltd., England. The instrument offers features necessary for the IR work in aqueous solutions such as high energy source, removal of water vapours, temperature stability and flow-through cells. Its design is based on the use of Grubb-Parsons Spectromaster chassis (Figure 5) which was rebuilt optically and electronicaly.

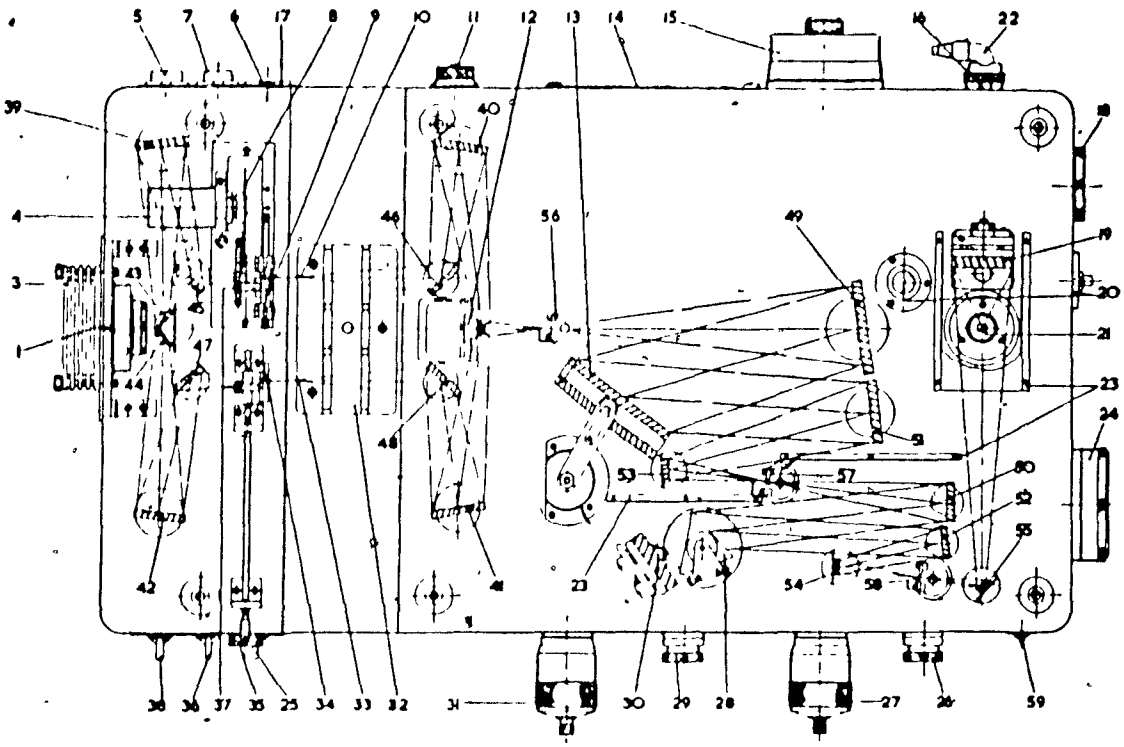
The following are the best features of the instrument which make it suitable for the low energy situations arising in the IR work in aqueous solutions:

1. Nernst filament is a high energy source producing the mid-IR radiation. It is mainly composed of powdered oxides of zirconium, thorium and cerium and can be used up to 2000$^{o}$K.

2. Specially designed gratings are capable to choose the desired wavelength with high accuracy. The first having 984 lines/cm covers 5 - 25 μm range in the first order. The second having 2,953 lines/cm covers the 2 - 5 μm range in the first order and 0.5 - 2 μm range in the second order. The double monochromator system consisting of Czerny-Turner grating, prism and Littrow mirror efficiently reduces stray light.

3. A highly responsive servo motor minimizes dead space at the null balance point. A high performance low noise solid state amplifier senses minute changes in signal.

Figure 1. Basic schematic of the Grubb Parsons Spectromaster, the base unit for the Spectroprocessor IV. (from Martin, A E., 1966)



Optical system of Spectromaster. 1 Position of Nernst, 3 Nernst housing (air-cooled), 4 Servo, 5 To electronics unit, 6 Fuses, 7 To barretter-box, 8 Comb cam, 9 Null-balance comb, 10 Reference cell position, 11 Phasing-adjustment, 12 Reciprocating mirror system, 13 Gratings, 14 Cover for wavelength speed-change gears, 15 Variable programme selector, 16 Earth terminal, 17 Mains input, 18 Thermostat, 19 Ellipsoid, 20 Dry air inlet, 21 Thermocouple, 22 To thermocouple transformer, 23 Light-shields, 24 Wavelength range selection drum, 25 Instrument on/off, 26 Slit programme manual/automatic, 27 Slit indication, 28 KBr prism, 29 Scanning speed selector, 30 Littrow mirror, 31 Wavelength indication, 32 Gas cell holder, 33 Sample cell position, 34 Trimmer comb (100 per cent), 35 Trimmer comb control knob, 36 Electronics on/off, 37 Comb clamping screws, 38 Servo switch, 39–42 Spherical mirrors, 43–48 Plane mirrors, 49, 50 Collimating mirrors, 51, 52 Focussing mirrors, 53–55 Plane mirrors, 56–58 Slits, 59 Wavelength pause button.

4. The digitally controlled stepping motor drive the grating on a 1:1 basis with the grating drum, i.e., 1,000 steps per grating micrometer revolution. The slits are driven on a 2:3 basis with the slit drum, i.e., 600 steps per slit micrometer revolution.

5. Short path length strontium and calcium fluoride cells are used having transmission cut-offs at 10 and 9.6 μm respectively. A flow-through device allows for pumping in the solutions into the cells without interfering air bubbles.

6. Losses of energy due to moisture are minimized by hermetically sealing the instrument. Its two ports are connected to the containers of dried molecular sieve through which air is being pumped The internal temperature of 37°C is maintained by solid state heaters.

Instrument logic and data processing

The Spectroprocessor IV is controlled by a Zilog MC-280 microprocessor and operated from a CRT terminal (Figure 6). The microprocessor has 64K memory, its one drive contains optional control programs and calibration files and the second drive is used for data storage The programs control the step - ping motors driving the slit and gratings and contain the command language RIO used to write new operational programs. The spectra can be plotted on a flat bed digital plotter (Wanatabe Digi-Plot, Northern Ltd, Natwick, England) capable of drawing the wavenumber or wavelength axis, plot the spectrum as the scan is in progress or from stored data.

Operation of the instrument.

One of the most important features of the Spectroprocessor IV is its ability to generate calibration programs Since water absorption varies through the IR region, obtaining a constant energy level through the entire spectrum is a major

Figure 2a. The Spectroprocessor IV apparatus.

(A) Stepping motor control;

(B) Solid state amplifier;

(C) Digital voltometer;

(D) Spectrophotometer;

(E) CRT display unit;

(F) Strip chart recorder replaced by x, y plotter;

(G) Disk drives;

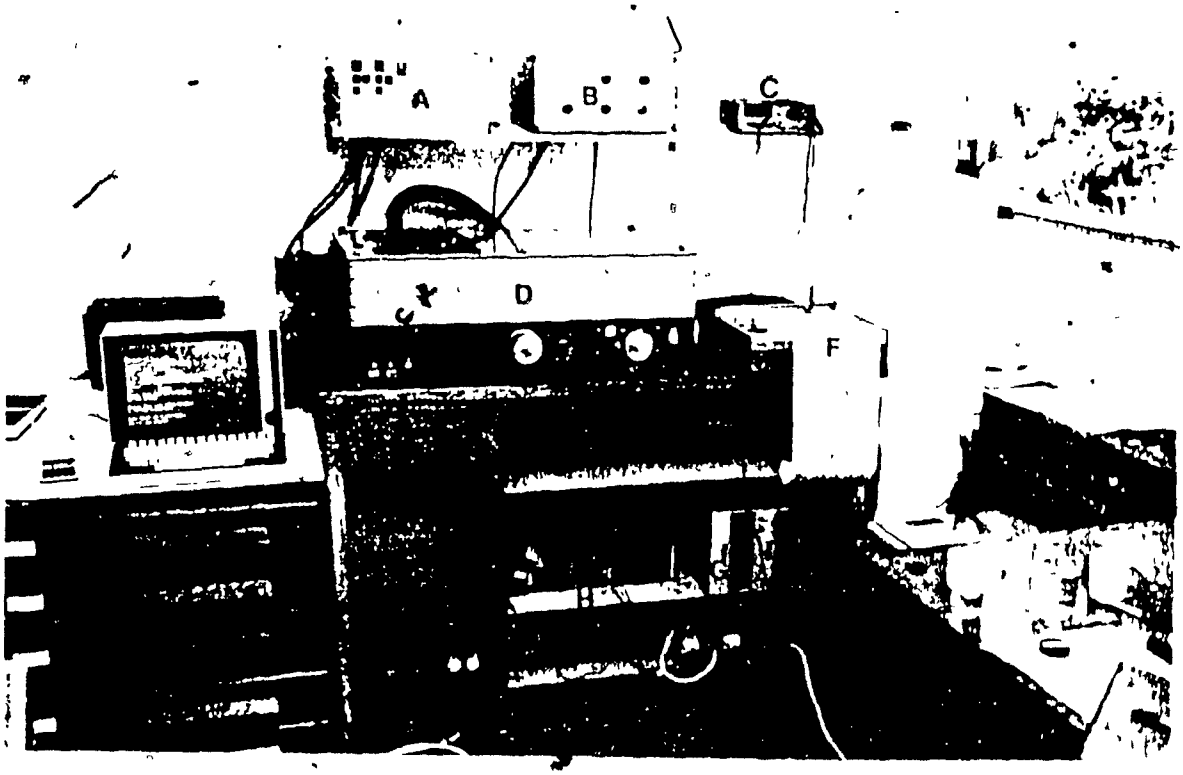
(H) Zilog microprocessor;

(I) Pump for desiccating instrument;

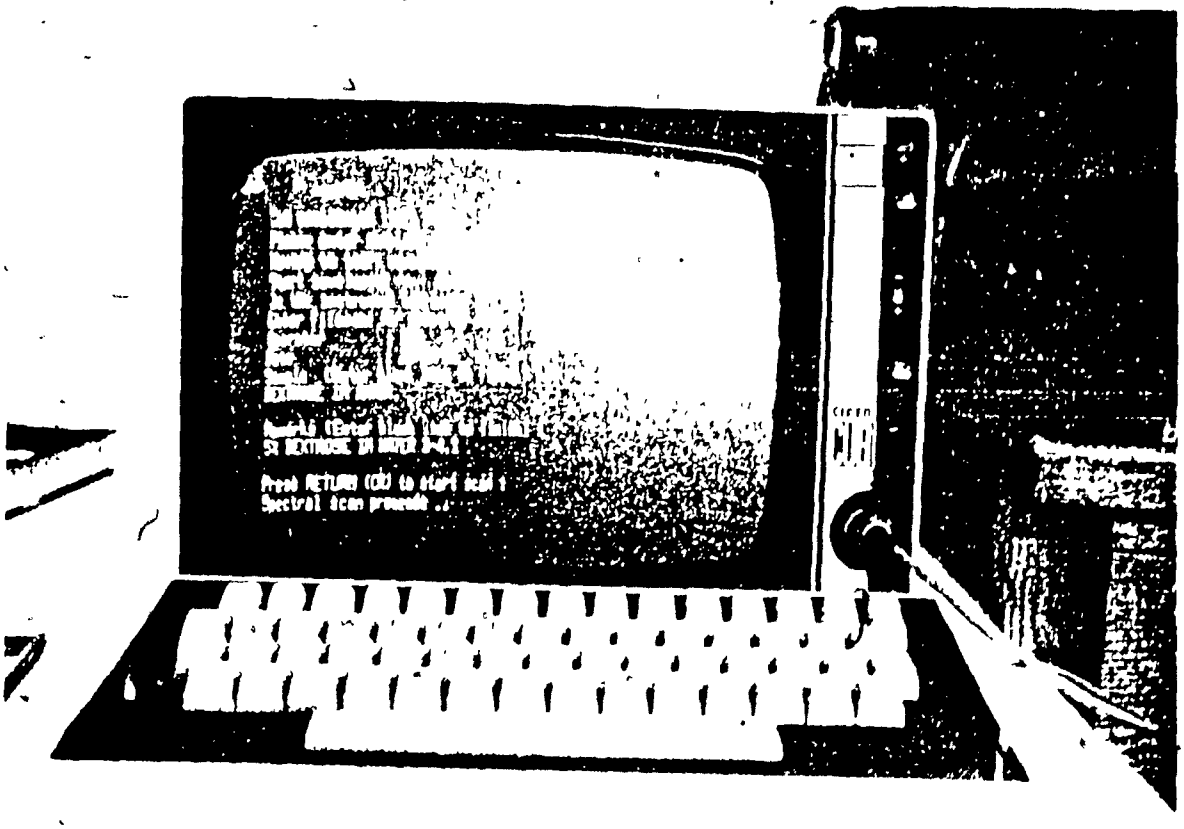
(J) Hard copy terminal for data output (not shown).

Figure 2b. CRT display unit.

A




B



problem. A calibration program delivers a constant energy level by automaticaly opening and closing the slit as the grating moves from one position to another (Appendix A). A calibration file is generated by scanning the solvent in a single beam mode and storing the slit settings on a disk for a desired energy level. This program can be run simultaneously with a standardized scanning program to give a constant energy scan of a sample in aqueous media.

The instrument also has capabilities to sample wavelengths, slit and other data during the run. It can also be programmed to suit the individual needs of the operator

The computer has to be oriented in the instrument's status to be able to execute the operational programs. This procedure called initialization was followed for all the programs used to run the instrument. A typical program to set-up the instrument for a single beam scan is presented in Appendix B.

Several operations required to set-up the instrument were carried out (Mills,1983):

1. Cells were assembled and scanned to determine their transmission characteristics. Cells were matched using the interference fringe method (Sutherland and Willis,1945) for the path length determination.
2. Beam balance was checked to ensure a drift of less than 1% over a 30 minute scan and noise level was set to a practical level by adjusting the gain.
3. Sampling and setting times were established to adjust scanning speed to response time.
4. To prevent loss of energy due to moisture, the instrument was hermetically sealed and its two ports were connected to the containers of dried molecular sieve. The internal temperature of 37$^{o}$C was maintained by solid state heaters.
5. The cell holder was plumbed with 0.1 cm i.d. stainless steel tubing to allow solutions to be pumped into cells without interfering bubbles.

On the daily basis, the following procedures involving the Spectroprocessor IV were generally followed:

1. Samples were prepared in deionized, distilled water, which was thoroughly degassed to prevent bubble formation in the instrument cells.
2. Temperature of the samples was rised to 37°C which corresponds to the inner temperature of the instrument.
3. Ammonium sulphate solutions were placed in the sample cell with the equivalent (% wt / vol.) sodium chloride solutions placed in the reference cell to eliminate the water displacement effect on the spectra (Thompson, 1966).
4. Before each ammonium sulphate scan, the instrument transmission level was set at 80% (Robinson, 1951) by placing distilled water in both sample and reference cell.
5. Three consecutive transmission readings were taken for each sample and the average value was used to calculate absorption value.
6. Distilled water was pumped through the sample cell prior to the next sample being run.
7. Sample and reference cell were cleaned on the regular basis with a cleaning solution made of 1 ml Triton X-100 / 1L and followed by distilled water rinse.

Summarizing, Spectroprocessor IV is a state of art research instrument, extremely versatile and useful in the analysis of aqueous solutions.

**5.2 The Multispec M.**

Multispec M (Figure 7) is a commercial infrared analyzer designed by Shields Instruments Ltd, York, England, specifically for rapid quantitative milk analysis (Biggs,1979). It differs from the conventional double beam, dual cell instrument by relying on a single cell, dual wavelength optical system. The original instrument was equipped with a fat (C=O group) filter pair at 5.73 and 5.58 µm, protein (CO-NH group) filter pair at 6.46 and 6.68 µm, lactose (C-OH group) filter pair at 9.61 and 7.68 µm and total solids ( C-H group) filter pair at 3.48 and 3.56 um, where the reference wavelenght serves as a background level of absorption. For this study the total solids filter pair was replaced the filter pair capable of measuring the ammonia ion (6.86 and 5.56 um).

The basic optical schematic for the Multispec M is presented in Figure 8. The cell of the instrument is alternately irradiated with sample and reference beam by a chopper The transmitted energy is focussed on the detector which generates a small signal due to the lack of balance between sample and reference beam. The action of null balance system is employed in which the servo motor drives an attenuating comb into the reference beam until the beams are balanced. Since an exponential relationship holds between component concentration and comb displacement (Transmission), the component's quantitation is based on the log of the transmission of the sample wavelength to a reference wavelength. To obtain a linear readout (Absorbance) of component concentration, the logarithm of the signal is obtained electronicaly.

In order to provide stable temperature for sample analysis, the interior of the instrument is maintained ay 37$^{0}$C, and the cell is enclosed in a heating block maintained at 40$^{0}$C. Since water vapour affect instrument stability, the

Figure 3. Basic schematic of the Multispec M exterior and its component parts. (from the Instruction Manual for the Multispec M, Berwind Instrument Group, Birmingam MI)

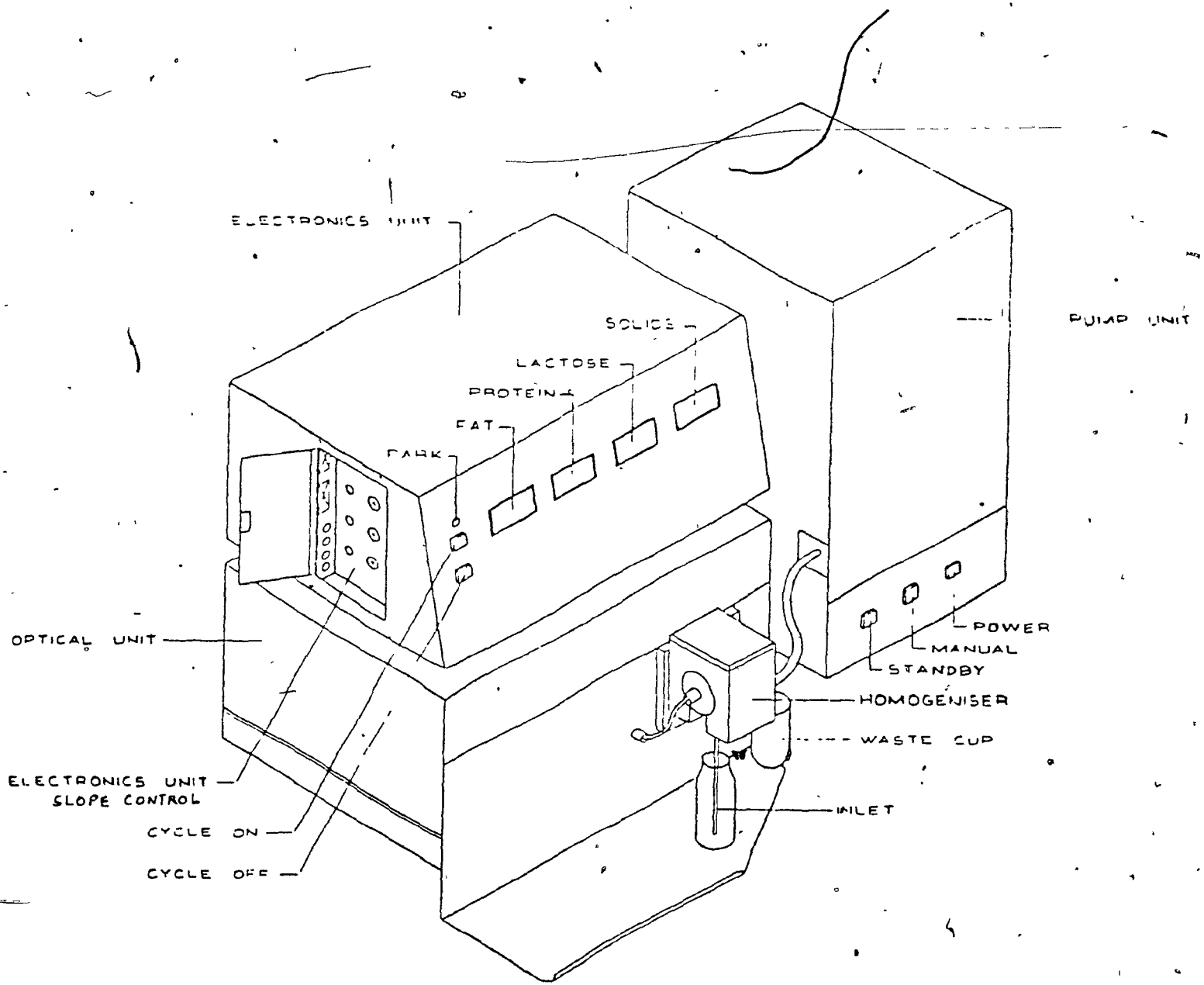
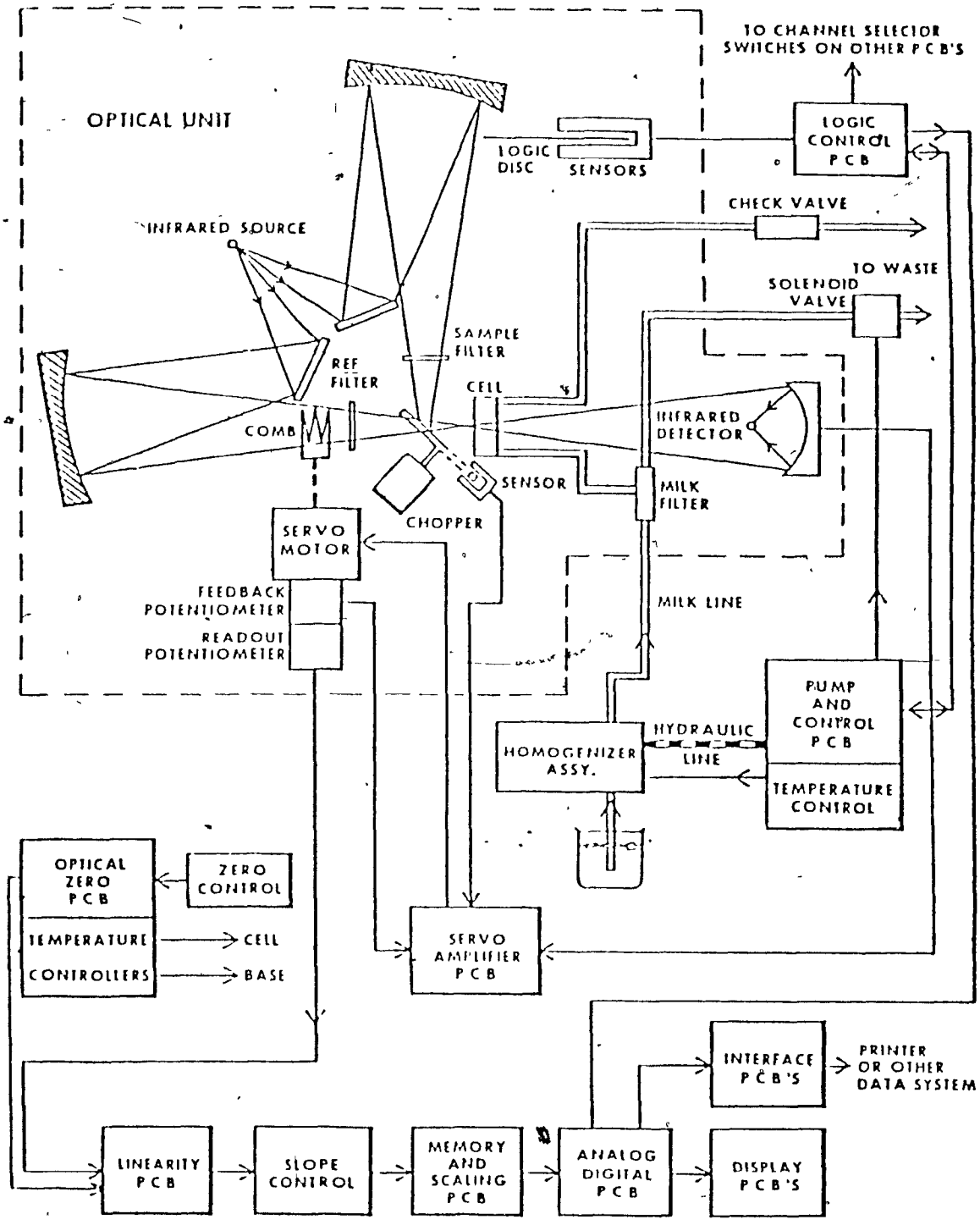

ELECTRONICS UNIT
SOLIDS
LACTOSE
PROTEIN
FAT
PUMP UNIT
DARK
OPTICAL UNIT
POWER
MANUAL
STANDBY
HOMOGENISER
WASTE CUP
ELECTRONICS UNIT
SLOPE CONTROL
CYCLE ON
CYCLE OFF
INLET

Figure 4. Basic optical schematic for the Multispec M.
from the Instruction Manual for the Multispec M, Berwind Instrument Group, Birmingham MI).


OPTICAL UNIT
TO CHANNEL SELECTOR SWITCHES ON OTHER P C B'S
LOGIC DISC
SENSORS
LOGIC CONTROL P C B
CHECK VALVE
INFRARED SOURCE
TO WASTE SOLENOID VALVE
SAMPLE FILTER
REF FILTER
CELL
COMB
INFRARED DETECTOR
SENSOR
MILK FILTER
CHOPPER
SERVO MOTOR
FEEDBACK POTENTIOMETER
READOUT POTENTIOMETER
MILK LINE
PUMP AND CONTROL P C B
HYDRAULIC LINE
HOMOGENIZER ASSY.
TEMPERATURE CONTROL
OPTICAL ZERO P C B
ZERO CONTROL
TEMPERATURE CONTROLLERS
CELL
BASE
SERVO AMPLIFIER P C B
INTERFACE P C B'S
PRINTER OR OTHER DATA SYSTEM
LINEARITY P C B
SLOPE CONTROL
MEMORY AND SCALING P C B
ANALOG DIGITAL P C B
DISPLAY P C B'S

instrument is hermetically sealed and the water vapour controlled by using a high surface area molecular sieve.

Instrument calibration

The instrument is calibrated to ensure the linearity of absorbance relative to concentration for a component of interest. The linearity for ammonia channel can be verified by analyzing a serial dilution of standard solutions containing ammonium sulphate.

The next step in the instrument calibration is the adjustments of the slope and optical zero controls to obtain the best correlation between the instrument readout and chemical data. The slope control sets the primary slope while the zero controls used to remove any intercept which can result from a small optical unbalance of the beams. The slope is adjusted to 1:1 ratio between instrument signal and chemical values and the optical zero is set with water in the cell. This procedure is used regularly to maintain the instrument linearity and a constant 1:1 ratio between instrument signals and chemical values

Multispec M provides us with both 'corrected' and 'uncorrected' signals. A single pure component in solution gives always 'correct' signal, but the main component signal in a mixture may deviate from Beer's law due to minor absorption by other components and the effects of water displacement. These interferences may be corrected by using secondary slope controls as described by Mills (1983).

Multispec M can be directly interfaced with Hewlett Packard 85 desk top computer capable of processing data and rapid calibration of the instrument providing the chemical values for sample calibration are available.

The basic procedures for Multispec M operation are presented below (Mills, 1983):

1. The zero setting on each channel was set using 42.5°C deaerated water.

2.The samples were preheated in waterbath at 42 5°C for 5 minutes.

3. The sealed containers were inverted three times (not shaken as this incorporates air) and presented to the instrument.

4. The instrument was run in the manual mode and each sample passed through three times, the signal recorded and averaged.

5. The instrument was purged using distilled water prior to the next set of samples being run.

6. After operating the instrument, a cleaning solution made of 1 ml Triton X-100 was run followed by distilled water. If the zero setting was not regained, the recorded values were adjusted in accordance.

# Chapter VI

# MATERIALS and METHODS

## 6.1 Chemicals

The reagents used in this research were as follows: ammonium sulphate crystals (Fisher certified ACS), sodium chloride crystals (Fisher certified ACS), sodium hydroxide pellets (Fisher certified ACS), sulphuric acid 95-98% (Fisher reagent ACS), hydrochloric acid 36.5-38% (Fisher reagent ACS), potassium sulphate powder (Fisher certified ACS), cupric sulphate crystals (Fisher certified ACS) and selenium metal powder (Sargent-Welch reagent grade). All the solu - tions were prepared in distilled, deionized water

## 6.2 Work on the Spectoprocessor IV

Measurment of cell path length.

Cells were matched using the interference fringe method (Sutherland and Willis, 1945) for path length determination. The method uses the interfe - rence fringes caused by the change in refractive index of the windows and air space between the windows of a sealed cell.

The sample cell was cleaned with water and ethanol and dried with air. The reference cell was removed and the spectrum was recorded in the 1-5 μm region versus air. The operation was repeated for the reference cell and the path length for both cells was calculated using the following formula

$$b = n / 2 (L_1 L_2 / L_2 - L_1)$$

where b is the thickness of the cell (μm), $L_1 L_2$ are the respective wavelengths chosen for fringe count and n is the number of fringes.

Development of constant energy calibration program.

A slit program was developed which would maintain constant energy over the sample scan in the 1-10 μm region. Single beam scans of water were run at 50% energy level in the 1-5 and 5-10 μm region. The program thus generated was stored in the calibration file to be run simultaneously with a standardized scanning program to give a constant energy scan of a sample in aqueous media.

Optimization of slit width and gain.

To enhance the instrument response and to assure the most accurate quantitation of ammonium ion, the optimal instrument conditions were deter - mined in regard to slit width and gain setting. A series of ammonium sulphate solutions (1-5% wt / vol ) was prepared in distilled water and pumped into the instrument. The transmission readings were taken at both ammonium sulphate absorption bands at the slit width settings between 0 68 to 1.35 mm with gain at 80% full scale. The experiment was repeated at gain settings between 70 to 100% full scale and at the maximum slit width of 1.35 mm. Both absorption bands were evaluated for their linearity and relative response to concentration at different slit width and gain settings. Various settle / sample time settings were also evaluated for the optimal reproducibility of the results by calculating the mean and the standard error of estimate for five consecutive absorption readings taken at different settle / sample settings.

Identification of the analytical wavelengths.

The first stage of this study involved establishing the spectral characte - ristics of ammonium sulphate in water. A 5% ammonium sulphate solution was prepared in distilled water and scanned in 1-5 and 5-10 μm range at the scan

speed of 5 µm / min and at the maximum gain. The bands obtained for ammo - nium and sulphate ion were identified and compared with the literature values.

Choice of the analytical wavelength

A series of ammonium sulphate solutions (0.0-0.5% wt / vol.) was pre - pared in distilled water to choose the most suitable band for the quantitative studies of ammonium ion. The solutions were scanned in the 1-5 and 5-10 µm range and the ammonium ion bands were evaluated for their linearity and relative response to concentration at 1:35 mm slit width and 80% full gain. The baseline method was used for quantitation. The linearity of the plots of absorp - tion versus concentration for ammonium ion was evaluated by linear regression and the relative response to concentration was assessed by considering the slope of the regression line between concentrations.

Choice of the quantitation method.

Two methods of quantitation of ammonium band were considered. The baseline method relies on the standard absorbance ($A = - \log 1/T$) at the maxi - mum absorbance wavelength while the dual wavelength method involves ratioing the transmission at the selected reference wavelength to that of the sample wavelength ($A = \log T_O / T_S$). Both methods were assessed by scanning a series of ammonium sulphate solutions (1-5% wt / vol.) and evaluating the linearity of the absorbance versus concentration plot as well as the reproduci - bility of the results.

Effects of pH on absorption.

The effect of pH on the absorption bands of ammonium ion was exa - mined. Serial weights of ammonium sulphate (1-5% wt / vol.) were dissolved in

distilled water and their pH adjusted using 1N hydrochloric acid and 1N sodium hydroxide producing solutions in the pH range between 3 5 and 8.8. A 5% ammonium sulphate solution of pH 9.5 was also prepared and scanned to demonstrate the appearance of an absorbance band due to aqueous ammonia. The solutions were scanned in the 5-10 μm range at the slit width of 1.35 mm and 80% full gain. The dual wavelength method was used for quantitation. The absorptivity of ammonium band at each pH level was determined as a slope of the plot of concentration versus absorbance using the linear regression method. The absorptivity values obtained were plotted against the pH values of ammo - nium sulphate solutions

Study of ammonium sulphate in dilute acids.

After studying the ammonium sulphate solutions in distilled water the next step was to examine its spectral characteristics in dilute acids. A carefully weighed amount (0.00-0.12g) of ammonium sulphate was placed in 30 mL Kjeldahl tube and dissolved in 3 mL of concentrated sulphuric acid. The pH of the solutions were adjusted to the 3-5 range by addition of 5N sodium hydro - xide containing metacresol purple indicator prepared according to the Hand - book of Chemistry and Physics (1978-79). During the addition of base the solutions were stirred and cooled in a specially designed Erlenmeyer flask to avoid excessive rise in temperature. Then the solutions were made to 25 mL volume with distilled water and scanned in 5-10 μm range at 5 μm / min with slit width set at 1.35 mm and gain at maximum. A blank solution prepared in an identical way was placed in the reference cell. The absorbance values obtained by the dual wavelength method were plotted against the ammonium ion concentration (% wt / vol.) and the linearity of the plot was evaluated by linear regression. Furthermore the maximum absorbance wavelength and the

absorptivity of the ammonium ion band exhibited in the dilute acid spectrum was compared to the values obtained from the ammonium sulphate spectrum in distilled water

Study of ammonium sulphate in simulated digest.

A Kjeldahl digestion is usually carried out using concentrated sulphuric acid with substantial levels of potassium sulphate added to increase the boiling point of a digest. A study was carried out to assess the spectral behaviour of ammonium ion in a simulated digest solution. A carefully weighed amount (1 25 g) of ammonium sulphate was placed in 30 mL Kjeldahl tube together with 2 5 g of catalyst mixture ($K_2SO_4$:Se:$CuSO_4$ = 100 6:1) and 5 mL of concentrated sulphuric acid (Buckee, 1974). The solution was boiled for 1 hour and the pH of the digest adjusted to the 3-5 range with 5N sodium hydroxide following the procedure developed for ammonium sulphate solutions in dilute acid. Then the volume was adjusted to 25 mL with distilled water and the solution was scanned in 5-10 µm range at 5 µm / min with slit width set at 1 35 mm and gain at maxi - mum. A blank digest solution prepared in identical way was placed in the refe - rence cell. The maximum absorbance wavelength and the absorptivity of the ammonium ion band exhibited in the digest spectrum was compared to the values obtained from the ammonium sulphate spectra in distilled water and dilute acid.

**6.3 Work on the Multispec M.**

Based on the results obtained with the Spectroprocessor IV, a filter pair was installed in the Multispec M instrument and the unit was assessed for its quantitative ability.

Study of ammonium sulphate in distilled water.

To determine the optimal slope potentiometer setting (signal multiplication) a serial dilutions of ammonium sulphate (0-1.0% wt / vol.) were prepared in distilled water and run at various slope potentiometer settings. The linearity of the plots based on the ammonium sulphate concentration (% wt / vol.) versus instrument pannel reading (Multispec M signal) was evaluated by linear regression.

Study of ammonium sulphate in acid solutions

The ability of the Multispec M to quantitate the ammonium ion in the partially neutralized acid solutions was assessed. A series of ammonium sulphate solutions (0-0.5 % wt / vol.) was prepared following the procedure developed for the analysis of acid solutions by the Spectroprocessor IV. The second series of ammonium sulphate solutions (0-0.5% wt / vol.) was prepared in an identical way without the cooling procedure during the neutralization step. The linearity of the plots based on the ammonium sulphate concentration versus the instrument's reading ( Multispec M signal) was evaluated by linear regression.

Comparision of the Multispec M method to the Kjeldahl procedure.

The ability of the Multispec M to quantitate the ammonium ion was compared to a modified titration procedure used in the micro-Kjeldahl method (AOAC,1984). A carefully weighed amount (between 0.0-0.6 g) of ammonium sulphate was introduced into a micro-Kjeldahl distillation aparatus, made basic and the ammonia was steam distilled into a 100 mL volumetric flask containing 50 mL of standardized 0.1N HCl. After distillation the solution was brought to volume and either back titrated into standard HCl or pumped through the filter

instrument calibrated in terms of % (wt / vol.) ammonium sulphate. The titration and infrared results were compared (Youden, 1975) using the mean, mean difference (MD) and standard deviation of differences (SDD).

# Chapter VII
# RESULTS and DISCUSSION

The first part of this research was done on the IR research spectrophotometer, the Spectroprocessor IV. The methodology developed on this instrument was then transferred into the commercial filter IR spectrophotometer, the Multispec M.

The cells of Spectroprocessor IV were closely matched using the inter - ference fringe method ( Figure 5). As a result, strontium fluoride cells (39.22 and 38.85 μm for sample and reference cell respectively) and calcium fluoride cells (60.99 and 60.89 μm for sample and reference cell respectively) were used in this work. The above path lengths were chosen because they repre - sented the best compromise between loss of energy due to water absorption and gain in sensitivity to ammonium sulphate concentration.

Since water absorbs strongly in the 1-10 μm region (Figure 6), a slit program was developed which would maintain constant energy over the sample scan in the 1-10 μm region. The calibration scan for water with its energy level and the slit profile is presented in Figure 7. The program gene- rated by running single beam scans of water in a 1-5 and 5-10 μm range (Appendix A), was stored with a standardized scanning program (Appendix B) to give a constant energy scan of a sample in aqueous solutions.

Ammonium ion infrared spectra in crystalline salts forms and in aqueous solutions were studied by the Thompson method (Thompson, 1966) with 0.7 μm cells. In addition, Miller and Wilkins (1952) compiled infrared spectra and chara - cteristic frequencies of crystalline ammonium ion. The unperturbed $NH_4^+$ ion is expected to have tetrahedral symmetry leading to four fundamental vibrations,

Figure 5. Interference fringe pattern for the calcium fluoride sample cell, recorded in the 1-5 μm range versus air.

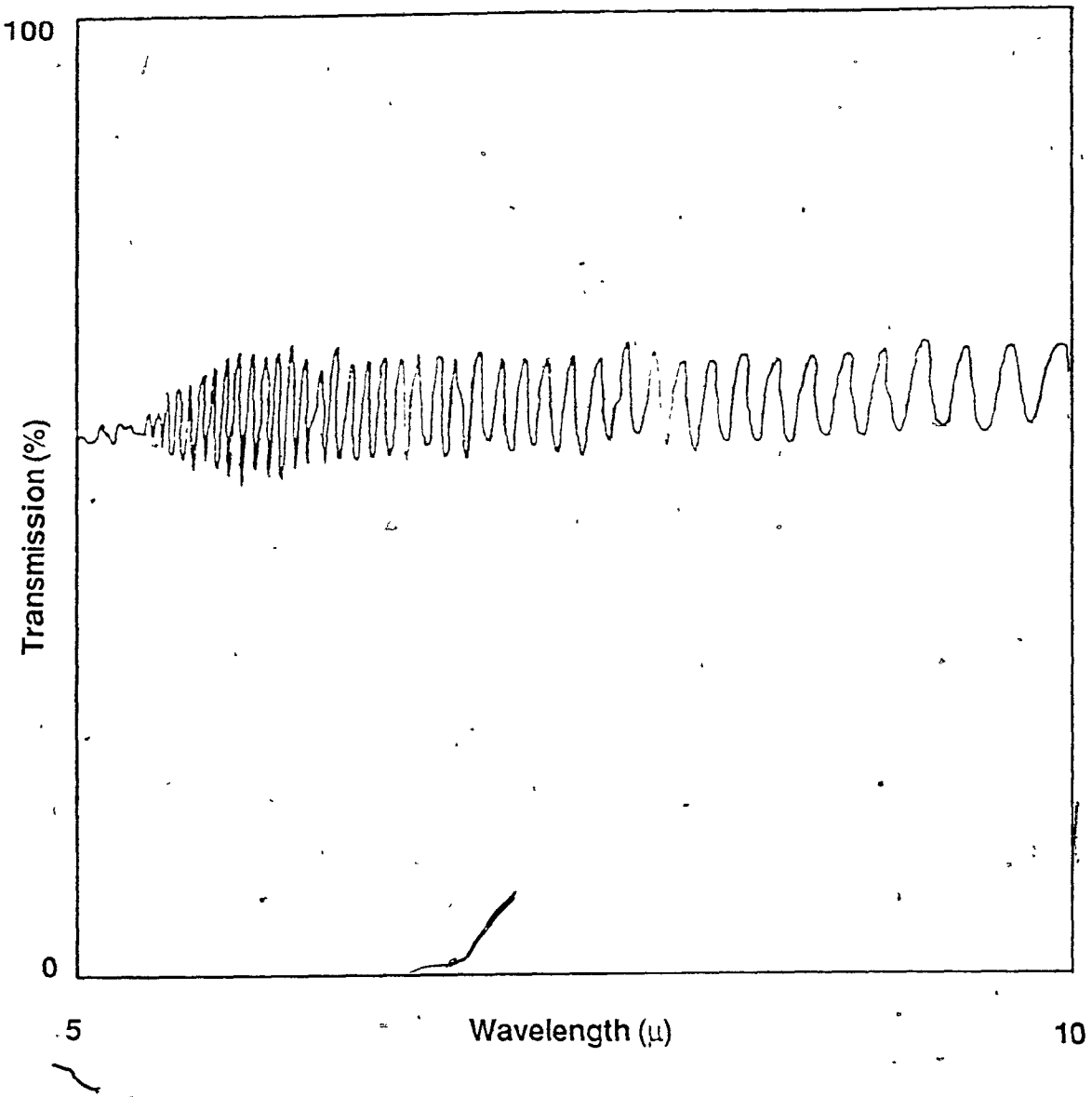

100
Transmission (%)
0
.5
Wavelength (μ)
10

Figure 6. A single beam scan of water over the 1-10 μm region.
Line A is the 1-5 μm region and Line B the 5-10 μm region.

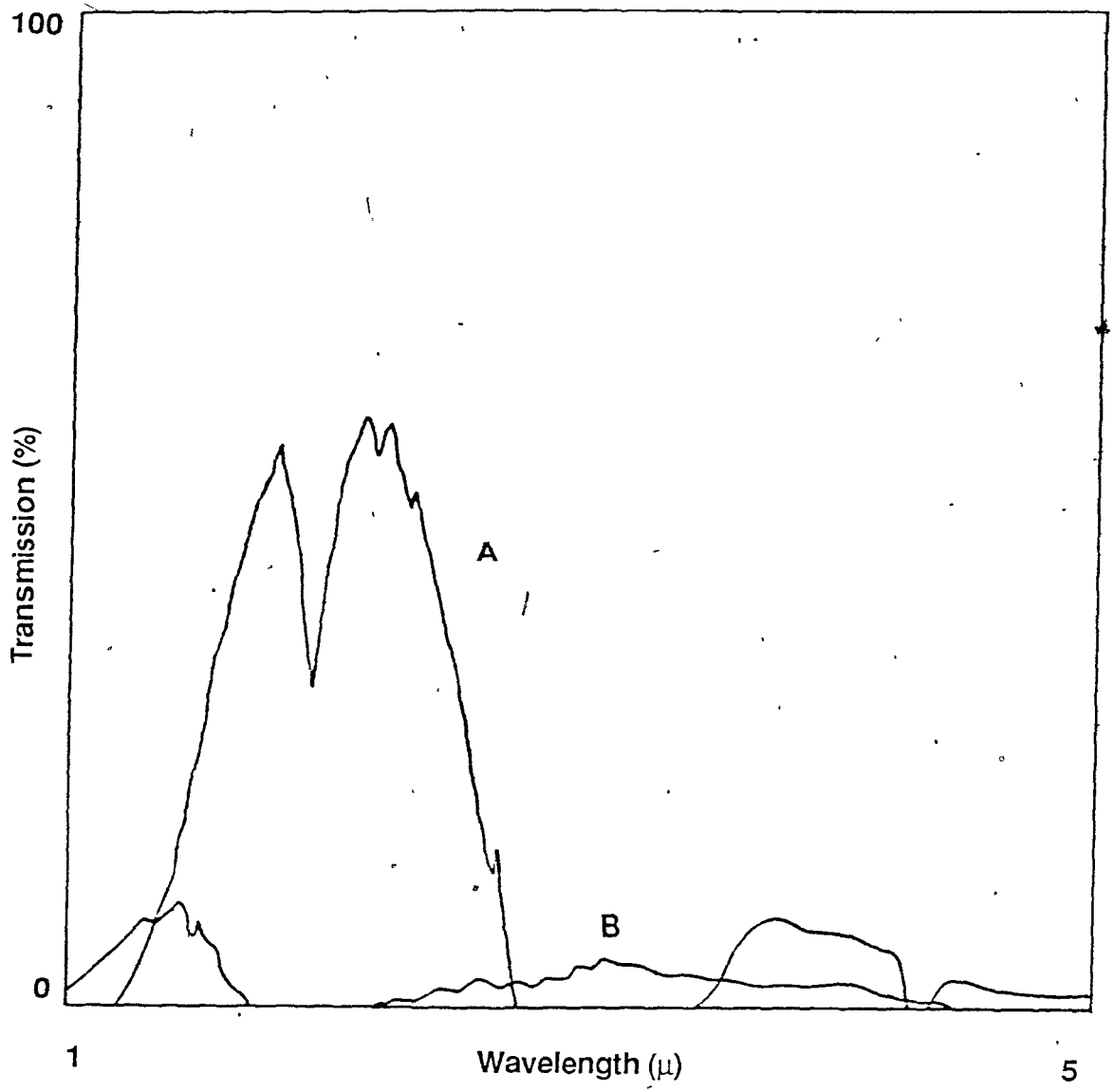
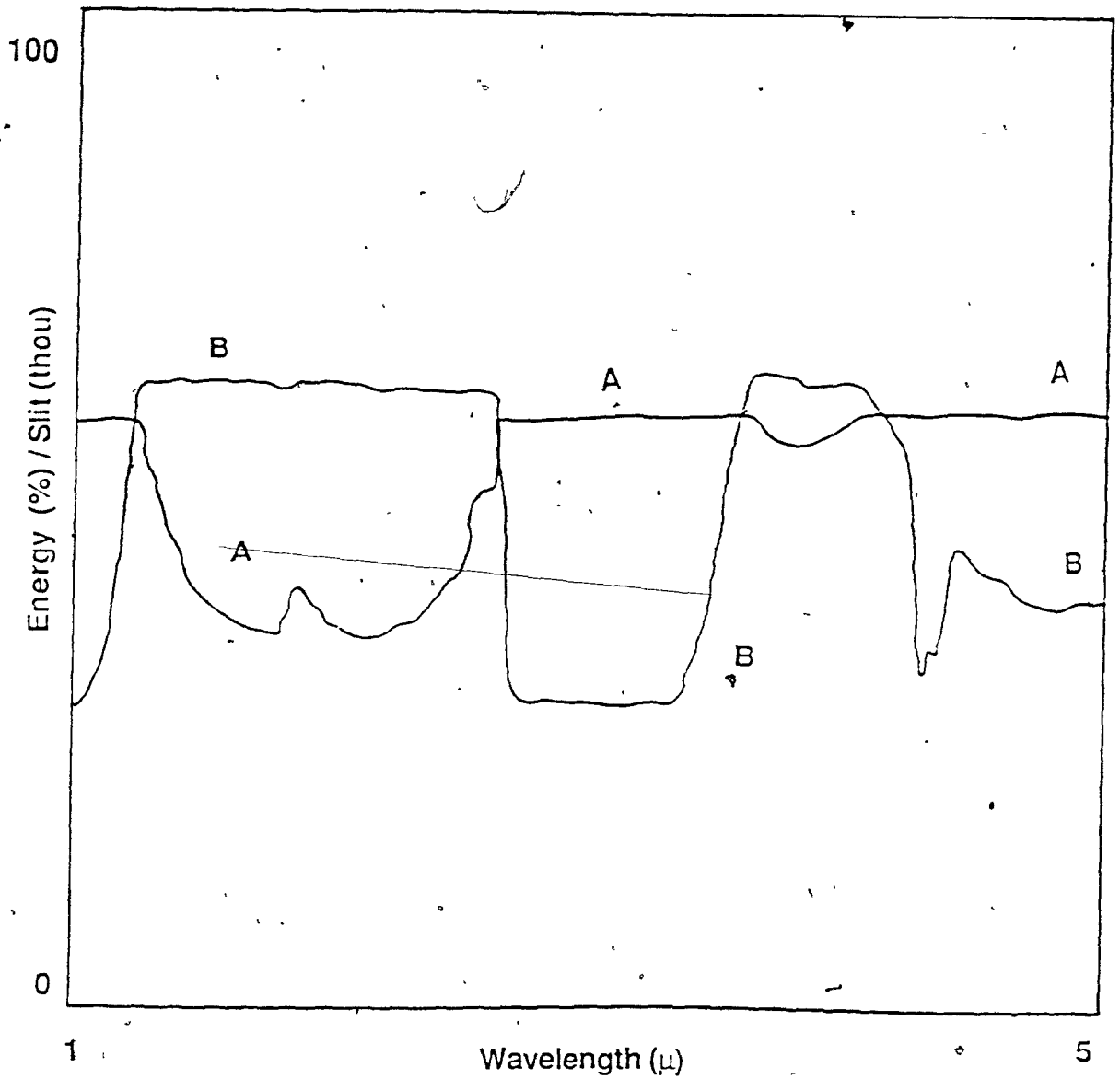

100
Transmission (%)
A
B
0
1
Wavelength (μ)
5

Figure 7. Calibration scan for water. Line A is the slit profile and Line B the energy level.

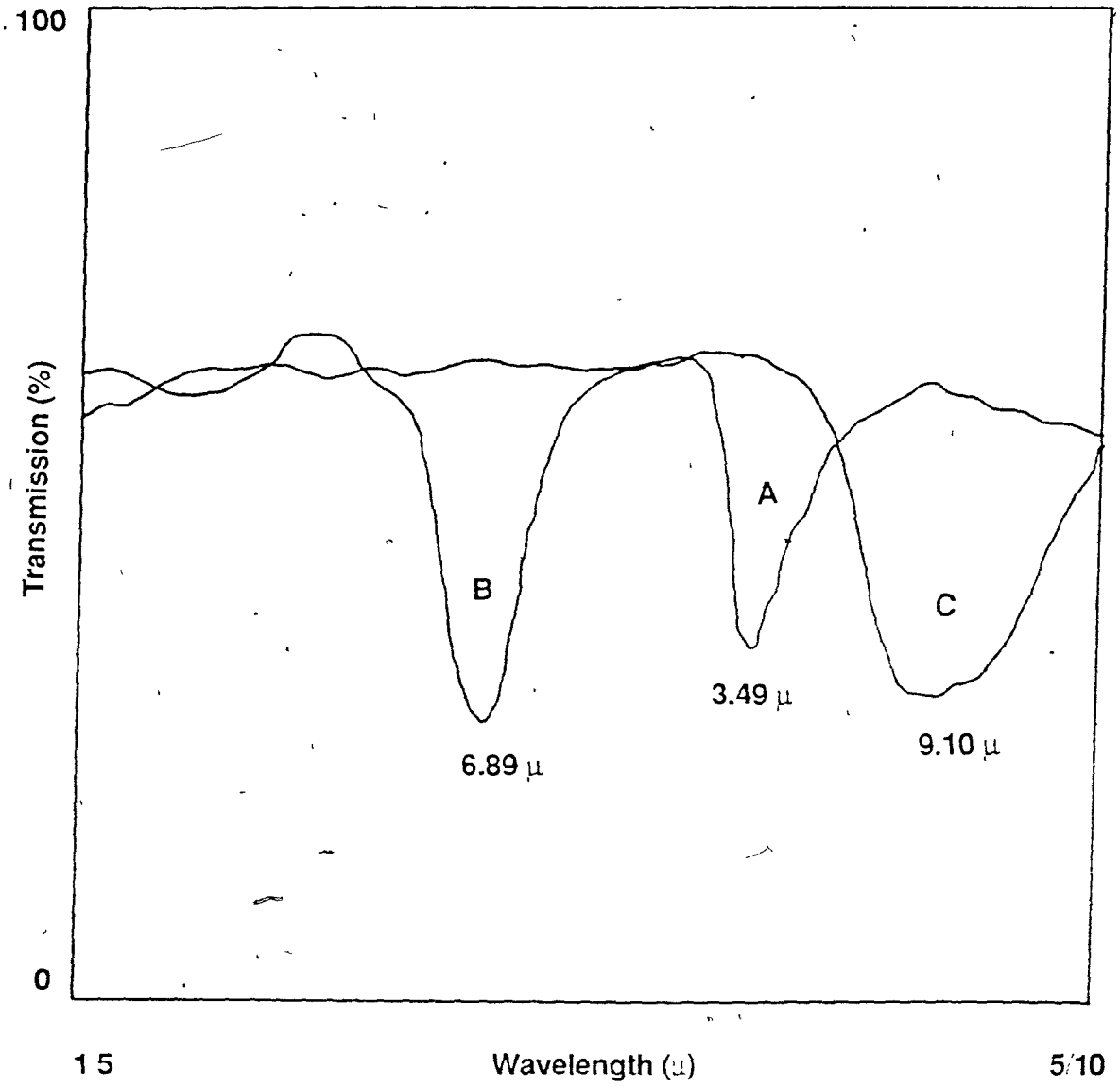

100
0
Energy (%) / Slit (thou)
1
Wavelength (μ)
5
B
A
A
A
B
B

two of which are infrared active (v3 and v4) having theoretical frequency values of 3350 $cm^{-1}$ (2.98 μm) and 1400 $cm^{-1}$ (7.17 μm) respectively In aqueous solution, however, the degeneracies are lost as hydrogen bonding to water occurs and two new (medium and strong) bands appear at 3040 (3 28 μm) and 2850 (3.35 μm) $cm^{-1}$ (Russell, 1965).

The ammonium sulphate spectra obtained from the Spectroprocessor IV with 40 μm strontium fluoride cells (Figure 8) were simple, showing none of the details characteristic of crystalline spectra or even that of solutions scanned using the 0.7 μm cell (Thompson, 1965). The ammonium sulphate spectrum features three peaks, the first at 3.49 μm, the second at 6 89 μm and the third at 9.1 μm. The 3 49 μm band is a composite peak due to hydrogen bonding interfering with $N^+$- H stretch, and any contributions expected to be seen from v3 is lost due to poor energy conditions The diffuse peak shifted away from the theoretical 3250 $cm^{-1}$ originates from the interaction of v2 + v4 in Fermi resonance with v3 (3040 $cm^{-1}$) and 2v4 strengthened by Fermi resonance with v3 (Waddington, 1958). The second peak at 6.89 μm corresponds to $N^+$- H bending (v4) and matches well with the values for ammonium ion frequencies presented by Thompson (1966). The third peak corresponds to the sulphate ion and is in reasonable agreement with the literature values for other sulphate salts (9.05 μm) (Miller and Wilkins, 1952). As a confirmation, ammonium chloride was scanned at pH 5.5 and the spectrum exhibited two bands associated with the ammonium ion. When ammonium chloride was scanned at pH 9.5, a third peak appeared having a maximum at 8 95 μm (1117 $cm^{-1}$) corresponding to aqueous ammonia (Manning, 1972).

Before quantitation of ammonium ion in solution was undertaken, the Spectroprocessor IV slit width and gain settings were optimized. The standard principles of Perry (1956,1970), which advocates the use of the maximum slit

Figure 8. A scan of aqueous ammonium sulphate (pH 5.5) over the range 1-10 μm , where A is $N^+$ - H strech of ammonium ion (3.49 μm), B is $N^+$ - H bend of ammonium ion (6.89 μm) and C is the sulphate ion (9.10 μm).


100
Transmission (%)
0
B
A
C
6.89 μ
3.49 μ
9.10 μ
1 5
Wavelength (μ)
5/10

width and gain setting necessary to provide sufficient energy with an acceptable noise level, were confirmed in this study. When the linearity of ammonium sulphate solution standard curves was evaluated, it was noted that the highest correlation coefficient (0 9997) was obtained for the widest slit width (1.350 mm) and it decreased gradually with the narrowing of the slit width. It was also found that the absorption coefficient (slope) decreased as the slit width increased (Table 5) These results were in agreement with the studies of Potts (1963) who observed that the absorption coefficient decreases only less than the first power of the spectral width, while the signal to noise ratio improves as the square of the slit width. Based on our results and the studies of Perry (1956) and Potts (1963), the slit width was set at 1.350 mm for the further studies.

Table 5. Slit width versus correlation coefficient and slope for ammonium sulphate solutions at 6.89 μm wavelength.

| Slit width (mm) | Correlation coefficient | Slope |
|---|---|---|
| 1.350 | 0.9997 | 0.0962 |
| 1.125 | 0.9947 | 0.1006 |
| 0.900 | 0.9907 | 0.1024 |
| 0.675 | 0.9904 | 0.1077 |

The optimal gain setting was found to be 80% full scale when the linearity of the ammonium sulphate solution standard curves was evaluated. It was noted that the correlation coefficient decreased from 0.9983 to 0.9931 as the gain increased from 80% to 100% full scale due to the higher noise level (Table 6). Whereas, in the case of slit width, the optimal gain setting for ammonium ion quantitation resulted in a lower absorption coefficient (slope)

for the ammonium band, 0.1002 for 80% versus 0.1157 for 100% of full gain.

Table 6. Gain versus correlation coefficient and slope for ammonium sulphate solutions at 6.89 µm wavelength

| Gain (%) | Correlation coefficient | Slope |
|---|---|---|
| 100 | 0.9931 | 0.1157 |
| 90 | 0.9950 | 0.1137 |
| 80 | 0.9983 | 0.1002 |

Furthermore, the effect of the settle / sample time on the stability of instrument response was examined (Table 7). As a result, the maximum settle/ sample time of 1000/100 msec was chosen for further studies to provide the highest consistency of transmission readings.

Table 7. Settle / sample time versus standard error of estimate for ammonium sulphate solutions at 6.89 µm wavelength.

| Settle / sample time | Standard error of estimate (%)[a] | |
|---|---|---|
| | 0.1% | 0.5% |
| 10/100 | 0.017 | 0.023 |
| 50/500 | 0.006 | 0.008 |
| 100/1000 | 0.004 | 0.005 |

[a] n=5

A study was carried out to choose the optimal analytical wavelength for ammonium ion quantitation. A series of ammonium sulphate solutions (0.0-0.5% wt / vol.) in distilled water were scanned and both analytical wavelengths were assessed for their linearity and relative response to concentration using the baseline method. Figure 9 presents the scans of a serial dilution of ammonium sulphate in distilled water (5 - 10 µm) range and Figure 10 presents the standard curves for the 3.49 and 6.89 µm wavelengths. Both curves were linear, having a correlation coefficient of 0.9982 and 0 9991 for 3.49 and 6.89 µm respectively but their response differed, with the band at 6.89 µm being about 2.0 times as sensitive as that at 3.49 µm. The 6.89 µm band was chosen for further work because of its superior response to the ammonium ion concentration and the fact that the 3.49 µm peak appeared to be a more complex, composite band.

A study was also carried out to compare the dual wavelength method to the baseline method for the quantitation of ammonium ion. Both methods gave accurate and consistent results (Table 8) but the dual wavelength method was chosen for further work because it is the same principle utilized by the commercial infrared filter instrument. Perry (1956) also recommended a two wavelength method for quantitative work since it eliminated such variables as light scattering, cell window fogging and differences in preparation of sample and reference solutions.

Figure 9. Scans of a serial dilution of ammonium sulphate in distilled water (5 - 10 µm).

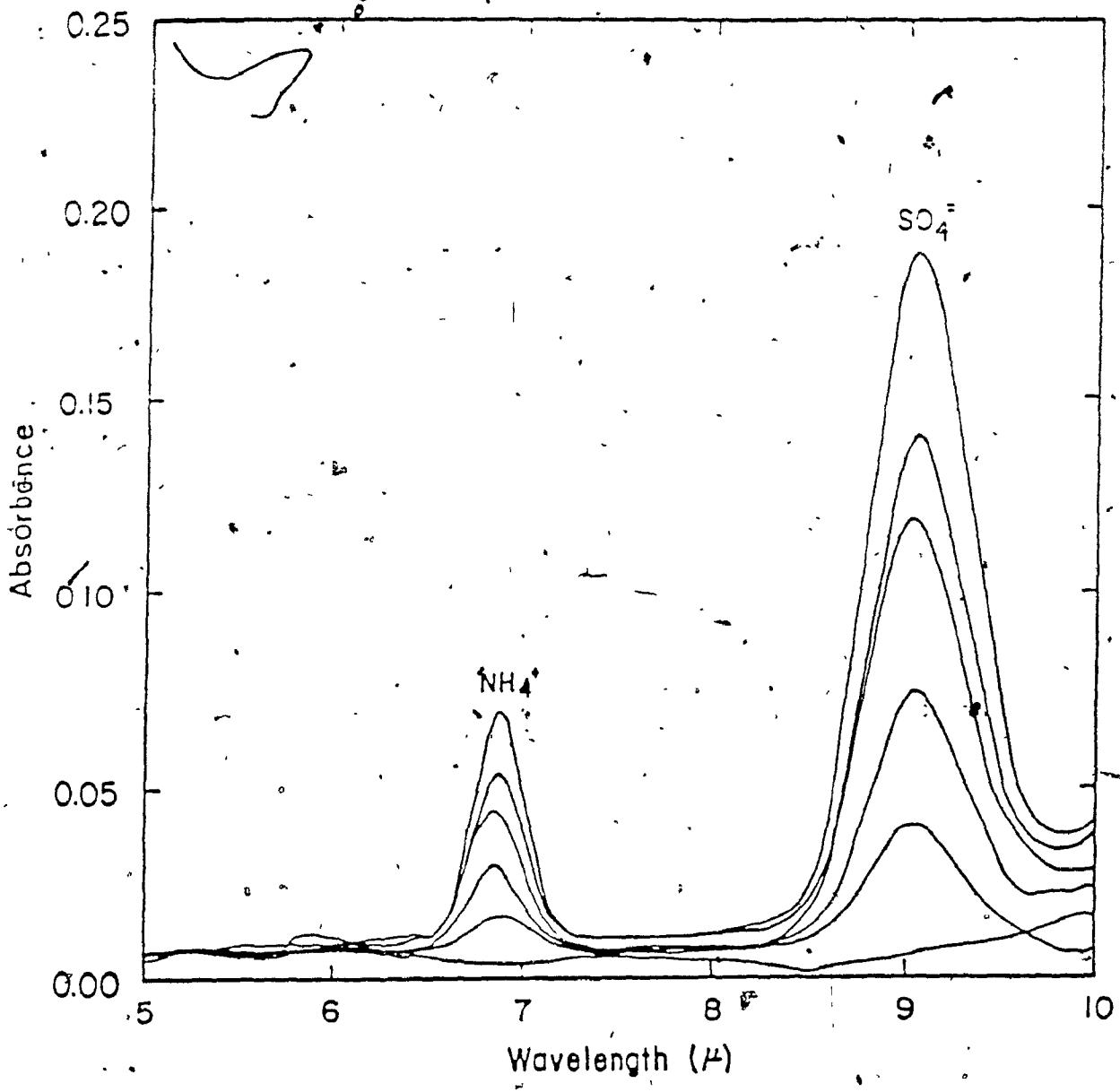

0.25
0.20
0.15
0.10
0.05
0.00
Absorbance
$SO_4^=$
$NH_4^+$
5
6
7
8
9
10
Wavelength (μ)

Figure 10. Plot of ammonium sulphate (% wt / vol) versus absorbance at 3.49 and 6.89 µm using the Spectroprocessor IV.

y = 0.109x + 0.105 (for 6.89 µm)
y = 0.058x + 0.114 (for 3.49 µm)

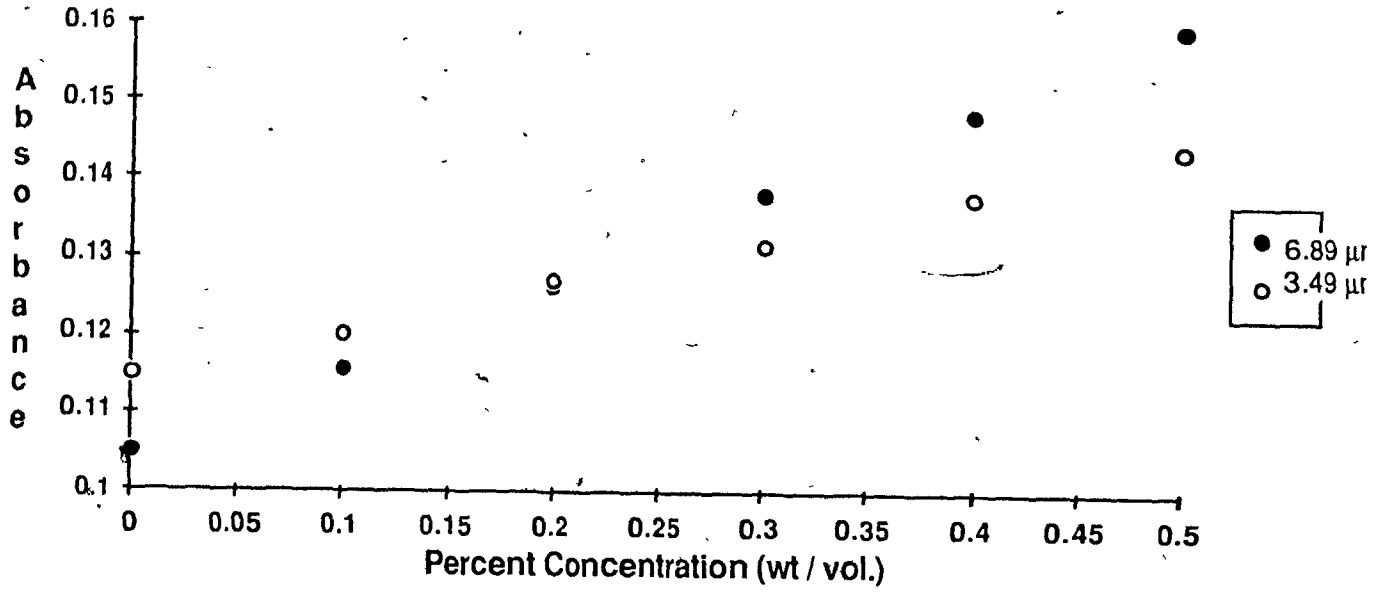


Table 8. Comparision of baseline and dual wavelength method for quantitation of ammonium ion at 6.89 µm.

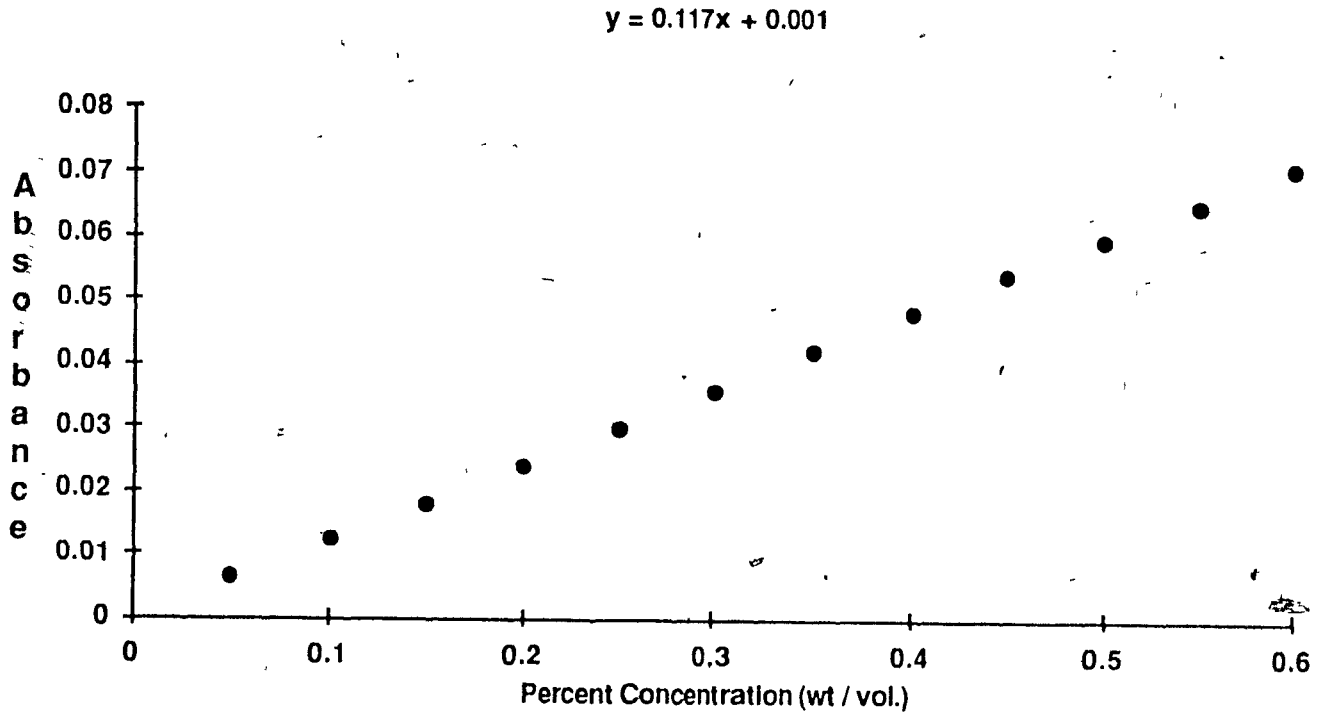
| Method | Correlation Coefficient | |
|---|---|---|
| | Mean[a] | S.D.[a] |
| Baseline | 0.9956 | 0.0054 |
| Dual wavelength | 0.9961 | 0.0023 |

[a] Based on 3 readings.

A reference wavelength has to fulfill the requirements of being near the sample wavelength and providing consistent readings independent from the sample absorbance. A variety of reference wavelengths were evaluated in the vicinity of the ammonium peak (6 89 µm). The 5.56 µm was selected since it provided the most consistent results and also coincided with the 5 56 µm filter available in the Multispec M instrument As a result, further quantitative work was done by the dual wavelength method utilizing 6.89 µm as the sample wavelength and 5.56 µm as the reference wavelength. Figure 11 presents a typical standard curve of ammonium sulphate (% wt / vol versus Abs.) where absorbance values were obtained by the dual wavelength method. The plot is linear having a correlation coefficient of 0.9931 and a standard error of estimate of 0.0150%.

Since the pH of the solution influences aqueous IR spectra (Goulden and Manning, 1967), the exact effect of this phenomenon on the quantitation of ammonium ion was evaluated. A series of ammonium sulphate solutions of increasing pH (3.5-8.8) were prepared. The slope of the plot of concentration (% wt / vol) versus absorbance was determined by linear regression and plotted

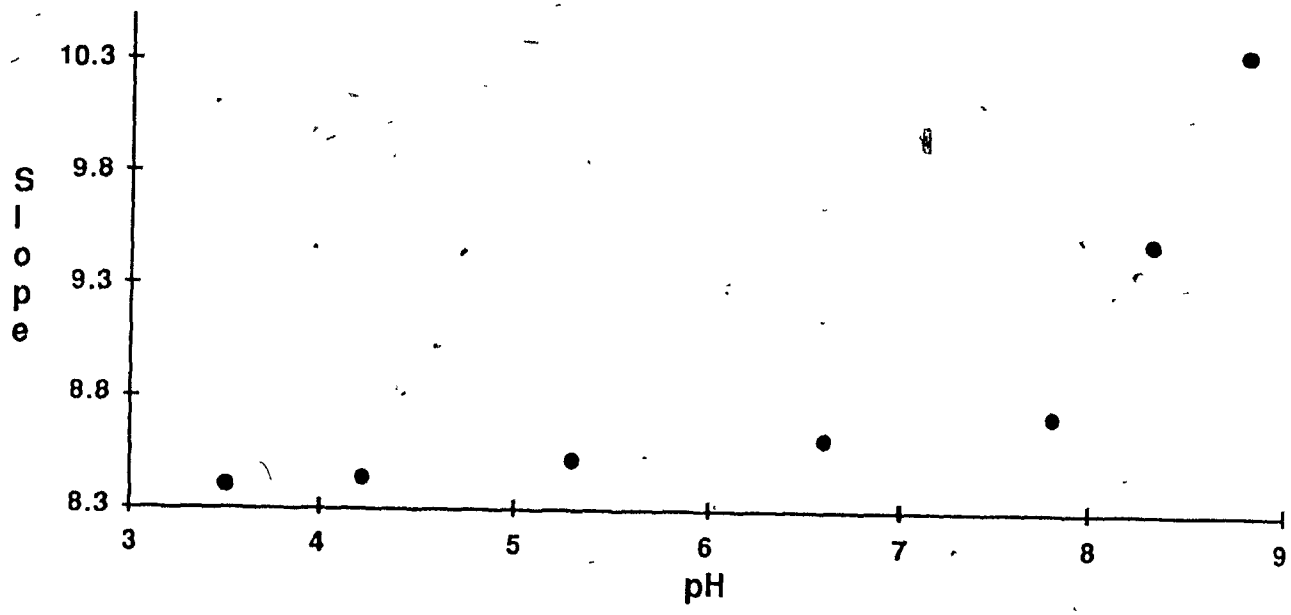
Figure 11. Plot of ammonium sulphate (% wt / vol.) versus absorbance ratio (6.89 μm / 5.56 μm) using the Spectroprocessor IV.


y = 0.117x + 0.001
Absorbance
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
0.1
0.2
0.3
0.4
0.5
0.6
Percent Concentration (wt / vol.)

against the pH of the solution Figure 12 presents a plot demonstrating the effect of pH on the slope of the standard curves. It can be seen that pH has a very minor effect over the range 3.5-8.0 but beyond the pH 8 the slope drops signifi - cantly. This observation is consistent with the shift in the equilibrium from $NH_4^+$ to $NH_{3(aq)}$ in the ammonium ion solution. As the pH of the solution is brought closer to the $pK'_a$ of ammonia ($pK_a$ = 9 4), the concentration of soluble ammo - nia increases rapidly with the simultaneous decrease in ammonium ion concen - tration (Williams and Williams, 1967). The effect of the pH was found linear over pH range of 3 5-8.0 and was assessed to be 0.0016% ammonium sulphate per pH unit. The errors in the concentration of ammonium sulphate expressed as a standard deviation over the pH range of 3-7 were calculated to be less than 0.1% of the mean and considered negligible These results were in agreement with Goulden and Manning (1967) who chose the optimal pH to be about 2 pH units removed from the $pK'_a$ value for their study of molar extinction coefficients of inorganic ions in aqueous solutions. It was therefore concluded that, to assure the accuracy of ammonium ion quantitation,the studies should be carried below pH 8. Solutions below pH 3 should be also avoided since they may,damage the cell windows of the instrument.

After examining the spectral behaviour of ammonium sulphate in water and the influence pH has on its quantitation, the next step was to study the spectral behaviour of ammonium sulphate in dilute acid. Close attention was paid to the temperature of solution during the neutralization process, since the $pK'_a$ of the ammonium ion-ammonia solution is temperature dependent (Bates and Pinching, 1950). Based on the results obtained by Bates and Pinching (1950) it was calculated (Henderson-Hasselbach equation) that the pH of ammonium ion-ammonia solution increases linearly by approximately one unit for every 25°C rise in temperature. As a result, the pH value 3 at 25°C is

Figure 12. Plot of the slopes of standard curves (% ammonium sulphate versus absorbance ratio) versus pH.


10.3
9.8
9.3
8.8
8.3
Slope
3
4
5
6
7
8
9
pH

equivalent to the pH value 3.86 at 50°C. In this study, a specially designed Erlenmeyer flask was used to cool the acid solution during the neutralization process. The temperature of the solution never exceeded 40°C which assured that no substantial losses of ammonium ion occured due to the pH shift The pH of the neutralized solution was kept within the desired pH range of 3-5 with the aid of metacresol purple indicator added to the base The metacresol purple indicator exhibits a red colour over the pH range 1 2-2 8, a yellow over the pH range 2 9-7.4 and a purple over the pH range 7.4-9.0 Therefore the reddish-yellow colour of the neutralized solution indicated that its pH was within the recommended range of 3-5 where loss of the ammonium ion due to its conversion to soluble ammonia gas is minimal

The spectrum of ammonium sulphate in dilute sulphuric acid was taken with the reference cell containing the blank acid solution This eliminated any possible errors due to the absorbance of other ions present and compensated for any water displacement effect. The spectrum of the ammonium ion in dilute acid was evaluated and as Figure 13 demonstrates it compares well to the spectrum of ammonium sulphate in distilled water having its maximum absorbance at a wavelength of 6.89 µm and showing similar absorptivity of the ammonium ion band, 0.1109 versus 0 0978 for dilute acid and water respectively. The standard curve obtained by plotting the concentration (% wt / vol.) of ammonium sulphate in dilute acid versus the absorbance was reasonably linear and it compared well to the ammonium sulphate curve in water having a correlation coefficient of 0.9981 versus 0.9991 for the acid solution and water respectively (Figure 14). The intercept of the curves differed since the instrument was standardized against distilled water. The results obtained indicated that, using the double-beam spectrophotometer, ammonium sulphate ion can be quantitated as accurately in dilute acid as in distilled water if the solutions

Figure 13. Scan of ammonium sulphate in acid solution over the range of 5-10 μm using the Spectroprocessor IV.

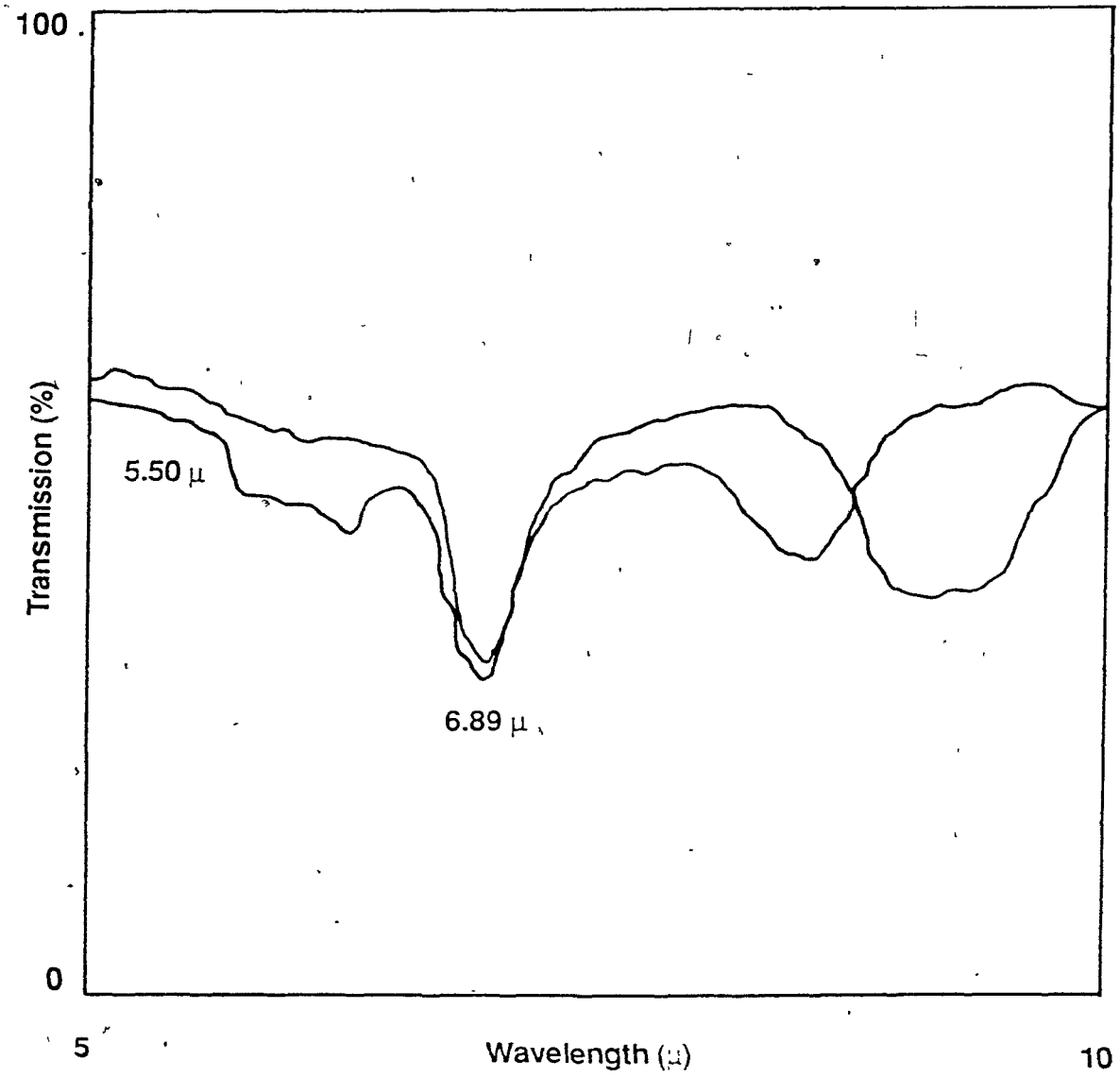
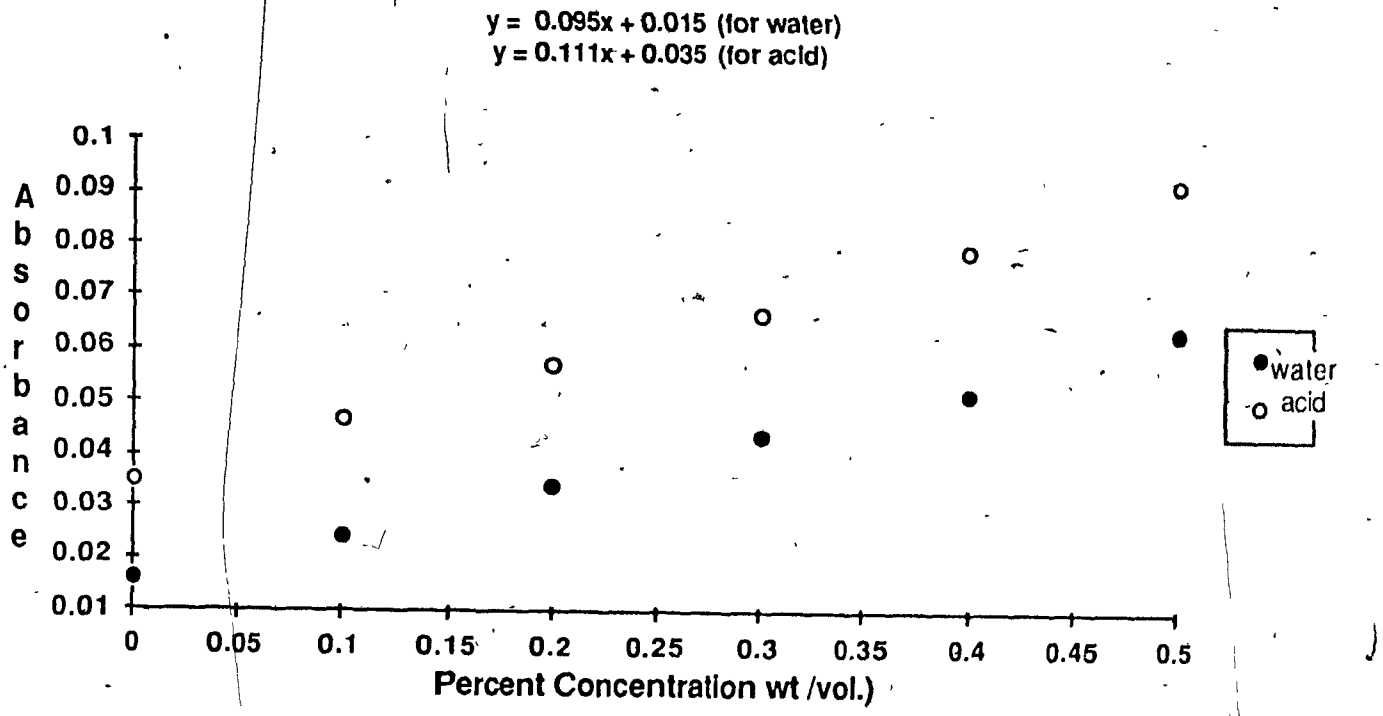

100
0
Transmission (%)
5.50 μ
6.89 μ
5
Wavelength (μ)
10

Figure 14. Plot of ammonium sulphate (% wt / vol.) in distilled water and acid versus absorbance ratio (6.89 µm / 5.56 µm) using the Spectroprocessor IV.


y = 0.095x + 0.015 (for water)
y = 0.111x + 0.035 (for acid)
Absorbance
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
0.45
0.5
Percent Concentration wt /vol.)
water
acid

are carefully prepared to avoid ammonium ion losses due to excessive temperature and higher than recommended pH.

Kjeldahl digestion requires substantial levels of potassium sulphate added to sulphuric acid to increase the boiling point of the digest. A study was carried out to find how the presence of potassium sulphate in digest solution affects the ammonium sulphate spectrum. Bradstreet (1954) stated that the critical point beyond which nitrogen is lost corresponds to an acid index value of 0.54. In this study, great care was taken in the preparation of the digest to provide the optimal ratio of sulphuric acid to potassium sulphate (acid index). Factors such as composition of the sample, acid lost on boiling and excess acid required to prevent the solidification of the final digest were considered. The catalyst chosen was the mixture of potassium sulphate, copper sulphate and selenium. Mercury was not considered because it forms ammonium complexes which have to be precipitated with sodium thiosulphate before the IR determination (Baker, 1954). Selenium may possibly cause losses of nitrogen if used in high amounts (Hiller, 1948) and the effectiveness of copper as a catalyst is lower than both mercury and selenium (Baker, 1939). Considering the above factors, a mixture of potasium sulphate, selenium and copper sulphate was chosen as the ideal catalyst. Hydrogen peroxide is used as an energetic oxidizing agent in many digestions. However, in this study hydrogen peroxide had to be eliminated since it caused bubbles in the instrument cell and consequently interfered with the transmission readings. The procedure used for the neutralization of the acidic digest was the same as for the dilute acid solutions. The use of metacresol purple indicator assured the required pH range of 3-5 of the final digest. Cooling of the digest during the neutralization process eliminated ammonium ion losses due to a shift of the pH at high temperatures.

Figure 15 presents a spectrum of the neutralized ammonium sulphate digest obtained on the Spectroprocessor IV. A major shift in the absorption maxima of the bands can be noticed due to ionic interactions and the absorption coefficient of ammonium ion band is also substantially decreased. The maximum absorbance wavelength for ammonium ion shifted from 6.89 µm in water to 7.04 µm in the digest solution resulting in incorrect quantitation at 6.89 µm wavelength. This erroneous reading would also be obtained for the reference wavelength, since the digest solution displayed a peak in the 5.5 µm region. Furthermore, the sulphate peak is substantially shifted to a lower wavelength, 9.10 µm for distilled water versus 7.91 µm for digest solution, and could interfere with the quantitation of ammonium ion. Based on these results, it was concluded that working directly with the digest would be extremely difficult partly due to spectral shifts and decreasing response, but more importantly due to displacement effects on the water spectrum background. Displacement effects can be compensated for in the dual - beam spectrophotometer but are difficult to eliminate in a single cell, dual wavelength instrument.

Based on the data collected with the Spectroprocessor IV, two interference filters supplied by Shields Instruments, England, were installed in the Multispec M in lieu of the Fat B filter pair. The filters transmission characteristics were balanced with wire gauze as indicated by their water background absorbances. The transmission characteristics of the filters are illustrated relative to ammonium sulphate in Figure 16. The sample filter has a center wavelength of 6.86 µm with a peak transmission of 74.3%, a half band width of 117 nm, and a noise level of <0.15%T. The reference filter has a center wavelength of 5.56 µm, peak transmission of 51.2%, a half band width of 86.8 nm, and a noise level of 0.3%T.

Figure 15: Scan of ammonium sulphate in digest solution over the range of 5-10 µm using the Spectroprocessor IV.

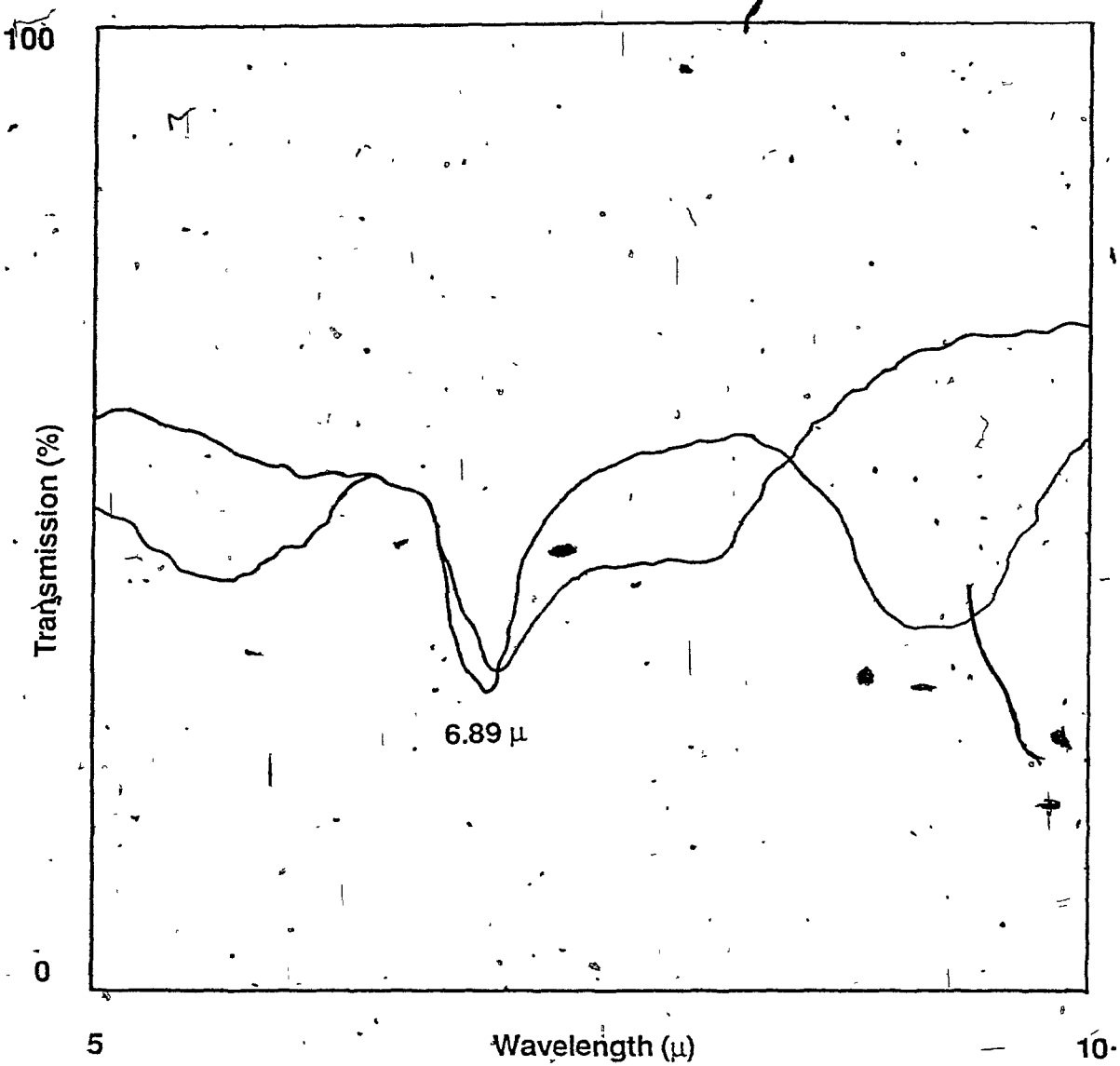
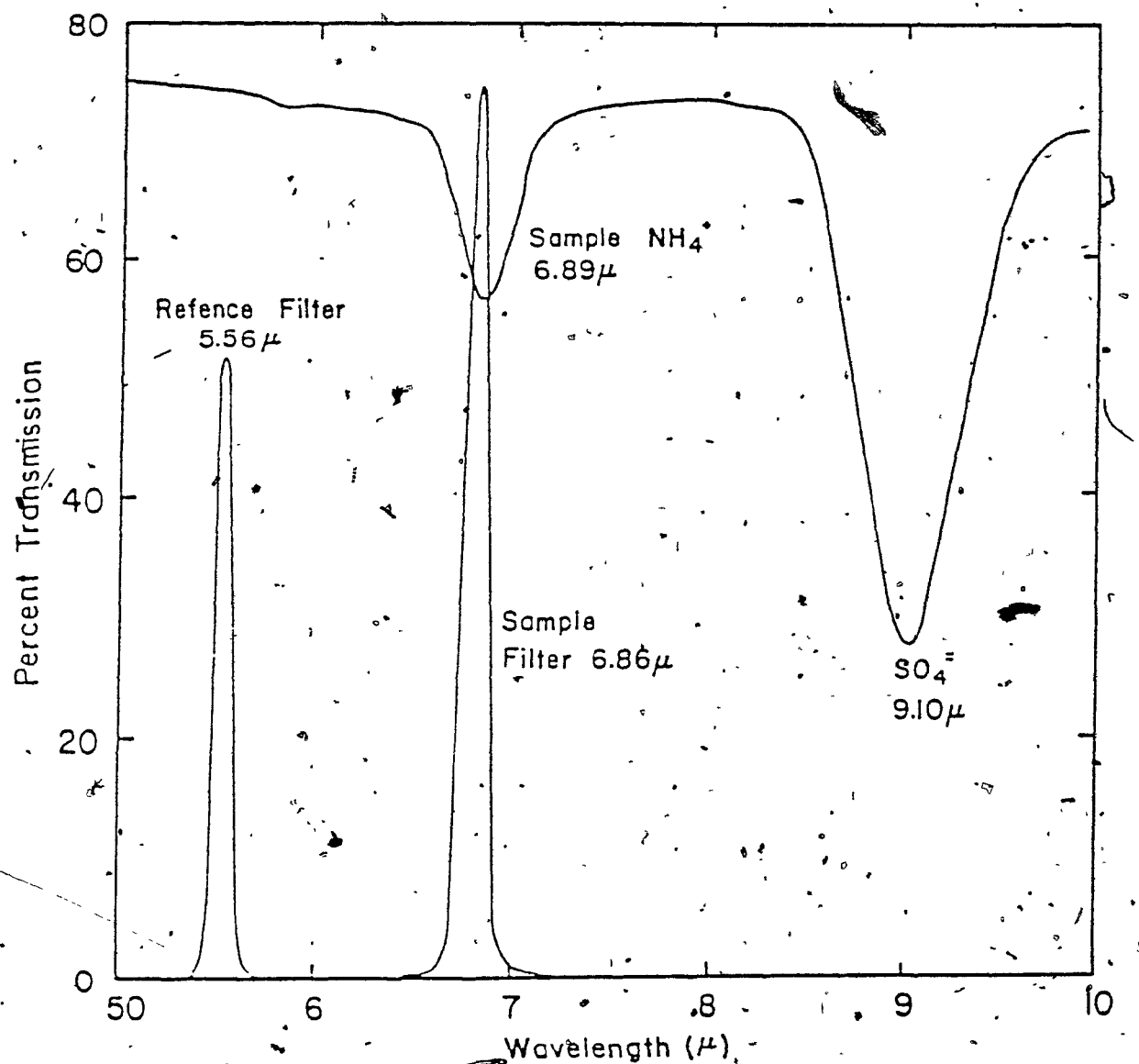

100
Transmission (%)
0
6.89 μ
5
Wavelength (μ)
10

Figure 16. Transmission characteristics of sample and reference filters installed in the Multispec M relative to a scan of ammonium sulphate solution.


80
60
40
20
0
Percent Transmission
Refence Filter
5.56μ
Sample NH4
6.89μ
Sample
Filter 6.86μ
SO4=
9.10μ
50
6
7
8
9
10
Wavelength (μ)

After zeroing the instrument with water, the study was carried out to establish the optimal slope potentiometer (signal multiplication) setting. Serial dilutions of ammonium sulphate were run on the instrument at various slope potentiometer settings and it was determined that with ammonium sulphate, the instrument could be run at a potentiometer setting of 9.0, about twice that used normally without generating excessive noise.

Figure17 presents a standard curve based on percent ammonium sulphate in distilled water as a function of the instrument panel reading using a slope of 9.0. The plot displays a slight deviation from linearity in the range of 0-1.0% solute producing a linearity coefficient of 0.9984 and a standard error of estimate (SEE) of 0.0195 The linearity improves as one limits the range to 0.6% solute producing a linearity coefficient of 0.9998 with a standard error of estimate reduced by a factor of four to a value of 0.0044 which indicates that ammonium sulphate can be quantitated with a reasonable precision up to 0.6% solute concentration.

A study was done to evaluate the capability of Multispec M to quantitate the ammonium ion in the partially neutralized acid solutions. As Figure18 demonstrates, the correlation coefficient for the "cooled" acid solution standard curve (0.9919) is much lower than the correlation coefficient obtained for the standard curve of ammonium sulphate in distilled water (0.9998). These results indicate that, at this moment, the ammonium ion can not be accurately quantitated in the partially neutralized acid solution because of the displacement effect caused by the ions present in the solution. As the studies on the Spectroprocessor IV demonstrated, the displacement effect could be compensated for in the dual - wavelength instrument, but are difficult to eliminate in the single cell instrument such as Multispec M. The results obtained for the "not cooled " acid solutions were very erratic (Figure 19) due to the fact that the temperature

Figure 17. Standard curve for ammonium sulphate for 0.0-1.0% on the Multispec M using the 6.89 and 5.56 µm filters.

y = 0.0248 + 0.1238x (for 0.0-1.0%)
y = 0.0032 + 0.1350x (for 0.0-0.6%)

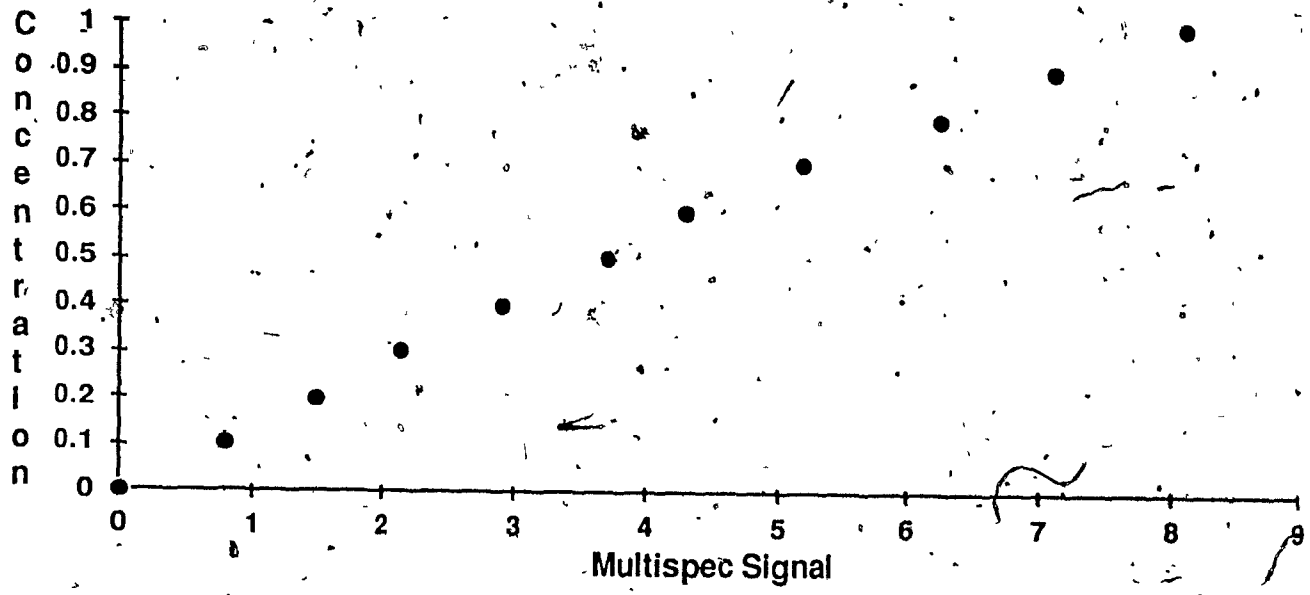


Figure 18. Plot of ammonium sulphate (% wt / vol.) in distilled water and a "cooled" acid solution on the Multispec M using the 6.86 and 5.56 µm filters.

y = 30.857x - 0.095 (for water)
y = 28.381x + 1.8829 (for acid)

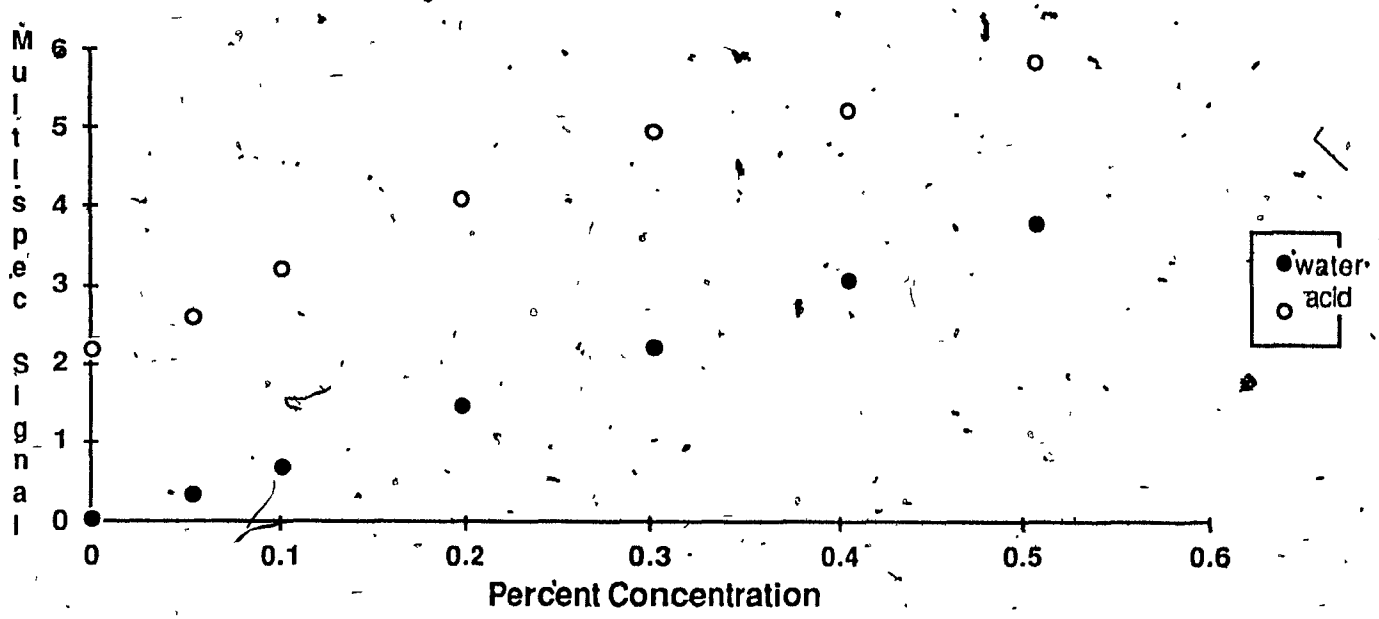
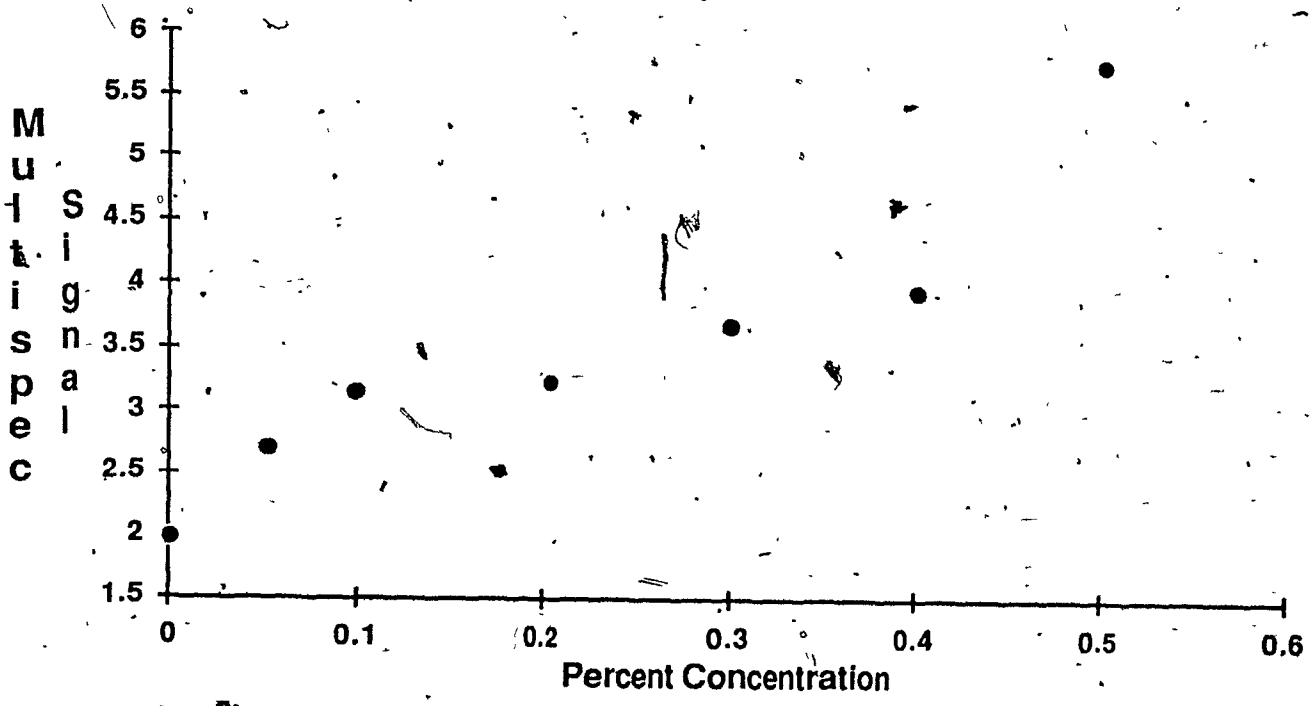


Figure 19. Plot of ammonium sulphate in a "not cooled" acid solution on the Multispec M using the 6.86 and 5.56 µm filters.


Multispec Signal
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
0
0.1
0.2
0.3
0.4
0.5
0.6
Percent Concentration

during the neutralization process reached approximately 80°C, 40°C higher than the recommended temperature. These results confirmed the studies done on the Spectroprocessor IV, which indicated that there is a substantial loss of ammonium ions at high temperatures.

A study was done to compare the accuracy of ammonium sulphate determination by the infrared spectrophotometric method and by the titrimetric method after distillation into standard acid. Two series of weights, 20 samples to a series, covering the range from 0.0-0.6% ammonium sulphate were analyzed by both methods. Table 9 presents the means by method relative to the mean of the series weight.

Table 9. Mean, mean difference (MD), and standard deviation of the difference (SDD) between prepared sample weight and result based on titration and IR analysis.

| Mean[a] Standard (g) | Mean of distillate (g) | MD | SDD |
|---|---|---|---|
| 0.2607 | 0.2593 (T)[b] | 0.0014 (T) | ± 0.0028 (T) |
| 0.2577 | 0.2578 (IR)[c] | - 0.0001(IR) | ± 0.0013(IR) |

[a] n=20
[b] Results for titration procedure.
[c] Results for infrared analysis.

The results obtained by infrared method were closer to the mean weight than the titration procedure results, and the standard deviation of differences (SDD) indicated that the infrared method had less variance relative to the weighted values of the individual samples in each series. These differences

were significant at the P = 0.05% level, and it can be concluded that the infrared method is somewhat more accurate than the titration procedure, which in itself is a primary method (van de Voort, 1986).

# Chapter VIII
# CONCLUSIONS

It is evident from this research that the ammonium ion can be successfully measured in simple solutions such as Kjeldahl distillates using the commercial infrared filter instrument, Multispec M. The study of the behaviour of ammonium ion in aqueous solutions, taking into consideration the influence of the pH and temperature on the infrared spectra, led to the development of a method producing accurate and reproducible results. The sensitivity of this method could be improved further by using wider cell pathlengths and by adjusting the sample filter passband to give a better response (Huffen,1985). The limited study of the ammonium ion in more complex matrixes such as dilute acids and dilute Kjeldahl digests elucidated a whole range of problems involved in this type of analysis. Ionic strength, displacement effects, dilution of ammonia solution, and wavelength shifts are the major ones which have to be addressed. The quantitation of ammonium sulphate in a dilute digest, by a double-beam spectrophotometer, would be feasible if the ionic content of the digest was lowered. A new microwave oven digestion technique utilizing only sulphuric acid under pressure could be considered as an alternative to the traditional Kjeldahl digestion procedure. Unfortunately, at this time, the quantitation of ammonium sulphate in dilute digests by a single cell commercial IR analyzer is not possible, due to displacement effects caused by the ions present in solution.

Presently, rapid measurment of the ammonium ion in Kjeldahl distillates may be possible if a low cost, simple and dedicated instrument designed for this purpose was available. If a suitable method was to be developed, a rapid, commercial IR analyzer integrated with a sulphuric acid microwave digestor,

could have the capability of analyzing sample digests directly and thereby replacing the well established Kjeldahl method for protein analysis.

The author hopes that this research contributed to the field of quantitative IR spectroscopic analysis in aqueous media and will help other scientists working on similar problems in the future.

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# Appendix A

```
BRIEF
SET COMPLEX
ECHO This routine sets up a calibration file over the 1-5u range@
ECHO and its companion program CAL5 can be used for the 5-10u range.@
ECHO @
PAUSE
ECHO @
ECHO A calibration file consists of a single beam scan in which@
ECHO the instrument adjusts the slits automatically to a specified@
ECHO energy level. The ability to attain the desired energy level@
ECHO is a function of slit range allowed and the gain setting.@
ECHO For aqeous solutions which have poor transmission, the gain@
ECHO should be set at the maximum, for other systems (solvents)@
ECHO the gain can be set at 9.0 at 2.2u SB.@
ECHO @
ECHO @
ECHO SETTING UP THE INSTRUMENT@
ECHO @
ECHO Set up for single beam operation by driving trimmer comb@
ECHO to blank out the sample beam.@
ECHO Switch over to single beam (SB) mode@
ECHO Put beam blanker switch on and move the servo comb to place@
ECHO the reference beam at the open end of the comb.@
ECHO Put the gain at maximum for aqeous systems.@
ECHO @
PAUSE
ECHO @
SET RANGE MEDIUM,SCALE 1.0 0.11764
SET SCANRANGE 1 5
SET PLOTON,GRATING 3,SLIT 150,SCANTIME 10
SET MAX_SLIT 300,MIN_SLIT 1
PARAMETERS
ECHO @
ECHO The slit should be at 150, the grating at 3.0u, are they?@
ECHO @
PAUSE
ECHO @
SET VERBOSE
CALIBRATE
BRIEF
GOTO SLIT 150
ECHO If you plan to do a calibration for the 5-10u range, switch@
ECHO the grating, but wait 30 sec before typing '@@ CAL5'.@
EOF
```

# Appendix B

```
:CAT

 FILENAME              DRIVE
 CAL1                    0
 CONVERT_DISK.S          0
 CAL5                    0
 SCAN1                   0
 SELECTEDU               0
 CAL2                    0
 D001                    0
 D002                    0
 SCAN5                   0
 DBSETUP1                0
 PROGRAMCATALOG          0
 D003                    0
 SFH2OCAL1MAXGAIN        2
 SFH2OCAL2MAXGAIN        2
 C                       2
 DO                      2

 AMMSULF50               2
 AMMSULF50A              2
 AMMSULF35               2
 AMMSULF35A              2
 AMMSULF25A              2
 AMMSULF25               2
 AMMSULF10               2
 AMMSULF10A              2
 AMMSULF5A               2
 AMMSULF5                2
 AMMSULF2.5              2
 AMMSULF2,5A             2

:PRINT AMSULF   MSULF50A

File : AMMSULF50A

Spectral data file.
Spectroprocessor version : 04 User :  2
Creation date : 29-Sep-83
Instrument range : COARSE.
Range of scan :  8.9700 to  9.1300 microns.
Operator : FRED

Sample  : SULPHATE PEAK

Sampling interval :  0.0010 microns
Actual number of data : 160
Calibration file : SFH2OCAL2MAXGAIN

Enter start wavelength >8.97 9.13
Enter stop wavelength >9.13
Enter sampling interval in microns >0.025
```