DEVELOPMENT OF A FIBRE OPTIC BASED SYSTEM FOR THE MEASUREMENT OF NITRATE IN WATER.

BY

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DEDICATION

To Mam and Dad and to Margaret, for her love,encouragement and patience during the course of this work.

ABSTRACT

The development of a fibre optic based system for the measurement of nitrate concentrations in water is reported. This system was designed to monitor nitrate concentration at levels appropriate to EC directives relating to drinking water quality.

An experimental system was constructed to investigate direct UV transmission measurements at two wavelengths as a possible basis for a nitrate sensor. It was found that a dual wavelength measurement scheme was suitable since nitrates absorb strongly in the 210nm region but have negligible absorbance in the 275nm region. Limitations in the experimental system such as long data acquisition time and ouput drift meant that the experimental set up was not suitable as the basis for a portable nitrate detection system.

A prototype optical fibre nitrate sensor for groundwater monitoring was constructed using a rotating filter wheel, lower attenuation fibre and a new electronic detection and signal processing system to overcome some of the limitations of the original experimental system.

The perfomance of the prototype system was characterised in terms of range, sensitivity, resolution, accuracy and response time.The sensor exhibited excellent perfomance using short lengths of fibre but range and resolution performance were degraded somewhat when long lengths of fibre were used. However, the sensor was still capable of determining nitrate concentrations in the range of interest set by EC directives over long lengths of fibre.

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CHAPTER 1

INTRODUCTION TO OPTICAL FIBRE SENSORS

1.1 Introduction.

Optical fibre systems are well established as a cornerstone of the telecommunications industry. The development of this technology has led to rapid advances in the design of optical fibres, detectors, light sources, couplers and other components. The last twenty years has seen considerable research activity in the area of optical fibre sensors based to a large extent on the components developed for the telecommunications sector. Optical fibre sensors have been used to measure physical parameters such as temperature, pressure, flow, displacement, current and magnetic fields [1]. There is also a growing interest in the use of optical fibre sensors for measurement and monitoring of chemical parameters such as pH and species concentration. The work reported here is concerned with the development of a fibre optic sensor for a particular species : nitrates in water.

1.2 Optical Fibres

Optical fibres generally consist of a core of high refractive index material surrounded by a cladding of lower refractive index. Optical fibres guide light by multiple total internal reflections at the core/cladding interface..While the material used is silica, recent advances in glass technology have enabled enhanced transmission in the near UV and mid IR spectral regions.Core sizes range from a few microns up to millimeters in diameter. In this work optical fibres are used simply as a transmission medium to a remote absorption cell. Accordingly, the parameters of interest are the spectral attenuation and numerical aperture. These are treated in chapter 3 and appendix A, respectively.

1.3 Optical Fibre Chemical Sensors.

A sensor may be defined as a device that is able to indicate continuously and reversibly the magnitude of a desired measurand. Optical fibre chemical sensors have many attractive features, some of which are not offered by more conventional sensors.

(I). Optical fibre chemical sensors can utilise many optical techniques that have been developed for routine chemical analysis. Many of these techniques offer high specificity and sensitivity.

(II). The low attenuation of optical fibres enables the remote in-situ sensing of species in

difficult or hazardous environments.

(III). The small physical size of optical fibres allows the development of very small and flexible fibre sensors. These can be used for looking at small sample volumes or for in vivo measurements in clinical chemistry and medicine.

(IV) Optical signals transmitted through optical fibres are free from electromagnetic interference. Optical fibres do not present an electrical hazard since they are non-conducting and this is very important in some medical applications where fibre sensors are placed inside the patient's body.

(V) The signals from everal optical fibre sensors can be multiplexed on to a single fibre and this can be linked to one signal processing unit with considerable cost savings.

In order to be successful outside the laboratory, optical fibre chemical sensors must be rugged, reliable and achieve at least the same standard of accuracy, repeatability and stability as conventional sensors.

1.4 General Classification of Optical Fibre Chemical Sensors.

Fibre optic chemical sensors fall into two categories as shown in fig. 1.1:

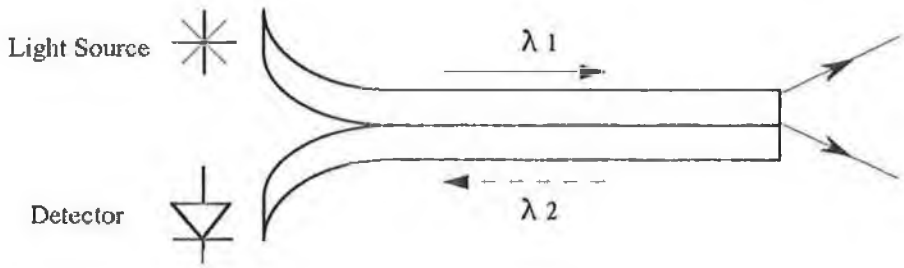
(a) Remote Fibre Spectroscopy (RFS)

Here the fibre acts as a simple light guide which separates the sensing location from the monitoring instrumentation. The fibre enables direct spectral analysis (eg. absorbance, fluorescence etc) to be performed on a sample at a distance [2].

(b). Fibre Optic Optrodes

In this case, the optical fibre is combined with specific reagent chemistry. At the distal end of the fibre specific reagents are contained in a miniature reservoir attached to the fibre tip and are separated from the sample by means of an appropriate semi-permeable membrane. Alternatively, suitable reagents may be immobilised directly on the fibre tip. Reagents are chosen to react sensitively and specifically to a particular analyte and the resultant absorbance (or fluorescence) change is a measure of the analyte concentration. [3].

I Remote Fibre Spectroscopy (RFS)



II Fibre Optic Chemical sensors (FOCS)
"Optrodes"

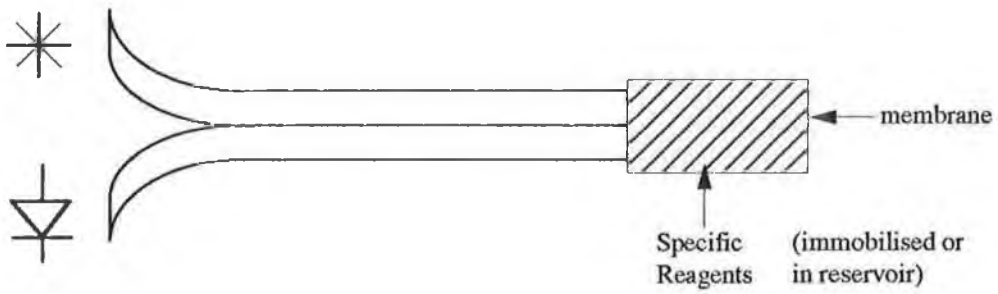


Figure 1. 1 General Classification of Fibre Optic Chemical Sensors

1.5 Drinking Water Supplies..

Drinking water can be abstracted from rivers, lakes, reservoirs and from underground sources called aquifers.Water percolates down through soil to the underlying rock where it continues to move downwards through pores and fissures to form underground reservoirs which can be tapped by drilling wells and boreholes. Water from these aquifers is known as groundwater and at present less than 2% of Ireland's developable groundwater is extracted. Of this, public bodies use 36%, industry uses 37% and the remainder is used for rural domestic supplies. Some midland and western counties have a very high dependence on groundwater - County Roscommon, for example, uses groundwater for about 80% of its water supply [4]. Traditionally, ground water has been of good quality because the slow percolation of rain water through soil and clay to the water table cleanses it of conventional hazards such as bacteria. There is a growing concern, however, that the increased use of pesticides and agricultural fertilisers can lead to contaminated groundwater and thereby have a detrimental affect on human health and on the natural environment. Recent awareness of this danger has stimulated requirements for better environmental practice. The implementation of EC regulations regarding water quality has given impetus to the need for measurements in the environment which previously had not been required. These factors have in turn generated a requirement for the development of new, reliable and cost-effective methods for measurement of environmental pollutants.

1.6 Sampling vs In-situ Monitoring.

Routine environmental monitoring usually requires frequent repetitive measurements of specific chemical species rather than a complete analysis. Consequently, the traditional high technology laboratory - based instrumentation such as gas chromatographs and mass spectrometers, which perform a complete analysis are not well suited to this purpose. In addition, skilled personnel are required to perform routine analytical procedures.Significant additional costs are expended on transporting samples to the laboratory. The transport and analysis of samples may mean a delay of days before results are available. This can give rise to doubts about sample integrity e.g. labile or volatile contaminants may have changed their concentrations considerably by the time analysis is performed. In the case of groundwater monitoring the type of pump used to remove a sample from a well for analysis can cause changes in sample composition [5].

Suction pumps may change the concentration of dissolved gases or volatile contaminants in the sample. Air lift pumps can change the pH of a sample by altering the CO_2 partial presssure of the sample.

The use of optical fibre chemical sensors for groundwater monitoring eliminates many of the problems listed above. Such sensors enable real time in-situ monitoring of a specific contaminant therby yielding rapid data without loss of sample integrity. The U.S Environmental Protection Agency has stated that optical fibre chemical sensors offer great scope for substantial cost savings over more traditional groundwater monitoring methods [6]. For example, withdrawal of groundwater samples for laboratory analysis typically requires drilling a 10cm diameter hole down to an aquifer whereas remote sensing with optical fibres requires only a 1.2cm hole. This leads to substantial savings in drilling costs alone [7].

The aim of this project was to develop compact, portable instrumentation to monitor nitrate levels in groundwater using optical fibre technology. The hazards of excess nitrate concentrations in the environment are explained in chapter 2.

CHAPTER 2

NITRATES IN DRINKING WATER

2.1 Nitrates in the Environment

Nitrogen in the form of Nitrates and Ammonium Ions (NH_4^+) is a very important nutrient for plants. They need nitrogen to make protein, as do all living things. Plants cannot use nitrogen directly from the atmosphere. Instead, they take it through their roots in the form of ammonium ions (NH_4^+) and as nitrates (NO_3^-) both of which are soluble in water. As plants take up nitrogen the soil becomes depleted. Water percolating through the soil also tends to remove or leach nitrate. Over the centuries farmers have learnt to counteract depletion of nitrogen from the soil by adding farmyard manure and by rotating crops. In recent decades farmers have taken to adding manufactured nitrogen fertilisers to the soil. This has led to a rapid expansion in intensive agriculture to such an extent that farming worldwide has become very dependent on artificial fertilisers. For example, in 1985 125 million tonnes of nitrogen fertiliser were used worldwide. In the 24 countries in the OECD, farmers used 32% more nitrogen fertilisers in 1985 than in 1970. In the rest of the world farmers used 120% more nitrogen fertiliser in the same period. [8]

2.2 Nitrate as a Hazardous Substance.

The revolution in agricultural production brought about by nitrogen fertilisers also has a down side. The excessive use of nitrates has led to an increase in nitrate concentration in groundwater which is a major source of drinking water in many areas. Excess concentrations of nitrate in surface waters is also an indicator of agricultural or sewage pollution.

Too much nitrate in drinking water can cause a blood disorder in babies younger than 3 months. The disorder is called methaemoglobinaemia or "blue baby syndrome". The infants lips and body take on a marked blue colouration. Bacteria, either in an unsterilised feeding bottle or within the child's gut, convert nitrate into nitrite (NO_2^-). The haemoglobin in the baby's blood takes up nitrite instead of oxygen and the result is that the baby suffers severe respiratory failure. Nitrite is an intermediate oxidation state of nitrogen, both in the oxidation of ammonia to nitrate and in the reduction of nitrate.

Mounting medical evidence suggests that nitrites may also cause cancer of the stomach and windpipe in adults. Nitrous acid which is formed from nitrite in acidic solutions can react with secondary amines (RR'NH) to form nitrosamines (RR'N-NO), many of which are known to be carcinogens. In China in the early 1980's, 140 out of every 100,000 males died from stomach cancer. Areas in which the death rate was high also had higher than average levels of nitrate in drinking water and vegetables. [8]

Nitrates also act as fertilisers for aquatic plants. If rain washes nitrates out of soil into streams, rivers, lakes and then into the sea in excessive quantities, they can boost the growth of algae and other aquatic plants. This enrichment is called eutrophication and it sometimes changes the balance of aquatic plants and animals so drastically that a particular species may be wiped out. Green algae respond very quickly to eutrophic conditions. When they grow rapidly on the surface they prevent light form reaching submerged plants, which may die as a consequence. Bacteria decompose the remains of any plants, algae and animals that sink to the bottom. This process uses up valuable oxygen and a vicious circle develops, drawing in all forms of aquatic organisms until rivers, ponds and lakes become devoid of life. The main cause of eutrophication in freshwaters is phosphate while the main cause of algal blooms at sea is nitrate. This nitrate enters the sea from rivers carrying agricultural run-off and from dumping of sewage. In the summer of 1988 toxic blooms in the North Sea almost wrecked salmon and trout farms off the coast of Norway. In the summer of 1990 toxic blooms were present off the coasts of Denmark, Yugoslavia and in the Irish Sea.[8]

2.3 Recommended and Mandatory Limit Values for Nitrate

The hazards to human health resulting from excess nitrate concentrations in drinking water has led the EC to issue directives on guide and maximum admissible concentrations of nitrate in surface waters which may be used as sources of public water supply. The directives state that drinking water should not contain more than 50 mg NO_3 per litre with a recommended guide concentration of not more than 25mg NO_3 per litre. Very often nitrate concentrations are expressed in terms of nitrogen (N) rather than NO_3. Accordingly, the recommended guide and maximum admissible values can be expressed as 5.6mg N l^{-1} and 11.3mg N l^{-1} , respectively [9]. Local authorities must comply with the European Community directive and this has led to a huge increase in nitrate monitoring requirements.

2.4. Current Methods for Determination of Nitrate in Water

Many methods for the identification and quantification of nitrates have been reported. These range from simple colorimetric methods to more sophisticated automated analyses. These can be grouped into three main categories [10] :

(1) Visible Colorimetry following reagent addition.

(2) Direct Ultraviolet light absorbance measurements.

(3) Ion Selective Electrodes.

The principal colorimetric method applicable to nitrate measurement involves reduction of nitrate (NO_3^-) to nitrite (NO_2^-) using a copper activated cadmium catalyst and determination of the nitrite concentration by reaction with sulphanilamide and N-1-napthylethylenediamine in HCl to produce a purple/red azo dye. The absorbance at 543nm of this dye is measured using a spectrophotometer and this is related to the sample nitrate concentration by means of a calibration curve derived from reference nitrate standards. A correction may be made for any nitrite present in the sample by analysing without the cadmium reduction step[11,15,16].

The nitrate ion absorbs strongly in the ultraviolet region and nitrate concentration in water can be determined by directly measuring the absorbance of the sample at 210nm on a UV spectrophotometer. Nitrate concentration is found by reference to a calibration curve derived from nitrate reference solutions [11,12,13,14,15,16]. However, a number of interfering species absorb in the 210nm region, the most serious being dissolved organic compounds,iron III and carbonates. A correction for organic interference can be made by measuring the absorbance of the sample at 275nm where nitrates do not absorb. Twice the absorbance at 275nm is subtracted from the absorbance value at 210nm to give a corrected value. This is an empirical correction which only works on samples where the organic content is relatively low. Iron and carbonate interference can be removed by acidification of the sample with hydrochloric acid. Because the nitrate ion exhibits a very high molar absorptivity (ε=1 x 10^4 liter $mole^{-1}$ cm^{-1}) [16], optical path lengths for a linear calibration curve are typically 1mm. Samples are usually diluted if a 10mm path length is used. A detailed description of absorbance and the Beer-Lambert law is given in appendix B.

The Nitrate Ion Selective Electrode consists of a silver/silver chloride electrode immersed in an electrolyte which is retained within the body of the sensor by an organophillic membrane. The membrane is impregnated with a liquid nitrate ion exchange material. The sensor develops a potential which is logarithmically proportional to the nitrate ion concentration in the surrounding solution. In use, the potential of the electrode is measured against a reference electrode of constant potential. The E.M.F. of the cell formed by the two electrodes is therefore proportional to the nitrate ion concentration of the sample [10,11]. Nitrate ion selective electrodes usually require the sample to be buffered to maintain the total ionic strength at a constant level, high enough to swamp the effects of variations in the sample ionic strength. The main operating problem with these devices is drift, although frequent recalibration with nitrate standard solutions will overcome this problem. They are also sensitive to interferents such as organics and detergents which may limit their utility for wastewater samples.

A number of researchers have carried out comparisons between the various methods used for nitrate determination. Miles and Espejo [14] found good agreement between nitrate values obtained by the direct UV absorption approach and a method involving a colorimetric reaction with 2,4 xylenol. They found the ultraviolet spectrophotometric method to be sensitive,precise and extremely rapid.In contrast, the procedure using 2,4 xylenol was long, complicated and involved using a number of reagents at different temperatures including toluene which is carcinogenic.

Avsec and Kosta [15,16] compared a wide range of nitrate determination methods including a number of different UV spectrophotometric techniques. They found that colorimetric methods based on direct nitration using such reagents as brucine, xylenol and sodium salicylate were subject to serious errors when applied to waters containing organic effluents. They proposed a method where UV absorption of nitrate at 220nm would be measured against a blank prepared from a second aliquot of the sample in which the nitrate had been reduced to ammonia by passing through a copper activated zinc column. This method succesfully compensated for most organic and inorganic interferents except nitrite which is found in extremely small concentrations in water. This method was also used successfully on groundwater samples to which surfactants had been added as artificial interferents.

They also investigated compensation for organic interferents by measuring absorbance at 275nm where nitrate does not absorb strongly. These results corresponded well to the

standard cadmium reduction technique.

The techniques described above are all laboratory-based methods. Some of them have been automated and adapted to provide on-line monitoring of nitrate concentrations in water. Maimo et al [17] and Clinch and Worsfold[18] have automated the cadmium reduction / azo dye reaction using computer controlled pumps to deliver samples to the analyser. The system of Maimo et al was capable of processing 60 samples per hour. The field monitor constructed by Clinch and Worsfold was used to sample river water at a rate of one sample every 30 minutes. This system was subjected to a nine month field trial and a complete 30 minute sampling interval record was obtained on only 34% of the days.This low figure was attributed to ancillary equipment failure including pump and switching valve failure [19].

The direct UV absorbance method has been automated by Sieger Ltd. [20] and DR Lange GmbH. [21]. Both systems use flow injection analysis where a sample is pumped to the analyser. The only reagent used is hydrochloric acid which is used in small quantities to clean the windows of the absorption cell. Thompson and Blankley [22] used continuous flow analysis by UV absorption. The inlet port to the absorption cell was covered by a dialysis membrane which minimised the effect of organic materials such as humic acids, and iron which do not diffuse freely through the membrane. This also avoided the necessity of filtering the samples of raw and waste waters prior to measurement. Only 5% of nitrate ions diffuse through the membrane and this meant that waters containing high levels of nitrate such as sewage final effluent did not have to be diluted to come within the linear calibration range of the instrument.

In conclusion, the automated systems described all have a number of drawbacks. They use pumps to move samples to the analyser and this can change the composition of the samples. Most use reagents which have to be replenished and must remain stable for long periods of time. The proposed fibre optic nitrate sensor described in chapter 3 monitors nitrate concentration in - situ and thereby avoids the need for pumps of any sort. It requires no reagents and gives rapid analyses. The operating principle behind the sensor is based on measuring the strong absorbance of nitrate in the 210nm region relative to the absorbance in the 275nm region where nitrate does not absorb.

CHAPTER 3

EXPERIMENTAL SYSTEM

3.1 Introduction

In order to investigate the feasibility of using optical fibres for in-situ remote sensing of nitrates in water the experimental system shown in fig.3.1 was constructed.
UV light from a Deuterium lamp is coupled into a monochromator by two fused silica lenses. The optical fibre is coupled to the monochromator via a SMA* connector and this fibre is used to guide UV light to the absorption cell. Another fibre takes light from the absorption cell to the detector - in this case a Hamamatsu R269 photomultiplier tube and C1050-01 photon counting module. The detector unit is connected to a counter circuit and computer interface. Software was written to scan the monochromator via a stepper motor interface and record light intensity levels as a function of wavelength. This data was then processed by other programs to provide absorbance data.

3.2 The Optical System

The 30W Deuterium gas discharge lamp (Hamamatsu L2196) provides a point source of UV radiation with a high output in the 200nm region [23]. Because of the dangers associated with UV radiation, the lamp was placed in a purpose built lamp housing with the output beam shielded by a 1'' diameter, 100mm long copper tube. A 25.4mm diameter ,100mm focal length fused silica lens (Spindler and Hoyer 06 3321) was used to collimate the output beam from the Deuterium lamp. The monochromator was an Optometrics SDMC-01 scanning monochromator of the Fastie-Ebert type with an f number of 3.9 and a focal length of 74mm. Light entering the monochromator at angles greater than the acceptance angle defined by the monochromator numerical aperture will not impinge on the grating and will increase background scattered light [24]. For efficient coupling of the light beam to the monochromator an f/3.9 lens would focus the light beam to the required acceptance angle of 7.3 degrees. A 25mm focal length f /1.16 fused silica lens was readily available, however, and this was used to couple the lamp output to the monochromator.

* Note : A SMA connector is a type of optical connector used for coupling optical fibres to light sources, detectors etc.

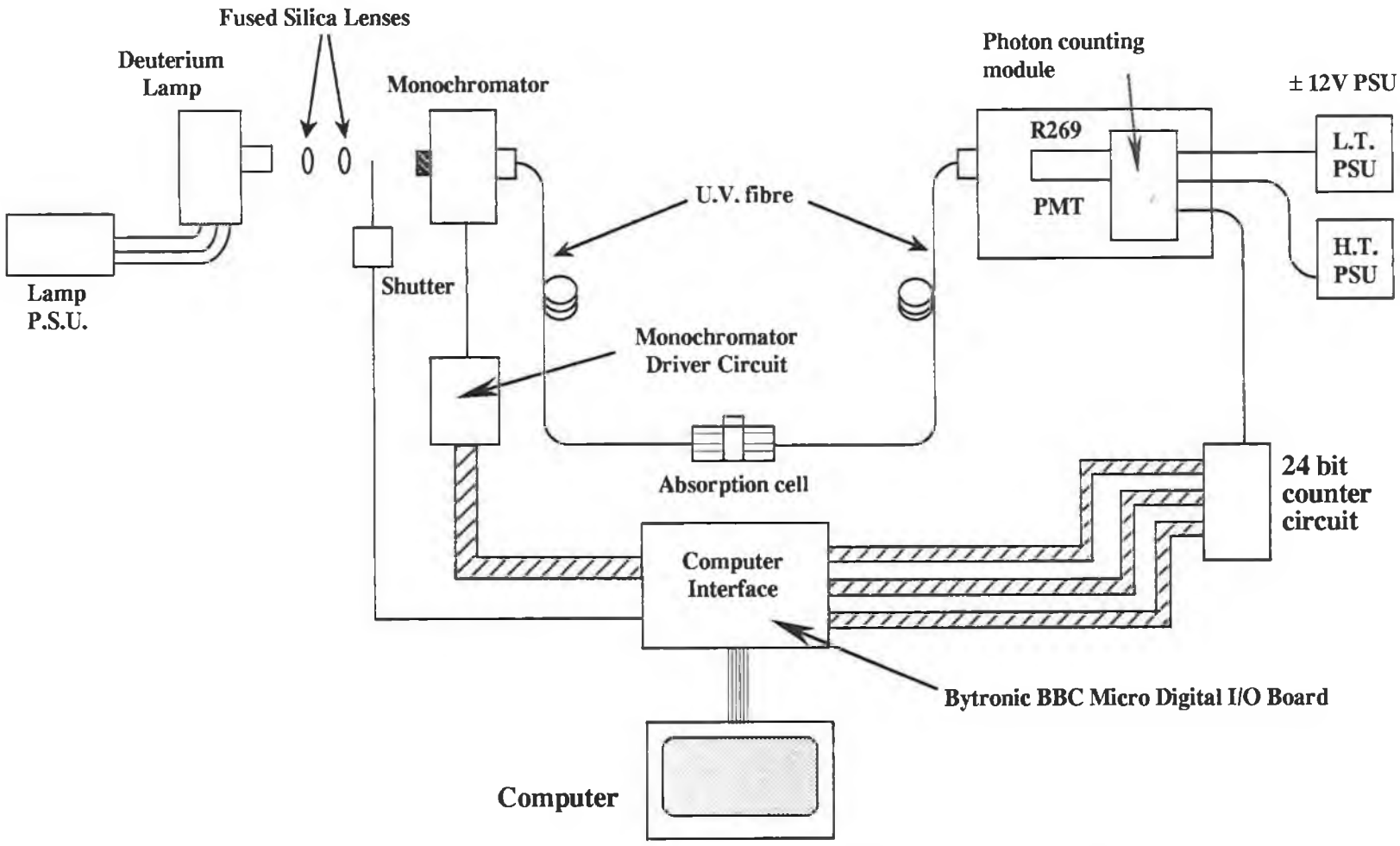


Fig. 3.1 Experimental System block diagram

The optical fibre used in this phase of the work was Fibreguide Industries Superguide PCS SPC 400B with a 400μm core diameter and a black Tefzel outer protective coating. Attenuation was quoted as 3dB per metre at 220nm [25]. However, the attenuation was considerably higher below 220nm as can be seen in fig. 3.2. The optical fibre was prepared for use by polishing the end faces on four grades of grit paper ranging in grit size from 30μm down to 0.3μm. This was accomplished by terminating the fibres with reusable SMA connectors and then placing the SMA connectors into a small hand polishing jig. The fibres were polished using a "figure of eight" motion on the grit paper which was well lubricated with water to prevent tearing. The fibre end faces were periodically examined under a microscope during the polishing process to ensure that an acceptable surface finish free from scratches was obtained.

The absorption cell consisted of an aluminium holder drilled to accept 10mm diameter perspex rods. These rods were drilled along their longitudinal axes on a lathe to accept the optical fibres which were held in place with cyanoacrylate adhesive. The absorption cell was designed so that the gap between the perspex rods could be changed and hence the absorbing path length of the liquid could be varied. For most of the experiments carried out the path length was set to 1mm as shown in fig. 3.3. The optical fibre numerical aperture was quoted as 0.4 in air. This is given by the equation $NA=n\text{Sin}\theta_{max}$ where n is the refractive index of the incident medium (usually air) and θ_{max} is the maximum acceptance angle of the fibre. For this fibre the maximum acceptance angle in air is 23.5°. If the incident medium is water (n = 1.33) then the maximum acceptance angle drops to 17.5°. If two fibres with NA = 0.4 and core diameters of 400μm are placed opposite each other 1mm apart in air then, only 9.8 % of the light transmitted by one fibre will be collected by the other. If the two fibres are placed 1mm apart in water then 15.25% of the light transmitted by one fibre will be collected by the other. Collimating lenses would increase this figure considerably. However , due to difficulities in obtaining small fused silica lenses of short focal length it was decided to use the fibres alone in the absorption cell. As explained in Appendix B, for low concentrations absorbance varies linearly with concentration. The short path length of 1mm was necessary because of the high molar absorptivity of the nitrate ion which causes the absorbance vs. concentration curve to deviate from a linear relationship if the path length

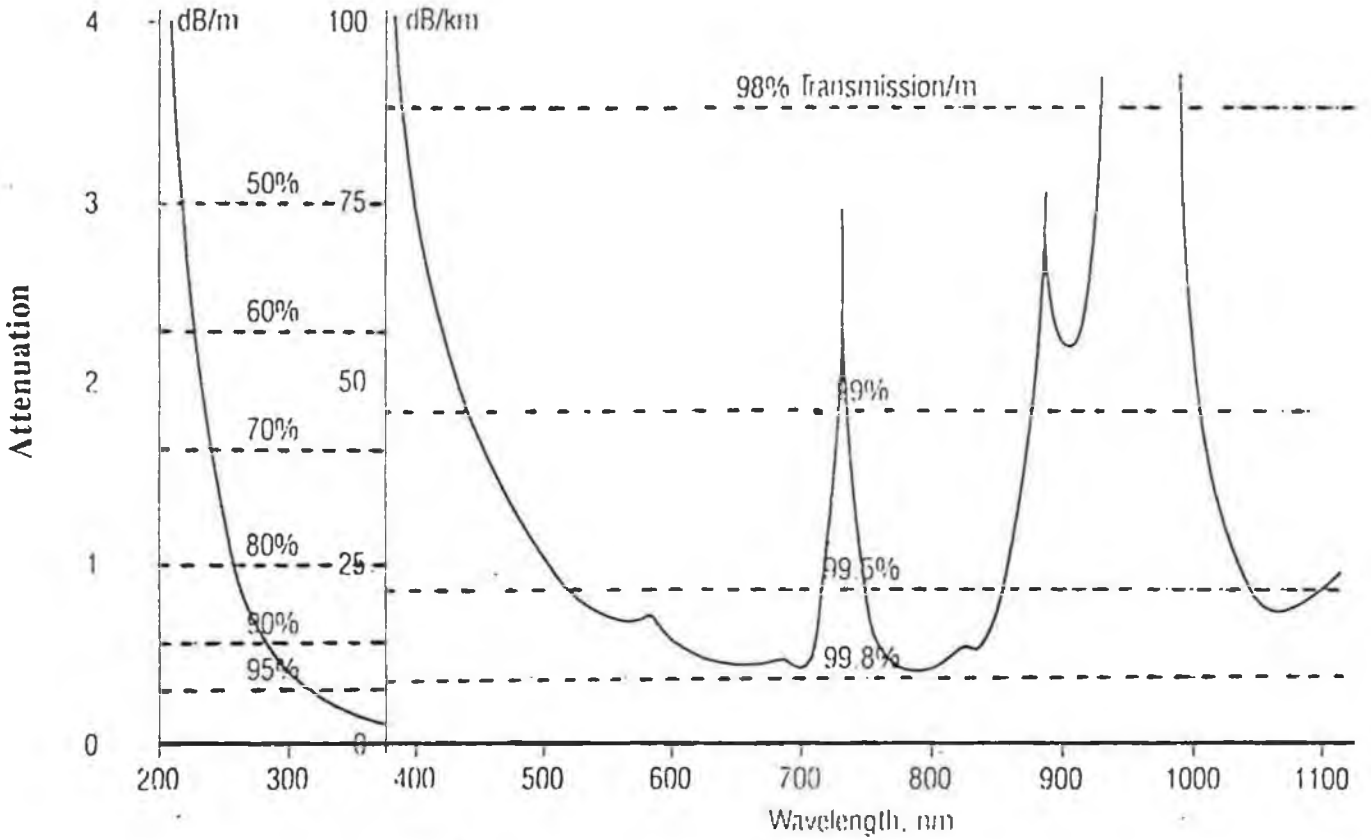


Fig. 3.2 Optical Attenuation vs Wavelength for SPC400B Fibre

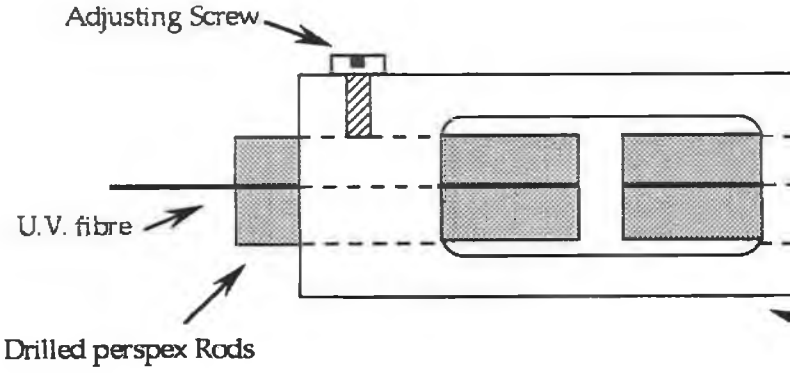
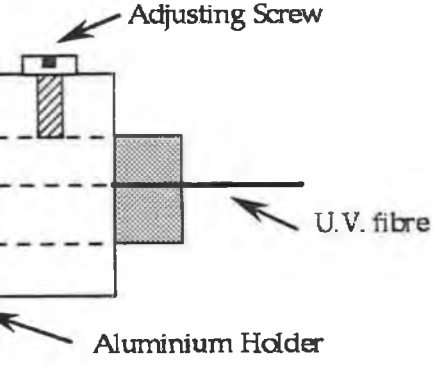


Fig 3.3 Absorption cell


Adjusting Screw
U.V. fibre
Aluminium Holder

3.3 The Optical Detection System

Due to the very high attenuation of the optical fibre in the region below 220 nm it was expected that photon counting techniques would be required to give an adequate signal to noise ratio. Photo multiplier tube detectors are generally used in photo counting systems. Some consideration was given to using avalanche photodiodes in a photon counting mode [26]. However , these were only available in the visible/ infrared region. The photomultiplier tube used in this work was a Hamamatsu R269 eleven stage head-on type with a bialkali photocathode and a UV glass window for enhanced UV response. It had a current gain of 2.1×10^6, a dark current of 5nA and a rise time of 12ns. The spectral response of the photocathode material is shown in fig. 3.4. [27]. The quantum efficiency of the photocathode was 10% at 210 nm. (i.e. for every 10 photons striking the photocathode only one electron is emitted from the surface). There were a number of photon counting systems commerically available.These ranged from large laboratory based instruments requiring computer control such as the Glen Spectra Spectrometer Controller and Data Acquisition System to stand alone instruments such as the Stanford Research Systems SR400 gated photon counter. While offering excellent performance, all of these systems were extremely costly and none of them were really suited to what would eventually become a compact, portable nitrate detection system. However, Hamamatsu manufactures photon counting modules designed for original equipment manufacturer (OEM) use. These are compact packages which provide a pre- amplifier, main amplifier, lower level discriminator and wave form shaping circuit together with a PMT socket at reasonable cost. The output consists of 30 ns wide TTL pulses which can be fed to an external counter circuit. The C1050-01 unit was chosen for this work as it is designed to accept photomultiplier tubes similar to the R269.

3.4 Electronics and Software Development.

A BBC microcomputer was required to control the movement of the grating in the monochromator via a stepper motor and also to perform signal acquisition from the photon counting module. This required an interface board which would provide the BBC micro with a link to the stepper motor driver board and to the counter circuit. A Bytronic Associates quad 6522 VIA interface board was purchased. This provided eight bidirectional eight bit interface ports and sixteen control lines together with decoding

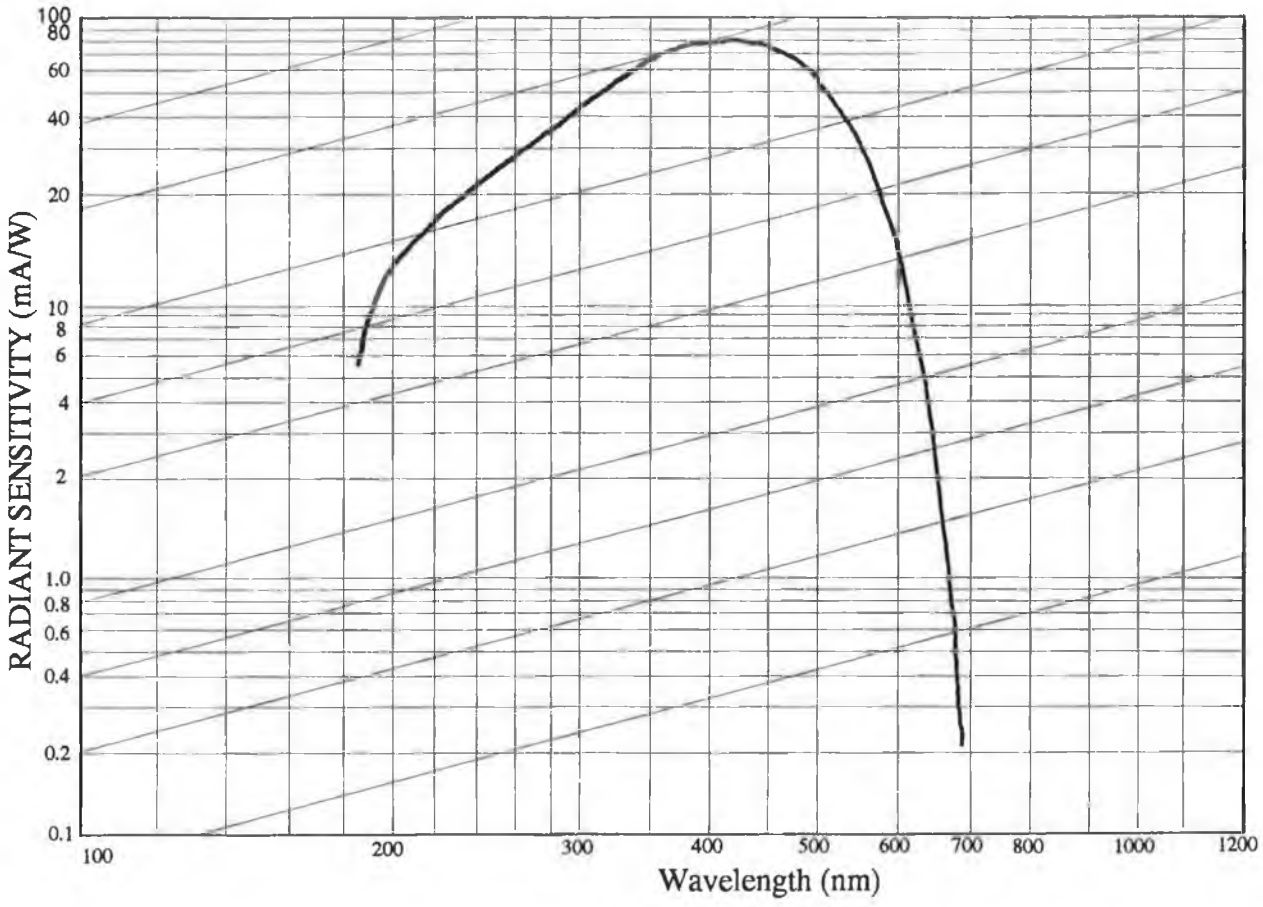


Fig. 3.4 Spectral Response of R269 Photocathode

circuitry to interface directly with data and address buses of the BBC micro.

Referring to the system block diagram (fig. 3.1), the C1050-01 photon counting module provides 30ns wide TTL pulses each time an electron is emitted from the photocathode of the R269 photomultiplier tube. A digital counter was constructed to count these pulses and this was linked to the BBC micro via the Bytronic Interface board. For background subtraction a shutter was placed in between the lamp and monochromator.This was a transistor-activated solenoid operated by a control line from the interface board. A circuit diagram is shown in Appendix C. The photomultiplier tube required high voltage biasing which was obtained from a C1309-06 high voltage module. This provided -1000Vdc when powered from a +15Vdc supply. The photon counting module required a $\pm$ 12Vdc supply and the counter circuit and interface board required a +5Vdc supply. All of these power supplies were built by the author and circuit diagrams are given in Appendix C.

The 24 bit counter was constructed using three 74ALS393 dual 4 bit counter chips daisy chained together to provide a 24 bit parallel output [28]. This was read by the computer as three eight bit words on ports A1, A2 and A3 of the interface board. Counter enable and counter clear functions were provided by control lines from the interface boards. The largest number of pulses capable of being counted was 2^{24} or 16,777,216.

The monochromator was scanned over a wavelength range by a built in stepper motor. The motor required 400 steps per revolution which moved the grating through 50 nm. Two optical limit switches in the monochromator housing to prevented the grating from being damaged in the event that the motor was overdriven. This was controlled by the BBC micro through one of the ports of the interface board and a purpose built stepper motor driver card. The driver card was designed so that the limit switches in the monochromator would cut off the power to the motor and signal an interrupt on the BBC micro in the event of the motor being driven too far. This function was also used in calibrating the monochromator with respect to zero order. The stepper motor driver card was provided with its own independent + 12Vdc supply. A circuit diagram is shown in Appendix C.

3.5 Electronic System Calibration.

The output from a photomultiplier tube contains a variety of noise pulses in addition to pulses representing photo-electrons. Straight forward pulse counting without some form of noise elimination will not result in an accurate measurement. The most effective approach to noise elimination is to investigate the distribution of pulse energies as represented by their amplitudes. When photo-electron pulses are compared to noise pulses the following observations can be made:

Pulses smaller in amplitude than photo-electron pulses are due to thermal electrons emitted from the dynodes in the PMT. These are smaller because in general they do not undergo the same number of amplification stages as the photo-electrons. However, dynode thermal electrons account for more pulses than any other noise source.

Pulses larger than photo-electron pulses are due to high energy particles such as cosmic rays or other radiation striking the glass tube. Light is emitted from the glass material resulting in the generation of a large number of photons. This multiple photon event creates a pulse of very high level. However, in terms of the total number of noise pulses, these large pulses are very few in number. A typical pulse height distribution of noise pulses in a photo multiplier tube is shown in fig. 3.5. Noise from the small dynode thermal pulses and from the large glass emission pulses can be eliminated by using pulse height discrimination where a voltage window is set so that only pulses emanating from the photocathode are passed to the counter circuit. Many sophisticated and expensive photon counting systems supply both an upper and lower level discriminator.

The C1050-01 photo counting module used in this work provided a lower level discriminator only. As can be seen from fig. 3.5 this is more than adequate for eliminating most of the noise pulses. Fig. 3.6 shows the pulse height distribution of a photomultiplier tube when a light signal is applied. The actual heights of the signal pulses vary about a central value with a statistical distribution as shown in the diagram. The discriminator level should be set in the valley between the dynode noise pulses and the pulses due to light falling on the photocathode. If the discriminator level is set too low then noise pulses will be counted resulting in an artificially high signal. If the

Fig. 3.5 Pulse Height Distribution of Noise Pulses in a PMT

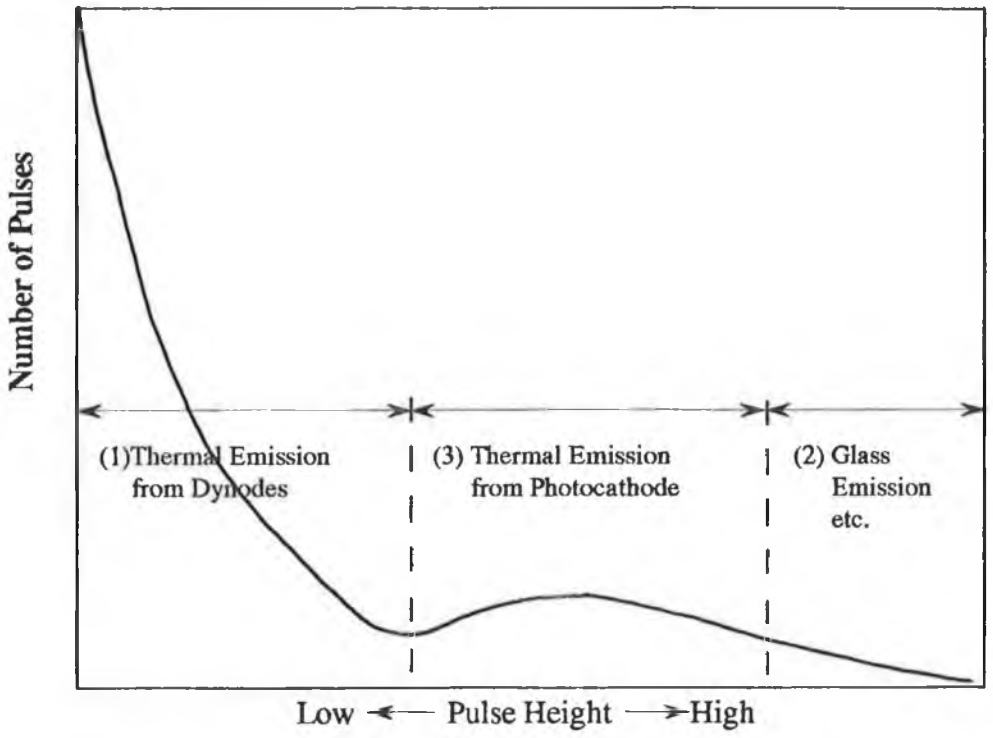
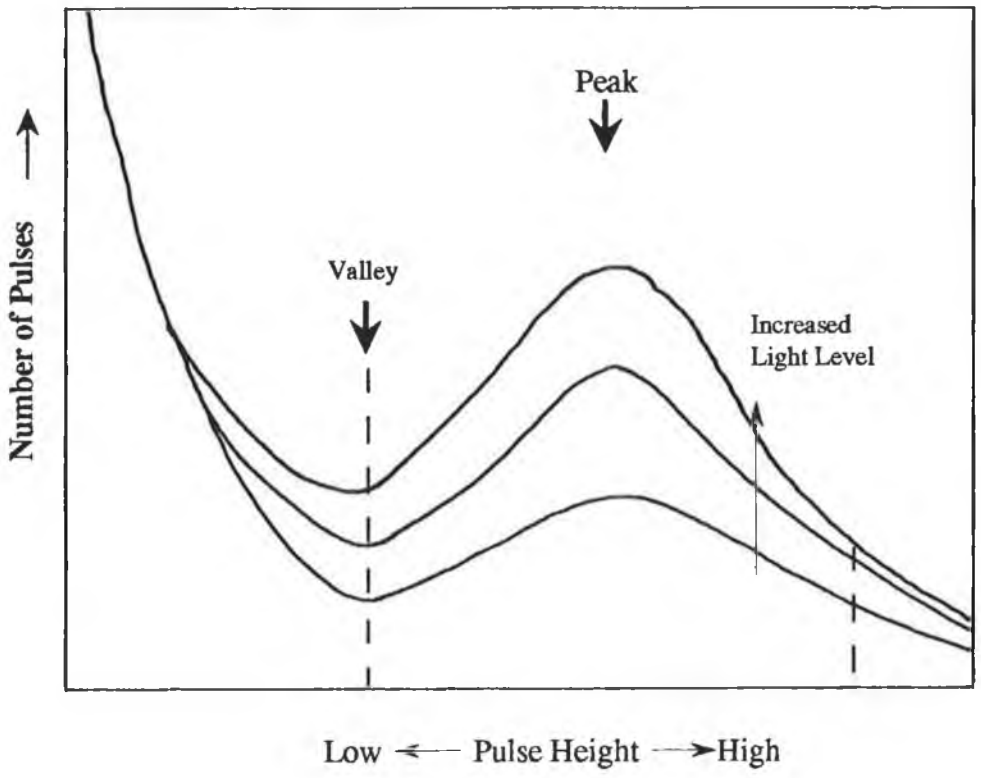


Fig. 3.6 Pulse Height Distribution of Signal and Noise Pulses in a PMT



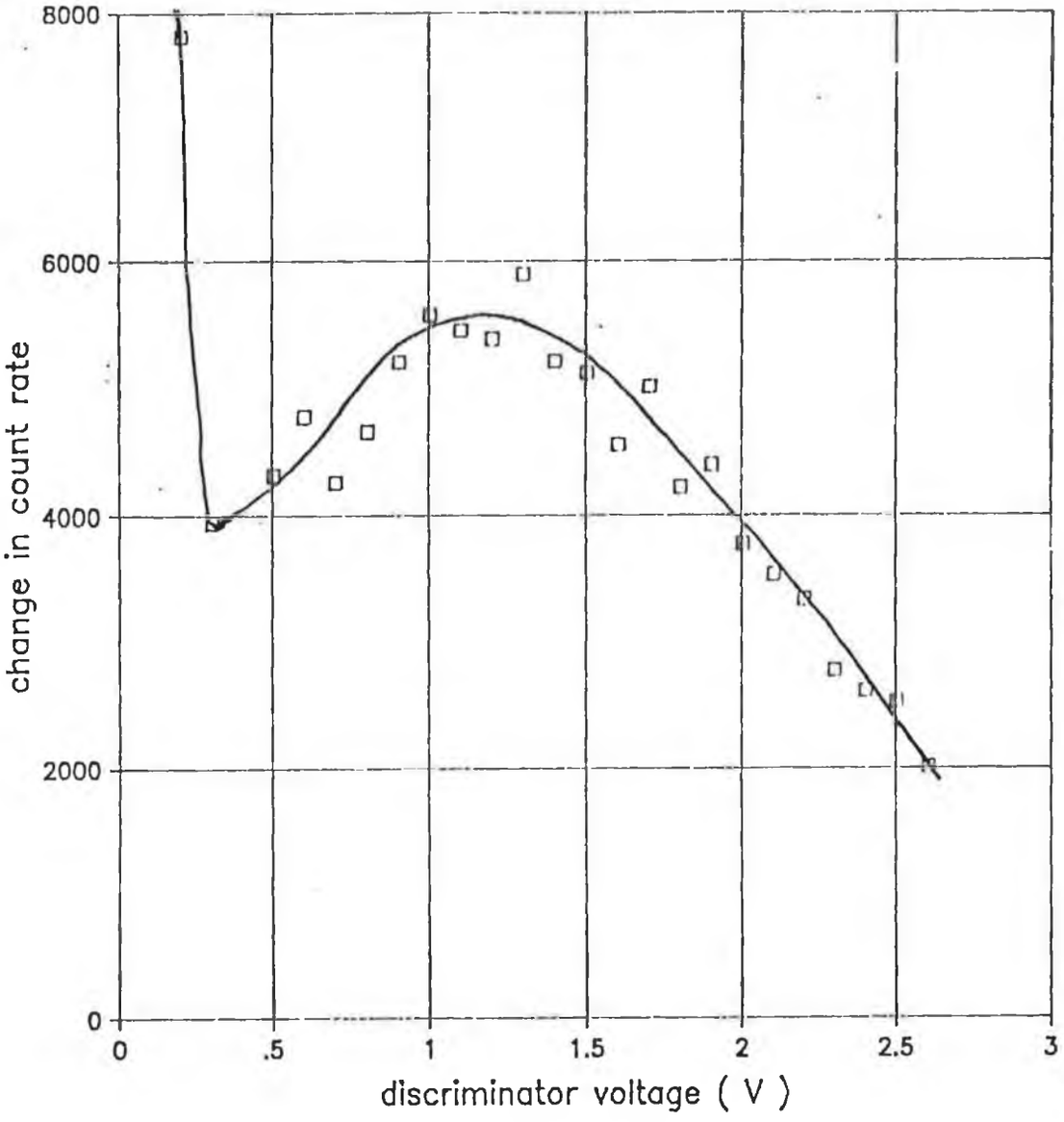


Fig. 3.7 Pulse Height Distribution for R269 Photomultiplier Tube

low an apparent count rate. The optimum discriminator setting for the C1050-01 photon counting unit was found by changing the discriminator level from 0 to 3V in 0.1V steps and plotting a graph of change in count rate vs. discriminator voltage as shown in fig.3.7. The count rate was obtained by opening the shutter to admit a low level light signal for 1 second and reading the value in the counter cicuit. From the graph shown in fig. 3.7 the best setting for the discriminator was found to be 0.35V.

3.6 Monochromator Calibration.

As stated in section 3.4 the monochromator has built in upper and lower optical limit switches to prevent the grating from being damaged by collision with its mechanical end stops. The wavelength range that can be scanned is defined by the separation between the two limit switches. For calibration purposes all wavelengths are defined with respect to zero order. This is the point at which all wavelengths are transmitted through the monochromator and as a result zero order is easy to detect as a definite peak in light intensity. Calibration consisted of running the stepper motor down to to the lower limit switch, then moving up one step at a time and recording the photon count rate at each step. The zero order intensity peak was found by comparing each count value with the preceding value. When zero order was found, the stepper motor would drive the monochromator to the correct starting wavelength for each scan. This calibration routine was incorporated into the data acquisition software which will be described in the next section.

3.7 Software Development

The experimental system software for this section of the work was written in BASIC to provide data acquisition, data analysis and graphical display functions in separate programs. All programs were written in subroutines called procedures (or Procs) and listings are provided in appendix D. The data acquisition program was called COUNT1J and a flow chart of this program is shown in fig. 3.8. This program provided spectra in the form of transmission curves for various nitrate solutions placed in the absorption cell. The setup procedure involved setting up the interface ports as inputs for the counter circuit and as an output for the stepper motor driver card. In this section the operator was asked to supply start wavelength, finish wavelength, wavelength increment, number of

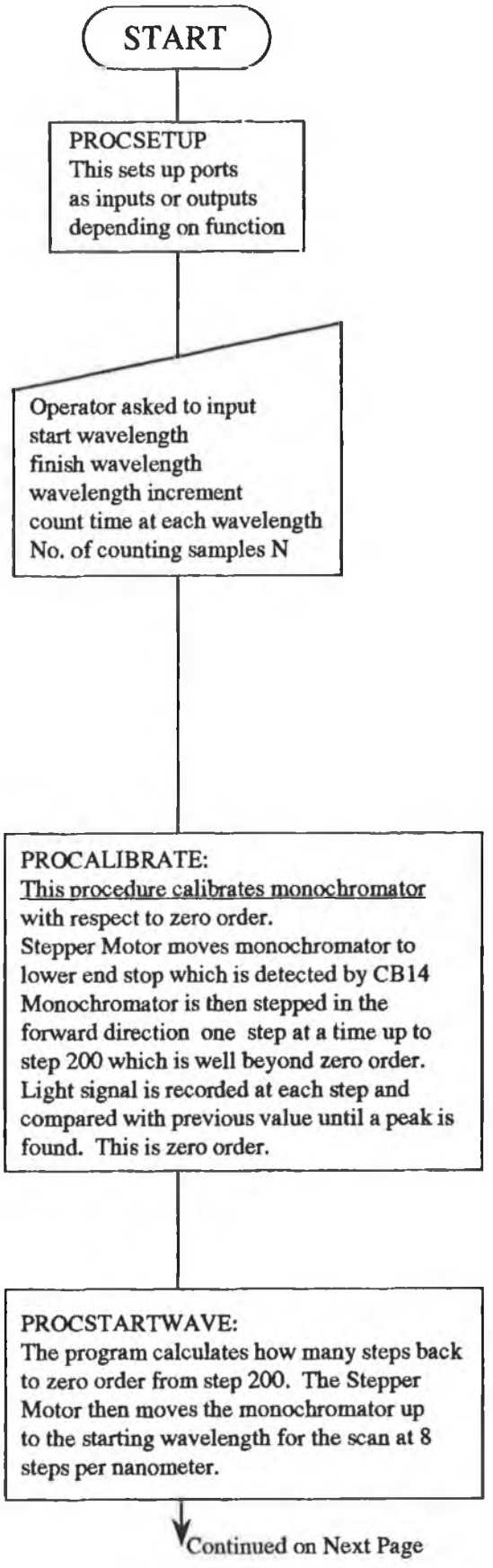


Fig . 3.8 a Flow Chart for Diagram Count 1J

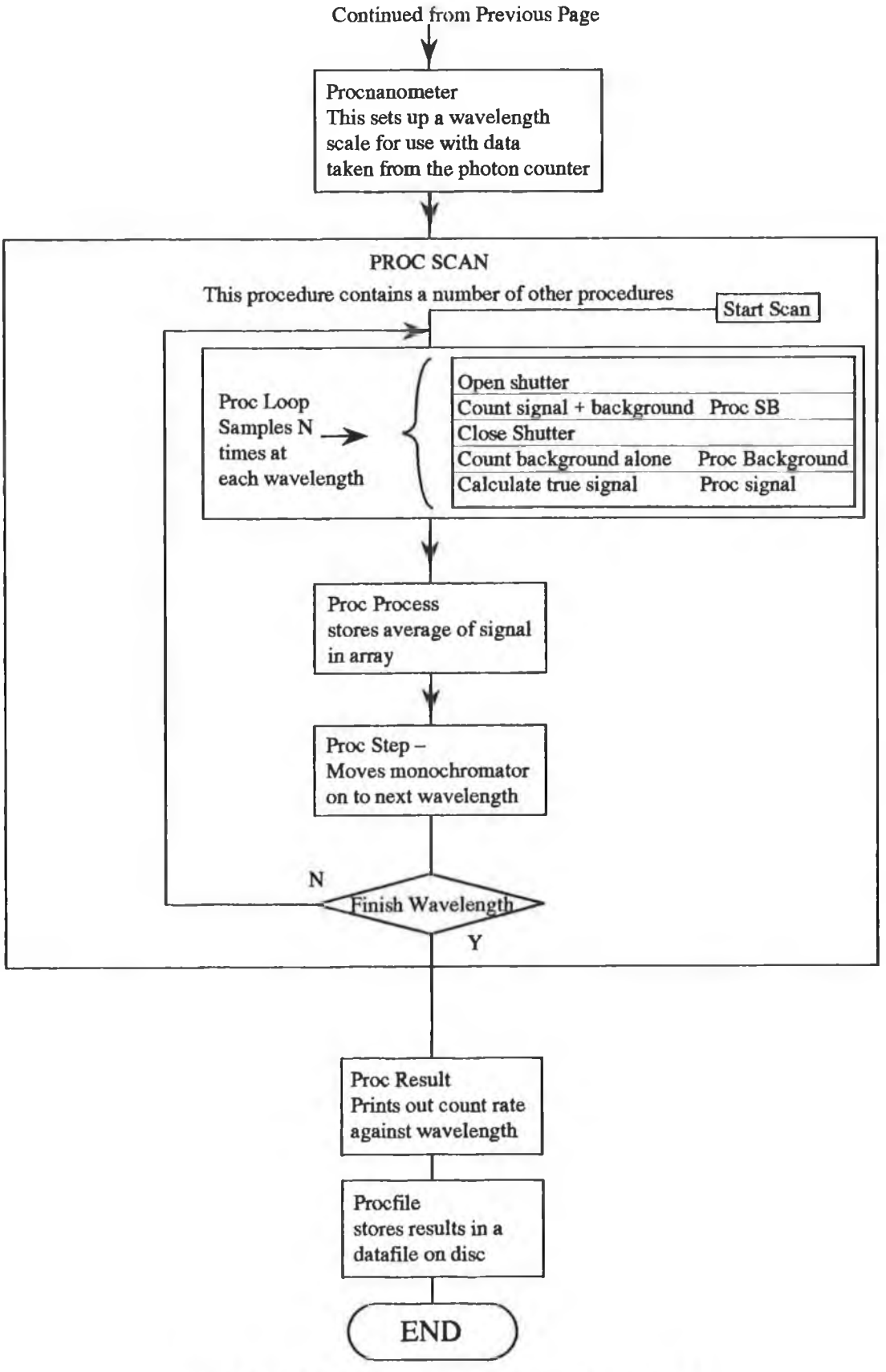


Fig. 3.8b Flowchart for Program Count 1J

samples to be taken at each wavelength and the count time for each sample ,T. The program then performs automatic calibration with respect to zero order and the monochromator is then set to the start wavelength for the scan. The heart of the data acquisition process is contained within Procscan. The monochromator is scanned from the start to finish wavelengths in increments of 1 nm as set by the operator. At each wavelength increment the shutter is opened and counting is enabled for a time interval T, usually 1 second. Following this, the counter is disabled, the shutter closed and the count value (signal + background) is stored in computer memory. The shutter remains closed but the counter is enabled for a further time interval T to count background alone. The "true signal" is obtained by subtracting the background counts from the signal + background counts. This process is repeated N times at each wavelength increment. Procprocess averages the signals obtained at each wavelength and stores the result in an array. Procstep then moves the monochromator on to the next wavelength where the whole process is repeated. Finally, when the finish wavelength is reached the count rate at each wavelength is stored on disc in the form of a datafile to await processing by data analysis software.

As stated above, the program COUNT1J obtained data in the form of a transmission curve over a wavelength range of typically 190nm to 290nm.in order to convert this data into an absorbance curve a program called ABSORB was written. This program calculated absorbance for various nitrate solutions with reference to a scan of pure water. This effectively removes the spectral contribution of the measurement system. This will be explained in the results section of chapter 4. The flowchart for this program is shown in fig. 3.9. The program takes data from two datafiles stored on disc, one of which is a scan of pure water. The program then divides every data point in the nitrate file by the corresponding point in the water file and stores the result in an array. Procscale finds the maximum value in this array and Procadjust divides every point in the array by this maximum value thus ensuring that the maximum transmission is scaled to 1. Procabsorb calculates the absorbance of each data point and procnwfile stores the absorbance data in a newfile so that it can be displayed by a graph plotting program called GRAPHM.

As will be explained in chapter 4 it was decided to investigate a simplified measurement scheme by measuring light intensity at two wavelengths only : at an absorbing wavelength (210nm in the case of nitrate) and a reference wavelength where the nitrate

ion does not absorb (275nm). A program called GETDAT2 was written to scan back and forth between these two wavelengths N times and record total light intensity and background light intensity at both wavelengths.The data was stored in a disc file to be analysed by another program called ANALYSE6. Listingsof both these programs are given in appendix D and flowcharts for GETDAT2 and ANAL6 are shown in fig. 3.10 and fig.3.11 respectively.

3.8 Conclusion

An experimental sytem to investigate the feasibility of using optical fibres for sensing nitrate concentration in water was constructed and calibrated.Software was written to obtain data in the form of transmission spectra and this was processed to obtain absorbance curves over the wavelength range of interest. Further programs were written to investigate dual wavelength referencing as a method for possible use in a prototype nitrate sensor. The results obtained with this system are detailed in the next chapter.

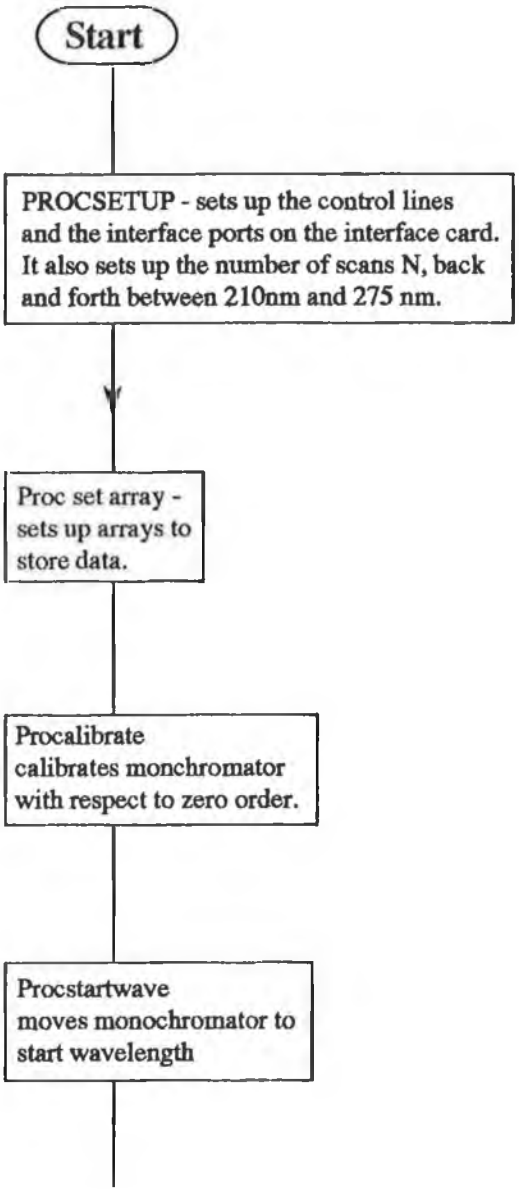


Fig . 3.10 (a) Flowchart for "Getdat 2" Program

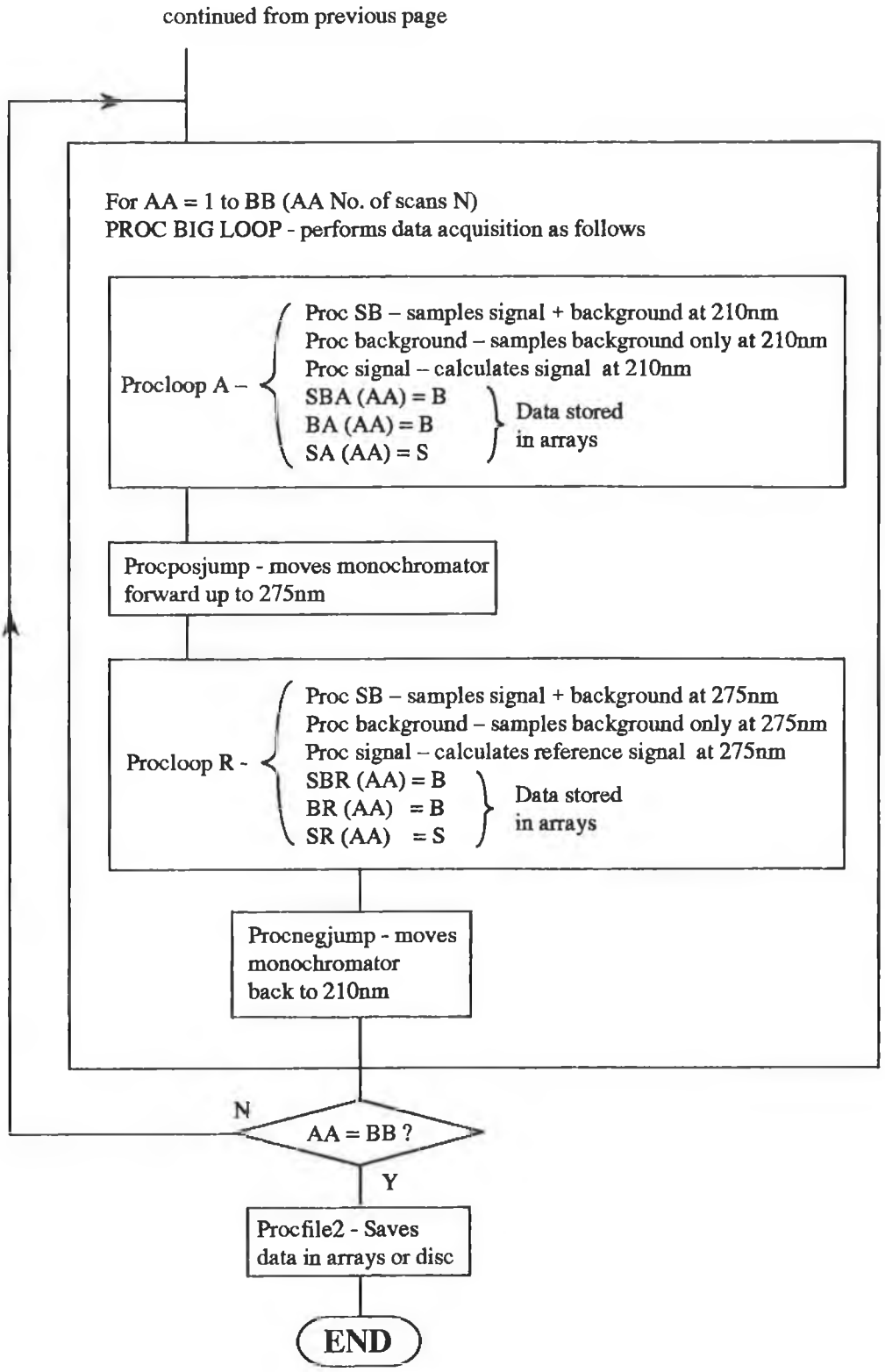


Fig . 3.10 (b) Flowchart for "Getdat 2" Program

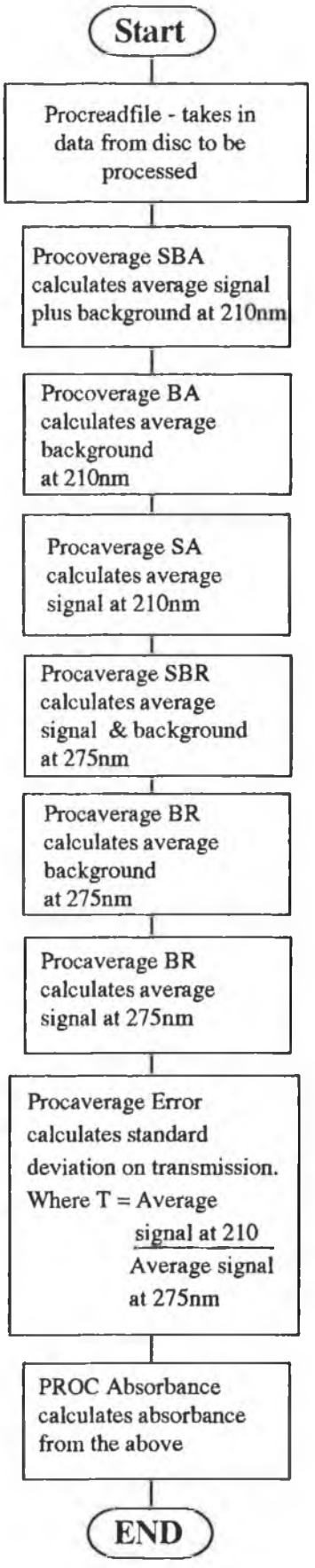


Fig 3.11 Flowchart for "ANALYSE6"

Chapter 4

Experimental Results

4.1 Introduction

The results reported in this section were obtained using the experimental setup described in chapter 3. A number of experiments were carried out to investigate the variation in nitrate absorbance with wavelength and the suitability of a simple dual wavelength measurement scheme.

4.2 Investigation of Spectral Dependence of Nitrate Absorbance

To investigate the variation in nitrate absorption with wavelength, a number of standard nitrate solutions were made up by serial dilution of a 100mg N l^{-1} stock solution. This was prepared by dissolving 0.7214g of Potassium Nitrate ($KN0_3$) in a one litre volumetric flask and making up to one litre with pure deionised water. The standard reference solutions had concentrations of 4, 5, 8, 10, 12, 16 and 20 mg N l^{-1} , respectively.

A transmission scan of pure water was recorded from 190nm to 290nm using the data acquisition program COUNT1J. Further scans of the reference nitrate solutions were taken using the same program and each scan was stored on disc in the form of a datafile. The raw data consisted of the number of counts at each wavelength recorded by the photon counting system. In effect this amounted to a transmission spectrum for each nitrate reference solution. Examples of these spectra for water and a 10mg N l^{-1} reference solution are shown in fig. 4.1 and fig. 4.2, respectively.

The number of counts at each wavelength is a function not only of the particular nitrate concentration , but also of the overall system spectral response. This consists of the response due to the lamp output, the monochromator throughput, the fibre spectral attenuation and the detector response. If a nitrate transmission spectrum is divided by the water transmission spectrum, however, the system response will be cancelled out.The transmittance, T, is defined as the fraction of incident radiation transmitted by the solution

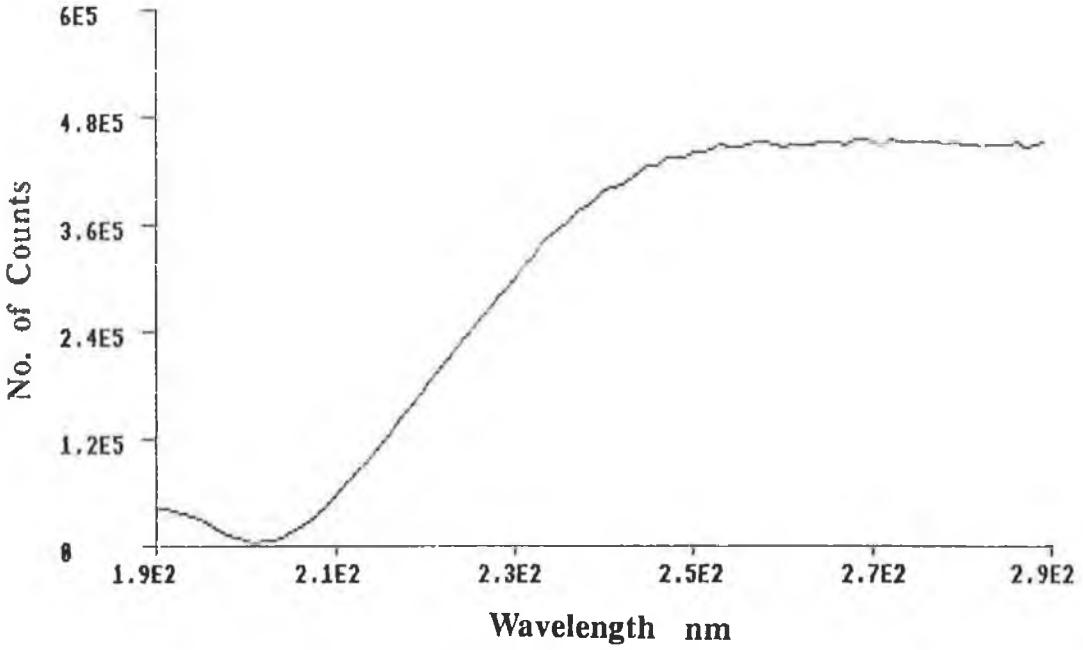


Fig.4.1 Transmission Curve for Pure Water

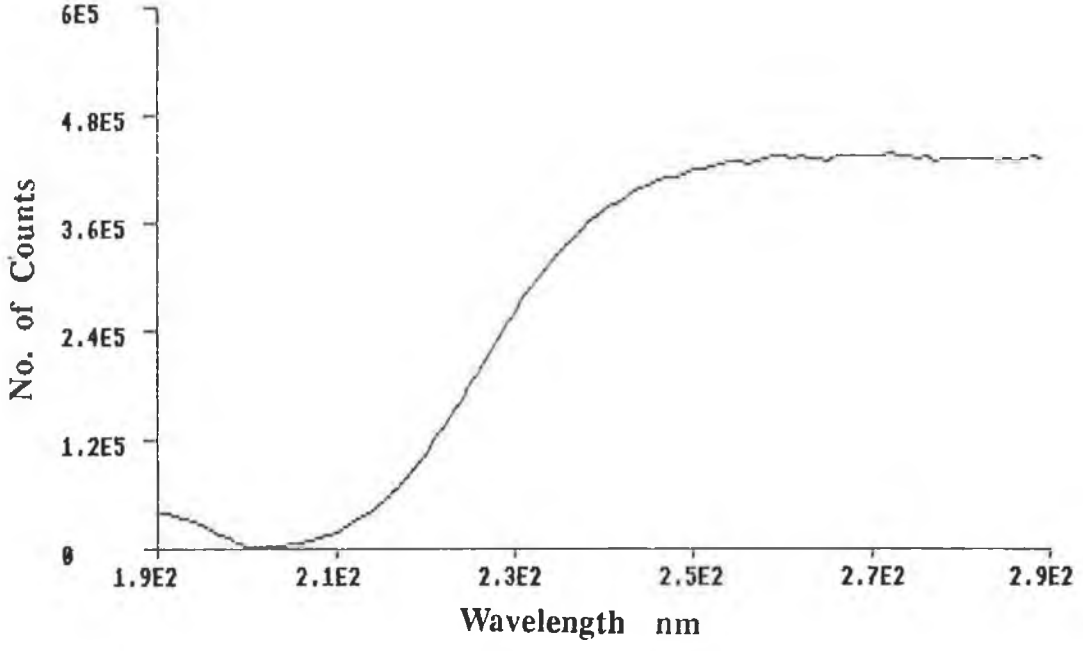


Fig. 4.2 Transmission Curve for 10mg N l^{-1} solution

$$T = I / Io \qquad \text{Eq. 4.1}$$

where Io is the radiant intensity incident on the solution and I is the radiant intensity transmitted by the solution. The absorbance A is related to the transmittance T by the equation:

$$A = \log_{10}(1/T) = \log_{10} Io / I \qquad \text{Eq. 4.2}$$

The program ABSORB (see chapter 3) was used to divide the spectra and obtain an absorbance curve for each nitrate solution with reference to pure deionised water. Typical examples of these absorbance curves are shown in fig. 4.3 and fig. 4.4. Clearly, the nitrate ion absorbs very strongly in the 210nm region with negligible absorption in the region from 270nm to 290nm.

This approach, which is essentially a fibre optic version of the UV spectrophotometric technique described in section 2.4, could be used to establish a nitrate concentration calibration curve. There exists a number of problems, however, which would preclude it from forming the basis of a viable sensor.. The first problem was drift in the peak absorbance value with time. This was due to changes in the lamp output and detector sensitivity between scans (i.e. the system response did not remain constant between the time the transmission scans for water and nitrate were taken.)

The second problem which is closely related to the first, was the long scan times involved. To count signal plus background and then background only over a range of 100nm in 1 nm steps takes at least 200 seconds if no averaging is perfomed at each wavelength. The above sequence has to be repeated for a pure water standard, so the total time taken before a result is obtained is about seven minutes.

4.3 Dual Wavelength Referencing

Having established that nitrates absorb strongly in the 210nm region but exhibit little or no absorbance in the 270nm to 290nm region, it was decided to investigate the approach of measuring nitrate concentration by ratioing the recorded light intensity at 210nm to the recorded light intensity at a non absorbing wavelength.The wavelength 275nm was chosen for this purpose because it can also be used to compensate for organic interferents when these are present in significant concentrations (This is discussed in greater detail in chapter 6). The light intensity at 210nm would be represented by I and the light intensity at 275 nm represented by Io in Eq. 4.1. This would enable measurements of nitrate concentration to be made without reference to a pure water standard and would result in

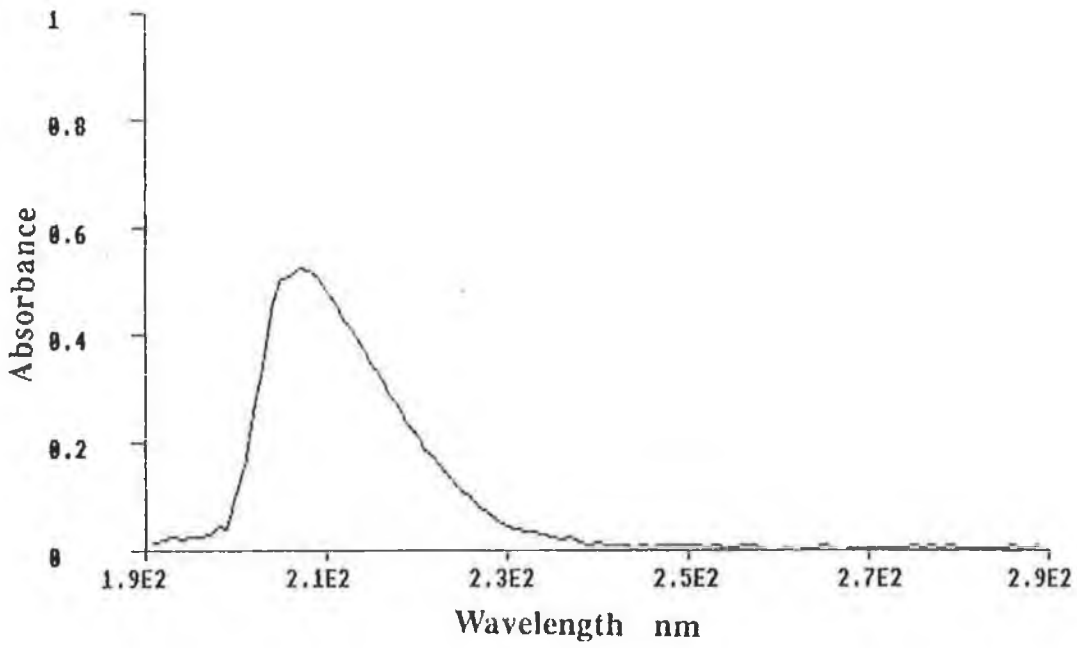


Fig. 4.3 Absorbance vs. Wavelength for 10mg N l^{-1} solution

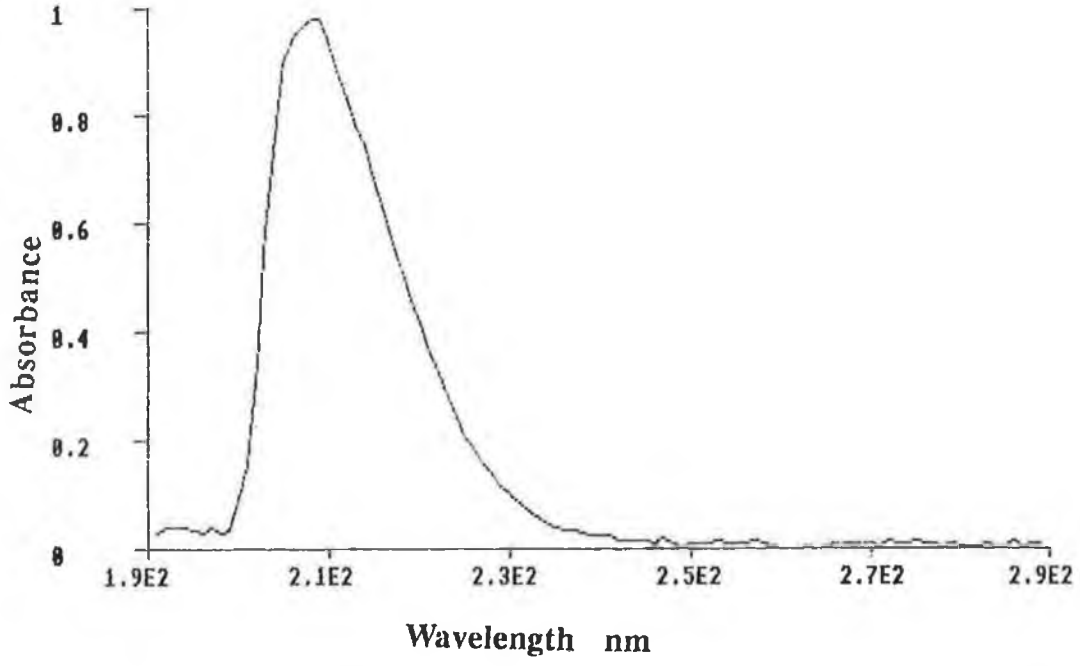


Fig. 4.4 Absorbance vs. Wavelength for 20mg N l^{-1} solution

faster analyses because of the relatively short scan times involved.It would also have the benefit of compensating for spectrally neutral drifts within the system such as aging of the light source and detector.The spectral response of the system at 210nm is very different to that at 275nm mainly because of the high attenuation of the fibre in the 210nm region (approx. $4dBm^{-1}$). Consequently, when A was calculated as in Eq. 4.2 the result was not a true absorbance expressible in absolute absorbance units, but rather a number displaced by a constant from the true absorbance value, i.e. when no nitrates are present the calculated value of A using this approach is non zero. For this reason the calculated result is referred to in the text as an absorbance factor.

As described in chapter 3 a program called GETDAT2 was written to execute this dual wavelength measurement procedure, often referred to as dual wavelength referencing. This program moved the monochromator from 210nm to 275nm and back N times. "Signal plus background" and "background" were recorded at both wavelengths for each nitrate solution that was placed in the absorption cell. To keep data acquisition time as short as possible, each data point was an average of three runs (N=3) from 210nm up to 275nm and back again. A total time of two and a half minutes was required to complete the process compared to seven minutes without any averaging for the previous spectral division approach. This data was analysed by a program called ANALYSE6 which provided an absorbance factor value for each nitrate solution. A calibration curve of absorbance factor vs. nitrate concentration is shown in fig.4.5. From the graph it can be seen that the absorbance factor exhibits linear behaviour over the range 0 to 20mg Nl^{-1}.One of the main limitations with this system was the very low light level at 210nm relative to the light level at 275nm. Any small fluctuations in the light level at 210nm resulted in a large change in absorbance factor. The main reason for this was the very high attenuation in the 210nm region of the SPC400B optical fibre used. The dual wavelength approach relies on the untested assumption that changes in the lamp output affect all wavelengths equally.

4.4 Conclusion

The experimental system was first used to show that nitrate has a strong absorption peak in the 210nm region with negligible absorption in the 275nm region.The system was then used to demonstrate that dual wavelength referencing using the above wavelengths was a valid technique for determining nitrate concentration in water.This technique was incorporated into the design of a prototype nitrate sensor which is described in chapter 5.

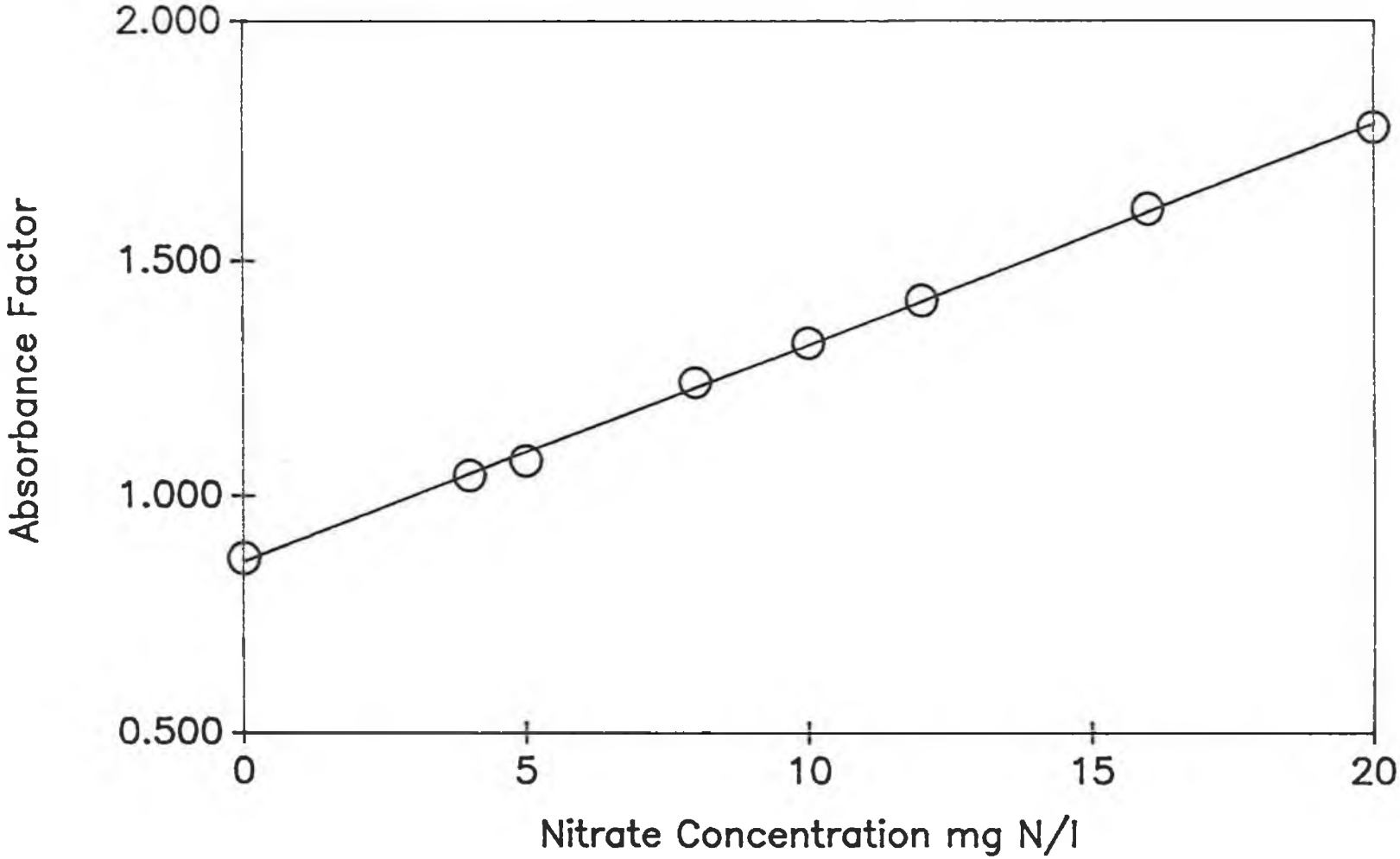


Fig. 4.5 Calibration Curve of Nitrate Standard Solutions

Chapter 5

Prototype Optical Fibre Nitrate Sensor

5.1 Introduction

Based on the results achieved with the experimental optical fibre nitrate sensor it was decided to proceed with the design of a prototype optical fibre nitrate sensor. With the ultimate aim of a portable field instrument a more simplified, compact system was constructed. The main features addressed were (i) data acquisition time and (ii) fibre attenuation and the detection system.

(i) Data Acquisition Time : The long data acquisition time of the experimental system (described in chapter 3) was due to two reasons. Firstly, the monochromator had to be scanned between the two wavelengths of interest ie 210nm and 275nm.The stepper motor took about thirty seconds to drive the monochromator between these wavelengths and for multiple scans this resulted in an unacceptably long data acquisition time when compared with other sensors outlined in chapter 2. Secondly, the photon counting system required a finite time (i.e.the count time was set to a one second interval) for a signal to be accumulated in the digital counter circuit.The overall response time was adversely affected by the use of two separate programs, one for data acquisition (GETDAT2) and one for data analysis and display (ANALYSE6), which had to be manually loaded and operated sequentially.

The long data acquisition time problem was addressed by replacing the slow scanning monochromator by a higher speed rotating filter wheel which performed spectral selection.The computer software was simplified by using a fast assembly language routine for data acquisition followed by data analysis and display functions in the same program. The finite count time problem was solved by replacing the photon counting system with an analog detection system as described below

(ii) Fibre attenuation and Light Detection System

As stated in chapter 4, one of the main limitations of the experimental set up was the very low light levels at 210 nm relative to the light levels at 275 nm. Any small fluctuations in light level at 210 nm resulted in a large change in absorbance factor. The main reason for this was the very high attenuation in the 210 nm region of the original SPC400B fibre

used.For the prototype nitrate sensor it was decided to use an optical fibre with the lowest available attenuation at 210nm (approximately 1.5 dBm^{-1}). Consequently, the higher light levels at 210nm could be detected using a sensitive analog detection system in place of the photon counting system.

5.2 An Overview of the Prototype Nitrate Sensor

A schematic diagram of the prototype optical fibre nitrate sensor is shown in fig. 5.1.The system utilised a dual wavelength approach in which a light signal at a nitrate absorbing wavelength (210nm) was referenced against a light signal at a wavelength where nitrate does not absorb (275nm).This was achieved by using two narrow band filters centred on 210nm and 275nm in a rotating filter wheel placed between the lamp and the launch fibre. The 210nm interference filter had a full width at half maximum of 16nm and a peak transmission of 18% while the 275nm filter had a full width at half maximum of 9.5nm and a peak transmission of 19%.

The analog detection system consisted of a R269 UV sensitive photomultiplier tube and a sensitive low noise preamplifier.The output from the preamplifier was fed to an analog to digital converter circuit via a "sample and hold amplifier" (SHA) which was synchronised to the rotating filter wheel. The SHA circuit effectively holds the signal at a constant value while an analog to digital conversion is performed. Signals corresponding to light levels at 210nm, 275nm and background were stored by the computer and the average of 250 signals used to provide absorbance factor values for the nitrate solutions placed in the absorption cell. Many of the components from the experimental system described in chapter 3 were utilised in the prototype sensor. These include the deuterium lamp, the absorption cell, the R269 photomultiplier tube, electronic power supplies and the interface board.

5.3 Optical System Design

The optical components of the prototype nitrate sensor are shown in fig. 5.2. Light from the deuterium lamp is coupled to the optical fibre by four lenses and two rotating filters which alternately transmit a narrow band of wavelengths centred at 210 nm and 275 nm. . The optical fibre takes light to the absorption cell while another fibre transmits light from the cell to the photomultiplier and amplifier.

For the prototype system described here the fibre used was Fibreguide industries Superguide G SFS600/660B UV-Vis optical fibre with a 600μm core diameter and a numerical aperture of 0.22. The attenuation at 210 nm is approximately 1.5 dB per metre as compared to ca. 4dBm^{-1} for the original fibre used. Fig. 5.3 shows the optical attenuation curve for the Superguide G fibre [25].

In order to transmit as much light as possible through the system, a 100 mm focal length lens of 25.4 mm diameter was first used to collimate the light beam from the deuterium lamp. The two narrow band optical filters had a diameter of 10 mm. A pair of lenses of focal length 50 mm and -30 mm, respectively were used to reduce the beam diameter to 10mm thus providing a parallel beam of light of the same diameter as the optical filters.Efficient transmission of light through the system requires that the f-number of the focussing lens match the f-number of the fibre.The relationship between numerical aperture and f-number is given approximately by [1]:

$$\text{f-number} = \frac{1}{2\,NA} \qquad \text{Eq.5.1}$$

A 25.4 mm diameter, 25 mm focal length lens was used to focus the light beam down on the fibre end face. The effective f-number of this lens was 2.5, however, since only the central 10 mm diameter portion was illuminated. Substituting this value into the above equation gives the effective numerical aperture of the lens as 0.2 which matches very closely the 0.22 numerical aperture of the fibre.

Equipment to measure the optical power output of the lamp directly was not available. An estimation of the optical losses in the system was calculated taking the case at 210 nm where optical losses were likely to be highest. With an approximate 4% reflection loss at each lens surface only $(.96)^8$ or 72% of the light is transmitted through the lens system. The filters only transmit 18% of this light at their centre wavelengths.As stated in chapter 3, in the absence of nitrate absorption only 15% of the light transmitted to the absorption cell is collected by the collection fibre for onward transmission to the detector. Neglecting fibre losses for the moment, only about 2% of the light that is launched into the optical system is actually received by the detector..The fibre attenuation is 1.5 dB per metre which means 50% of the light is lost every two metres.

5.4 The Electronic System

A number of changes were made to the signal detection circuitry compared to the original experimental system described in chapter 3. The circuit diagram of the new prototype nitrate sensor is shown in fig. 5.4. The photon counting module and counters were replaced by an analog amplifier module (Hamamatsu C1556).The R269 photomultiplier tube was mounted directly onto the amplifier module to prevent noise pick up from long leads.The amplifier was connected to a 12 bit analog to digital converter (ADC) via a sample and hold circuit (SHA).The AD585 SHA device holds the analog voltage level constant while the AD574 ADC performs a conversion. The ADC was configured to accept a 0 to 10 V full scale input signal to directly match the output of the amplifier. Spectral selection was accomplished by a custom made filter wheel shown in fig.5.5. For convenience this was mounted on a Scitec optical chopper which provided a variable speed motor to turn the wheel together with a slotted opto-switch to provide timing pulses. The two interference filters with centre wavelengths at 210 nm and 275 nm respectively were diametrically opposed on the wheel. Slots were cut along the outer edge of the wheel so that the ends of two of the slots were directly in line with the centres of the filters.These slots were used to initiate a "hold" of the optical signal followed by a data conversion on the analog to digital converter. Two other slots over opaque areas of the filter wheel enabled subtraction of background light. A small reflective strip was placed on the filter wheel as shown and this was used to trigger a reflective opto-switch. This provided a synchronisation pulse so that data was collected in the correct sequence on every rotation of the filter wheel.The electronic system was under computer control and the data acquisition sequence was as follows:

I A synchronisation pulse was obtained from the rotating filter wheel via the reflective opto-switch and a control line on the interface card.

II .A short pulse from the slotted opto-switch on the Scitec optical chopper indicated that the 210 nm filter was in line with the fibre and initiated a conversion on the read / convert line of the ADC.

III The ADC status line went low which caused the AD585 SHA device to hold the signal from the PMT amplifier. It also signalled the computer to be ready to receive data.

IV When the ADC conversion was finished the status line went high and this enabled

the computer to read in valid data which it stored in the appropriate memory location.

(V) As the filter wheel continued to rotate, another pulse from the optical chopper indicated that an opaque area of the disc was in front of the fibre and data corresponding to a background light value was stored in another memory location.

This sequence was repeated for the 275 nm filter and a further background blank. The computer repeated the above sequence when synchronisation pulses were received for a total of 250 rotations of the filter wheel.The stored data was then processed to give an average absorbance value as explained in the next section. A timing diagram for the data acquisition process is shown in fig.5.6.

5.5 Prototype System Software

A program called FILT6F was written to perform the data acquisition sequence described in section 5.4 and to process the stored data in order to calculate an average absorbance value.

A flowchart is shown in fig. 5.7 and a listing of the program is provided in appendix D.The data processing functions were performed as follows:

(1) From the data stored in the arrays the program first calculated the average signal at 210nm (AS210), the average signal at 275nm (AS275) and two background signals corresponding to the blank areas on the filter wheel (ABACK1 and ABACK2).

(2) The program then calculated the standard deviations, s on the above quantities using the equation [31] :

$$s = \sqrt{\left[\sum \frac{x^2}{n} - (\bar{x})^2 \right]} \qquad \text{Eq.5.2}$$

where x is the measurand value, $\bar{x}$ is the average value and n is the number of samples used to calculate the average values, in this case 250.

(3) Because the average signal value is obtained from 250 individually recorded signals, the estimate of the true signal value will be more reliable than in the case where say, only 5 recorded signals were used. To take account of this, the standard deviation of the data can be modified to a form called the standard error (S E) which is given by :

$$S.E = \frac{s}{\sqrt{n}} \qquad \text{Eq 5.3}$$

The program calculates the standard errors on the average signal at 210nm, the average

signal at 275nm and the two background signals, respectively.

(4) The program then calculates the background adjusted signals at 210nm and 275nm by subtracting the average background levels.

TSIG=AS210-ABACK1

TREF=AS275-ABACK2

(5) The program then calculates the relative optical transmission by dividing the adjusted signal at 210nm by the adjusted signal at 275nm.

TRANS=TSIG/TREF

(6) The standard error on the transmission is calculated at this stage followed by the relative absorbance value given by:

$$A=-\mathrm{Log}_{10}(\mathrm{TRANS})$$

(7) The standard deviation of the absorbance value is given by [32]:

$$S.D_{abs} = \left| S.D_{TRANS}\left(\frac{-0.434}{TRANS}\right) \right| \qquad \text{Eq.5.4}$$

(8) Finally, the calculated absorbance value and associated error are displayed on the screen and a hard copy obtained on the printer.

Measurement data acquired by this prototype system are presented and discussed in the next chapter.

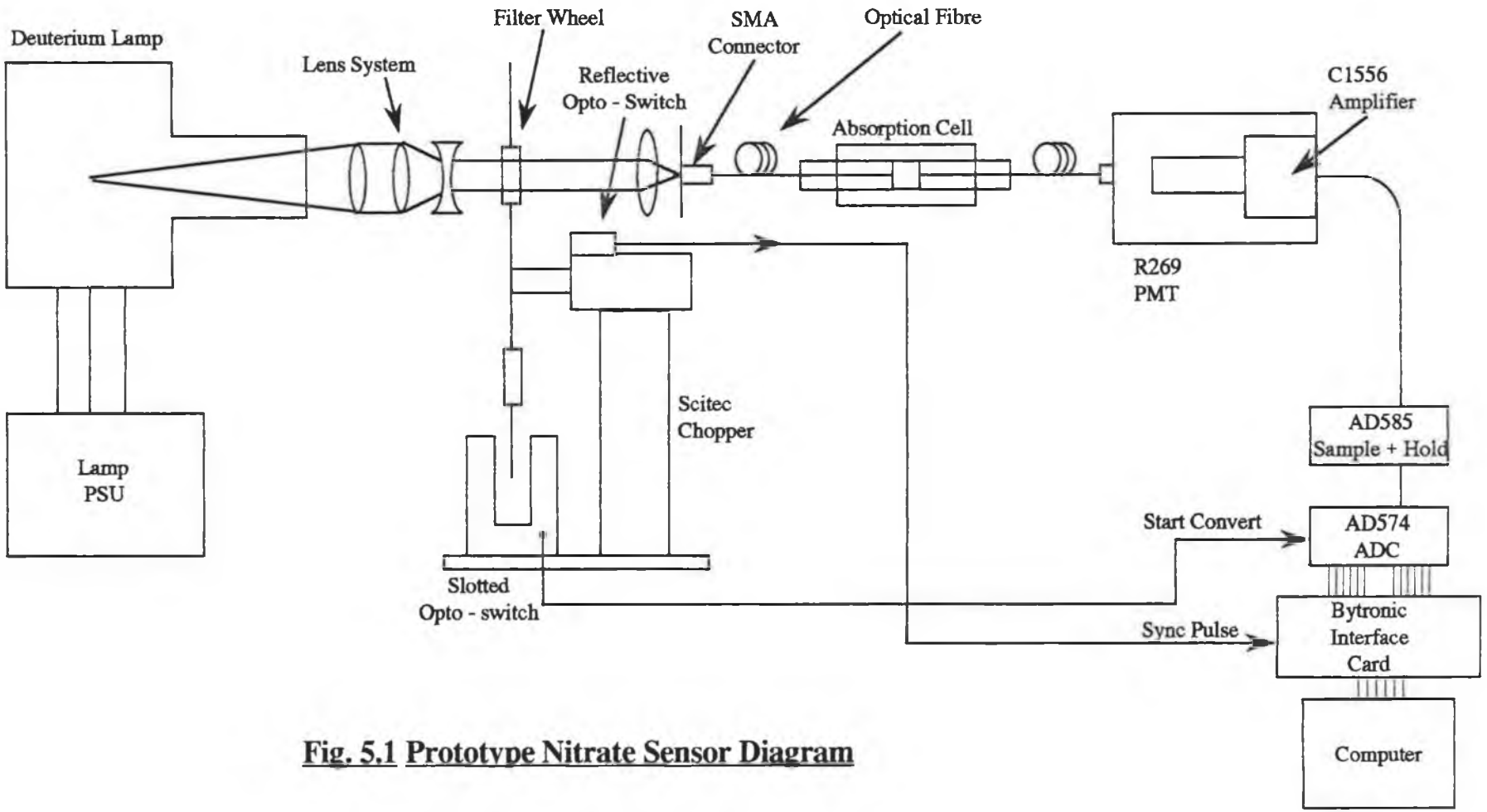
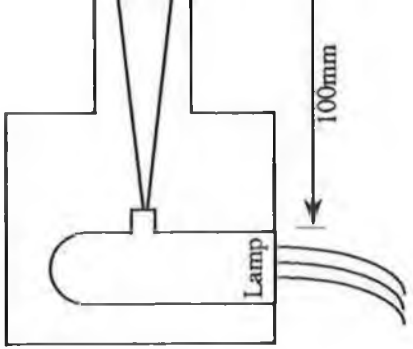


Fig. 5.1 Prototype Nitrate Sensor Diagram


100mm
Lamp

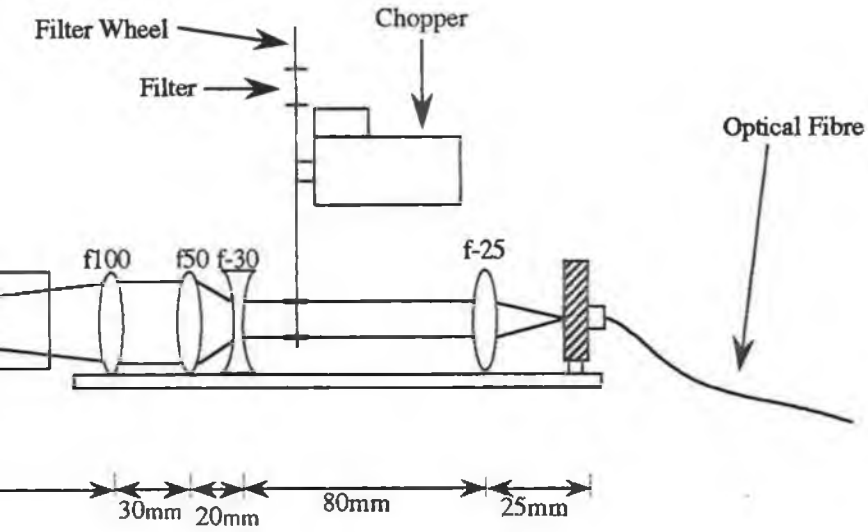


Fig. 5.2 Optical System

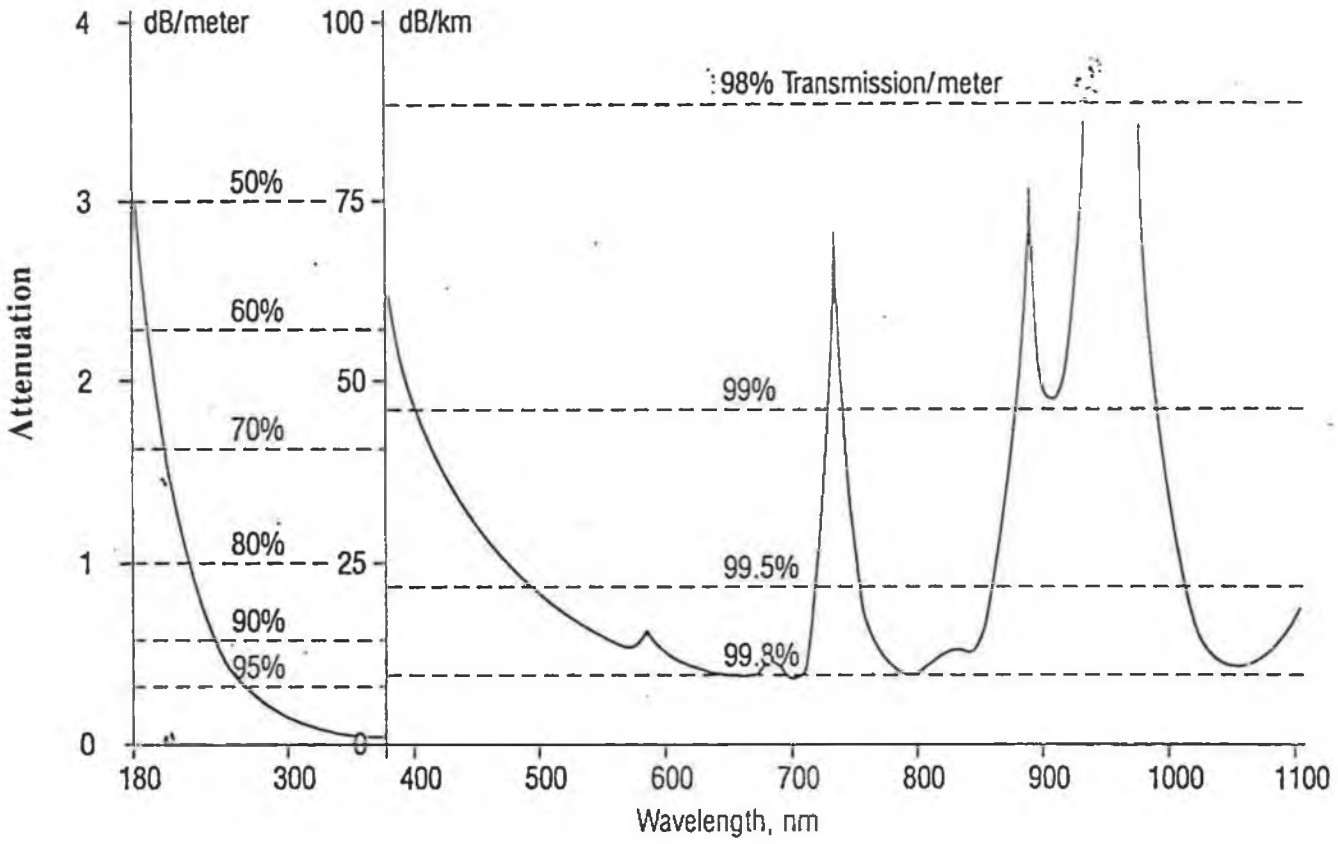


Fig. 5.3 Optical Attenuation vs. Wavelength for Superguide G Fibre

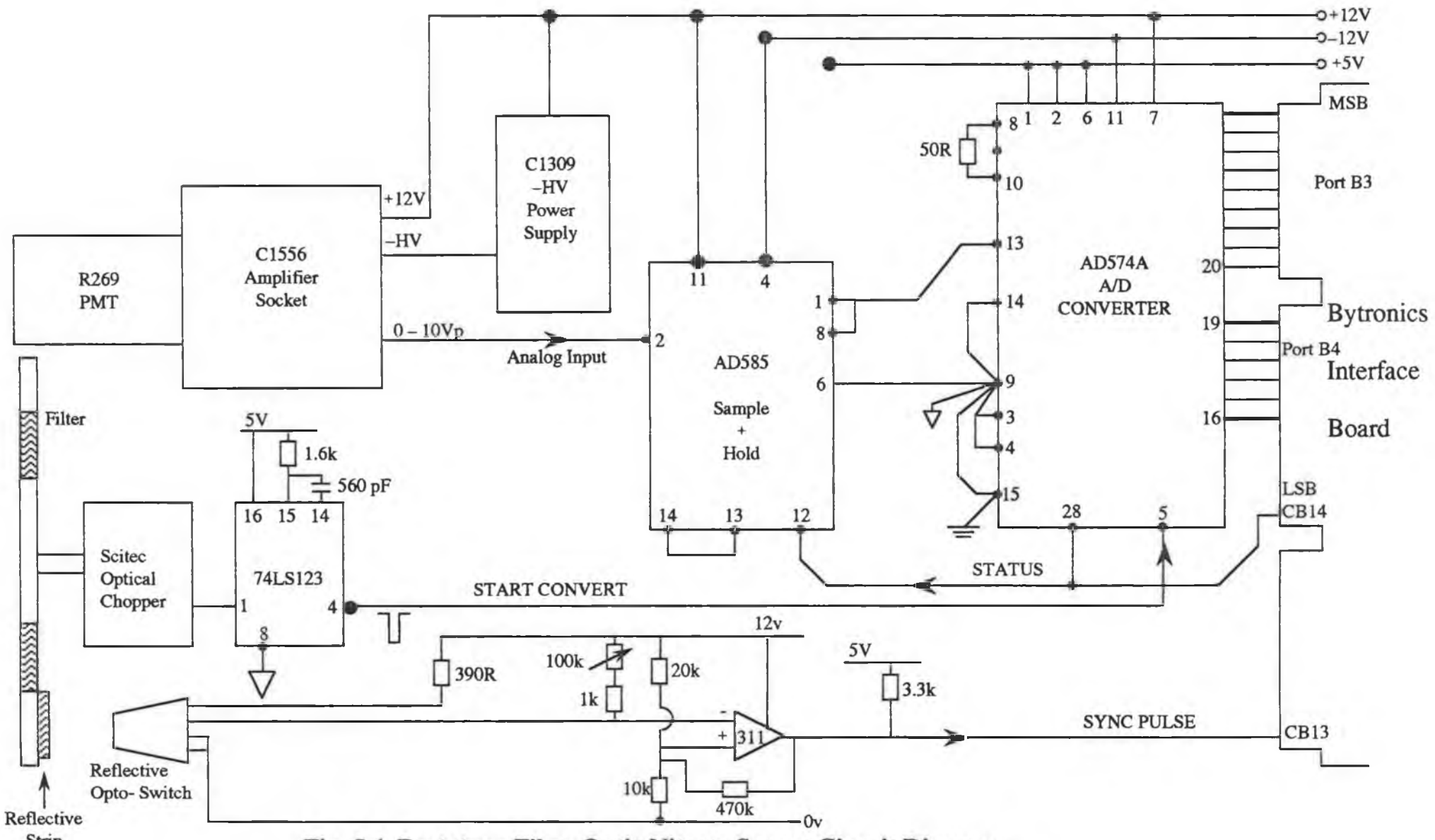


Fig. 5.4 Prototype Fibre Optic Nitrate Sensor Circuit Diagram

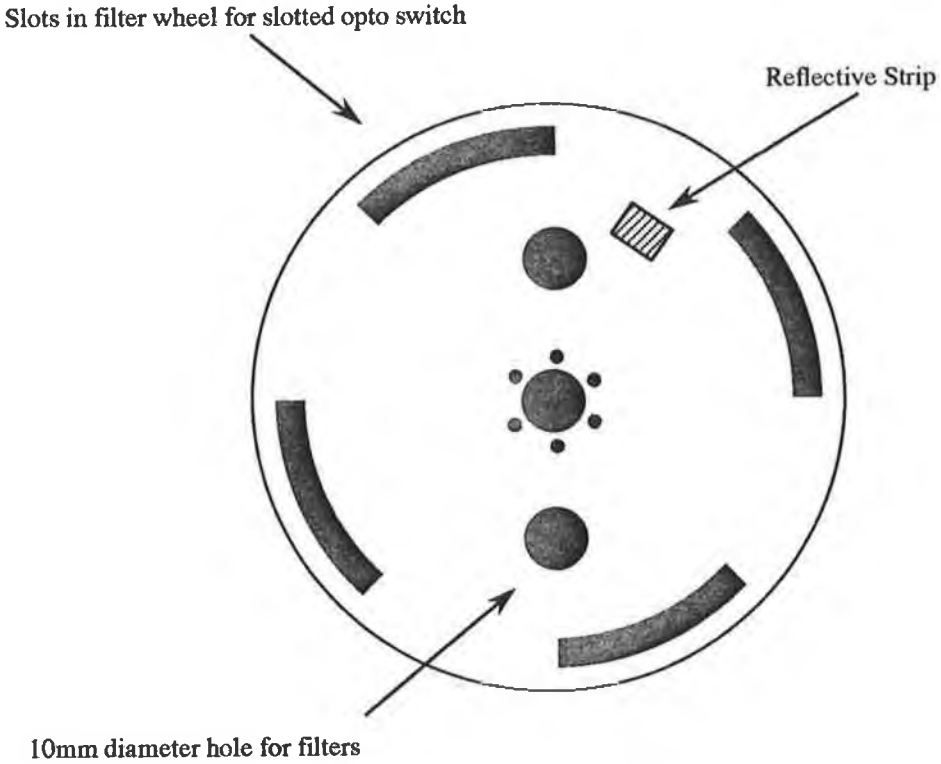


Fig 5.5 Filter Wheel

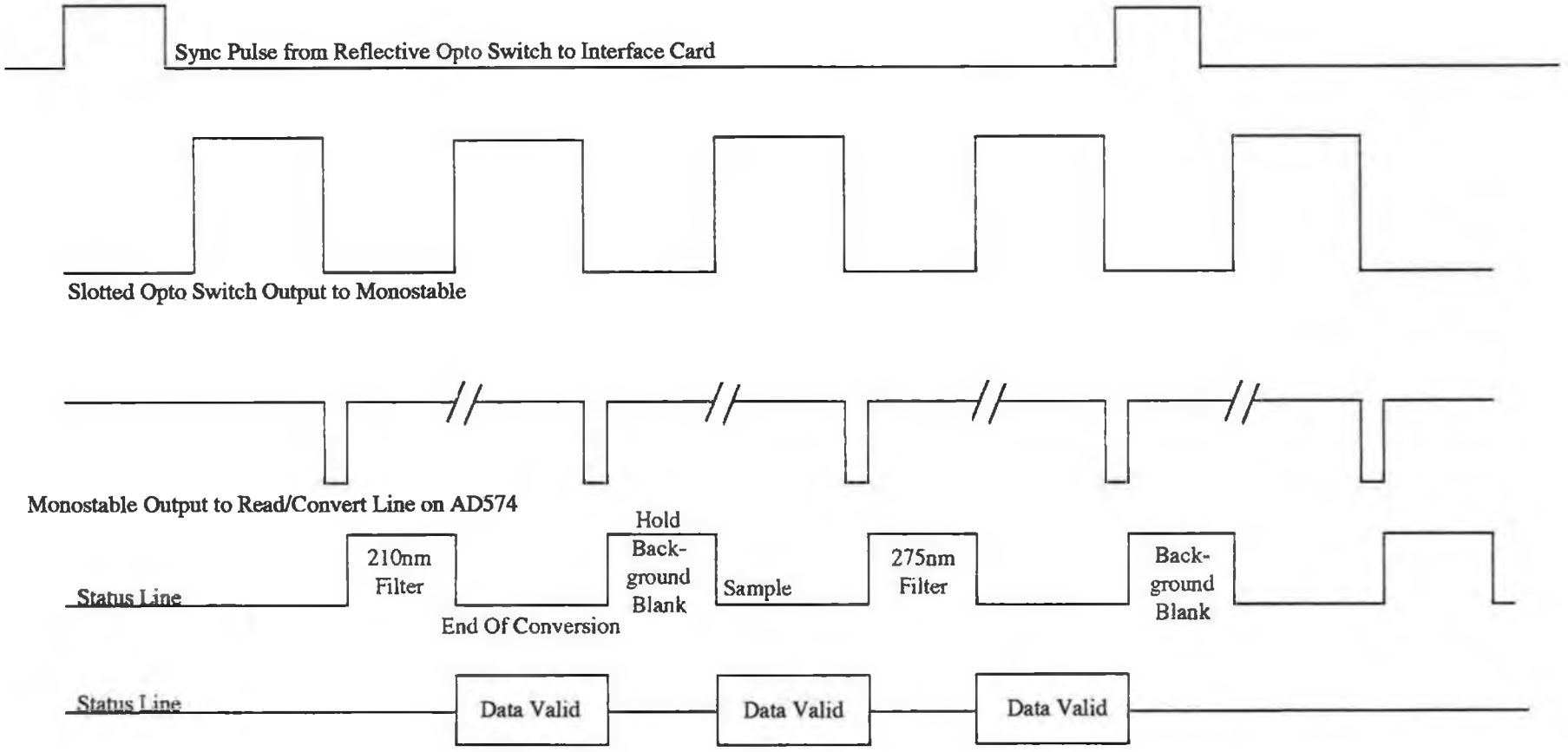


Fig. 5.6 Electronic Timing Diagram (Not to Scale)

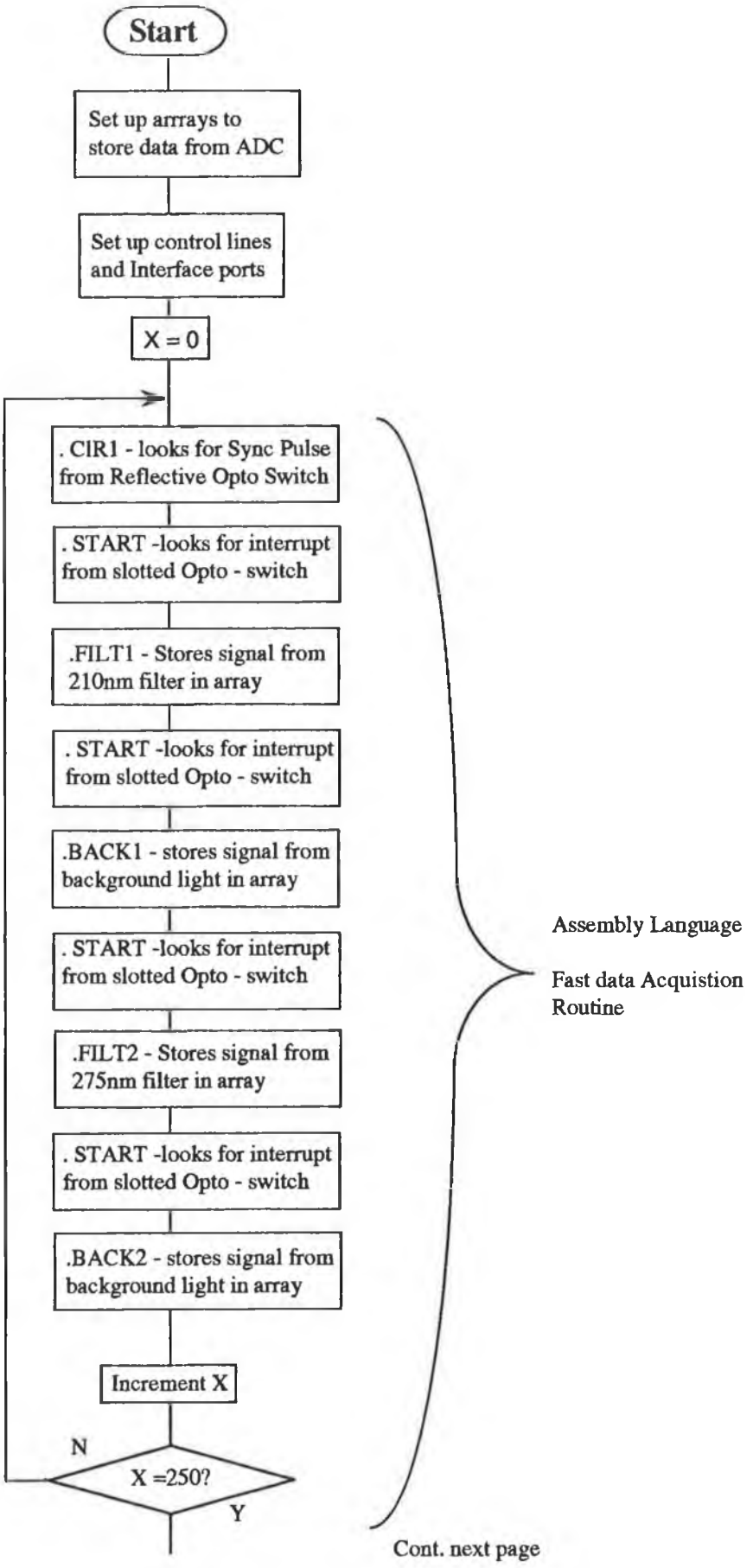


Fig 5.7(a) Flowchart for Program FILT6F

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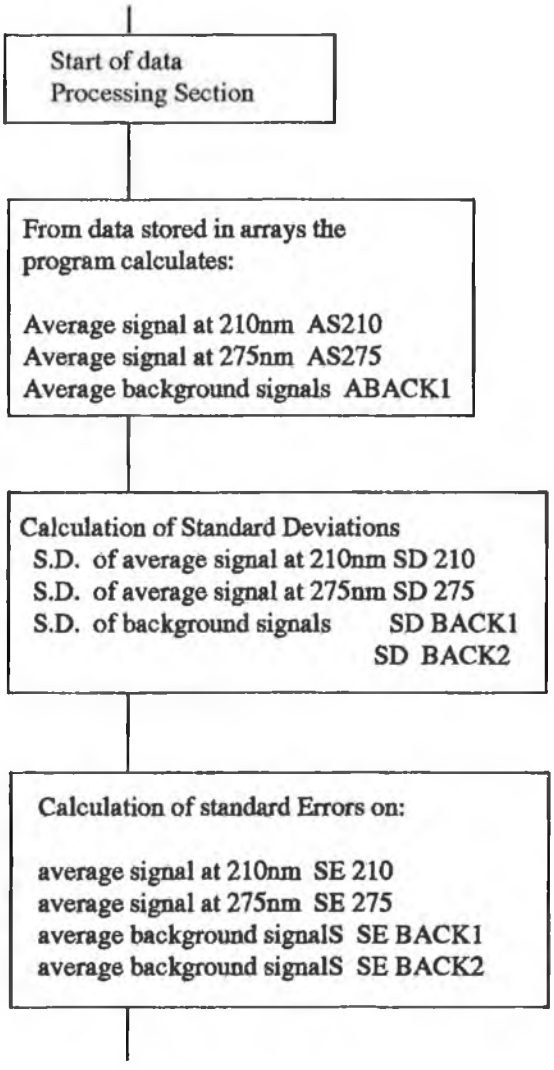

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Fig.5.7 (b) Flowchart for Program FILT6F

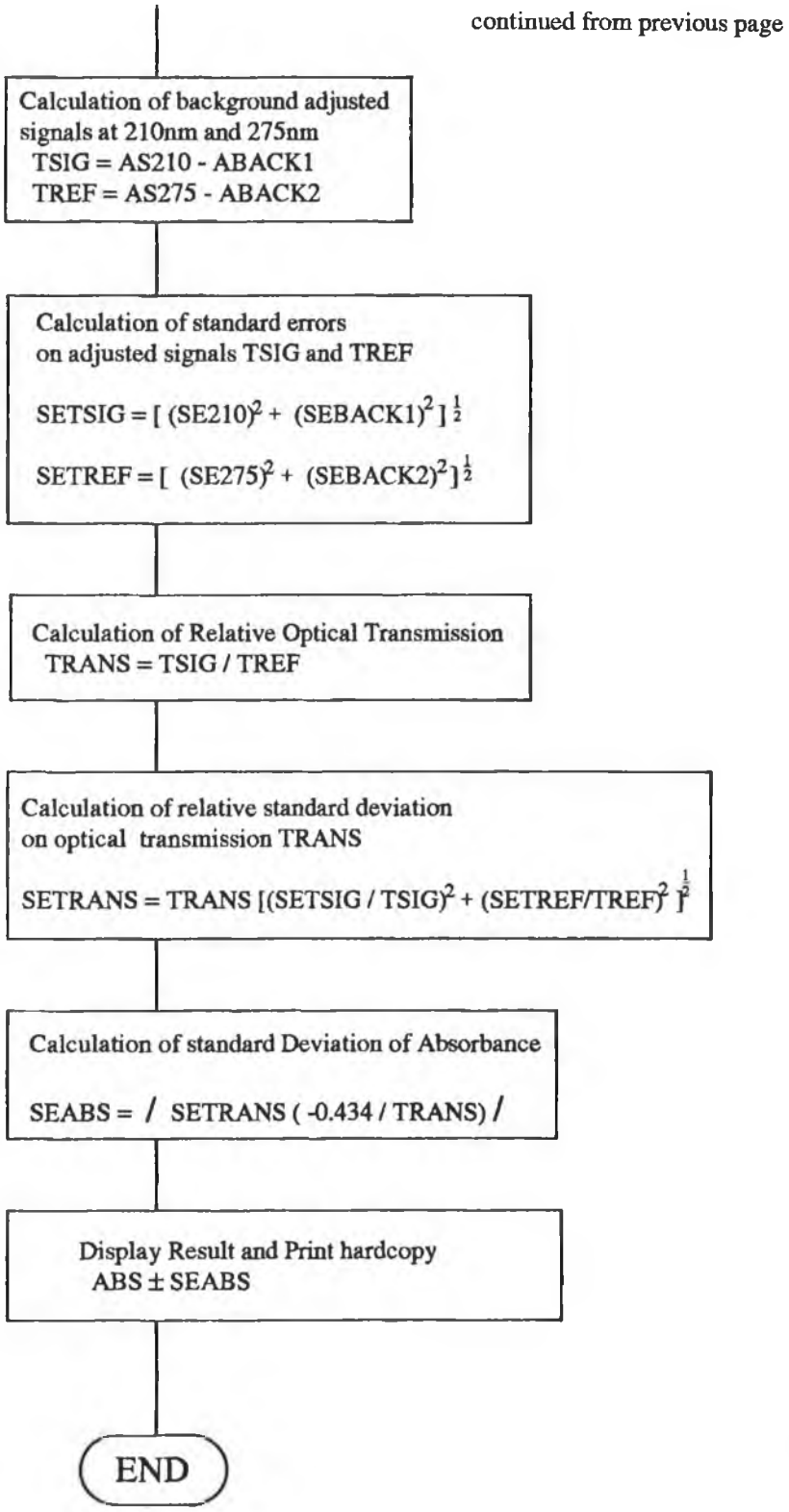

Fig. 5.7 (c) Flowchart for Program FILT6F

Chapter 6

Prototype Sensor Results

6.1 Introduction

A prototype optical fibre sensor for determination of nitrate in water was constructed as described in chapter 5.The experimental results obtained with this sensor fall into two categories.The first category deals with the characterisation of the sensor in terms of parameters such as range, resolution, accuracy, repeatability and limits of detection.The second category is concerned with the influence of interfering species such as nitrites, humic acids, ironIII and calcium carbonate on the accuracy of the sensor.

6.2 Experimental method

The operation of this sensor is based upon the principle that unknown nitrate concentrations can be determined by reference to a calibration curve derived from the measured absorbance factor values of reference nitrate solutions. The first stage in the sensor characterisation process was to establish a calibration curve. A number of standard nitrate solutions was made up by serial dilution of a 100mg N l^{-1} stock solution. This was prepared by dissolving 0.7214g of Potassium Nitrate in deionised water. The standard reference solutions had concentrations of 4, 8, 12, 16, 20, 26, 30, 36 and 40mg N l^{-1} , respectively. This range includes the EC guide level of 5.6mg N l^{-1} and the maximum admissable concentration of 11.3mg N l^{-1}. Each reference solution was placed in the absorption cell and the absorbance factor together with the associated standard error was recorded for each solution in turn. As explained in chapter 5, the absorbance factor was calculated from the measured transmitted signals at 210nm and at 275nm. For reasons detailed in chapter 5 the absorbance factor is not a true absorbance expressable in absorbance units but a number displaced by a constant from the true absorbance.The absorbance factor was derived from 250 independent measurements i.e. 250 rotations of the filter wheel. For this stage of the work a total length of 2m of optical fibre was used to guide light to and from the absorption cell. Initial readings indicated that the photo-

multiplier tube detector was saturated due to the high intensity light guided by this short length of fibre. The final 25mm focal length lens used to launch light into the fibre (see fig. 5.3) was defocussed in order to reduce the light intensity incident on the detector. For subsequent work on longer lengths of fibre the lens was replaced in the best focus position.

The calibration curve data are given in Table 6.1 below. These data were entered into a commercial graph plotting computer program (Sigmaplot) which also performed a polynomial curve fit to the data. The graph is shown in fig.6.1 and the curve fit coefficients are shown in Table 6.2.

Table 6.1 Calibration Curve Data

Concentration (mg N l^{-1})	Absorbance Factor	Standard Error
0	- 0.0478	0.0045
4	0.1566	0.0046
8	0.3444	0.0046
12	0.5205	0.0047
16	0.6735	0.0048
20	0.8153	0.0049
26	0.9768	0.0047
30	1.0714	0.0050
36	1.1665	0.0053
40	1.2247	0.0052

Table 6.2 Polynomial Curve Fit Coefficients for Calibration Curve

$$Y = A_0 + A_1x + A_2x^2$$

$$A_0 = -0.04957$$

$$A_1 = 0.05431$$

$$A_2 = -5.5022.10^{-4}$$

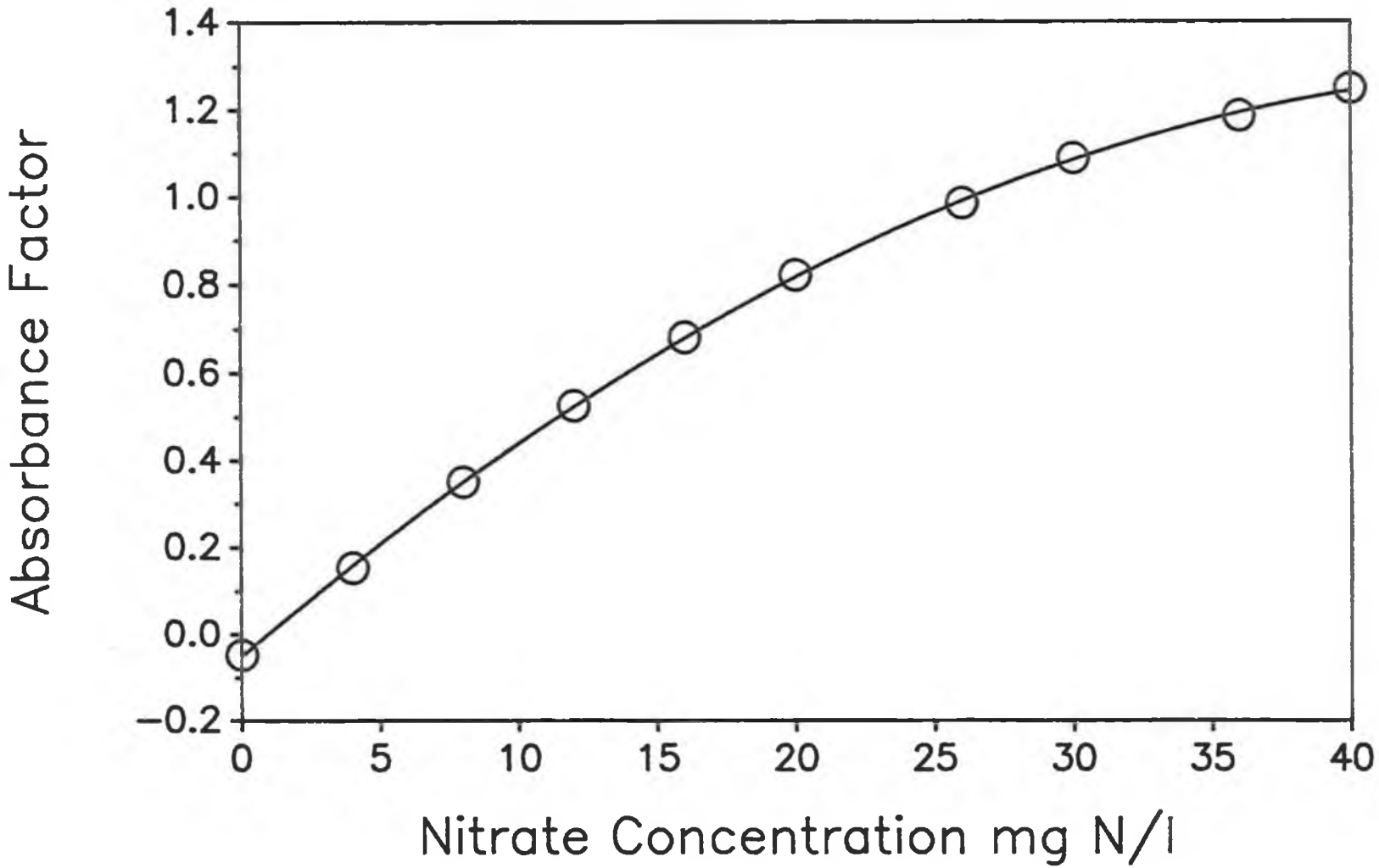

Fig. 6.1 Calibration Curve for Prototype Sensor

A number of features of the calibration curve require comment. The negative absorbance factor value at zero nitrate concentration (i.e. pure water standard) was due to the fact that the deuterium lamp output has a spectral peak in the 210nm region.With no absorbing species present in the absorption cell the light intensity at 210nm was higher than that at 275nm and ratioing these signals gave rise to a negative absorption factor.
Error bars representing the standard error values given in table 6.1 were calculated but these were smaller than the available data point symbol size on the graph plot.

6.3 Sensor Characterisation

(i) Range : The range of the sensor may be defined as the range of measurand values over which the sensor gives an unambiguous output signal. From the calibration curve shown in fig.6.1 it can be seen that the output of this sensor is unambiguous right up to the highest concentration tested, 40mg **N** l^{-1}.Thus, the range of this sensor is 0 to 40mg N l^{-1}. For a portable instrument this calibration curve would be stored as a look-up table and a computer algorithm used to find the nitrate concentration corresponding to the measured absorbance factor.
The curve is almost linear in the 0 to 20mg N l^{-1} region. However, the calibration curve exhibits a high degree of non linearity from about 20mg N l^{-1} up to 40 mg Nl^{-1}. This non linearity can be accounted for by the fact that the Beer-Lambert Law is successful in describing the behaviour of dilute solutions only. At high concentrations the refractive index and absorptivity of the solution are not constant but may deviate considerably from their values at low concentrations. Furthermore, the derivation of the Beer-Lambert law assumes monochromatic radiation (see appendix B). However,this sensor utilises two narrow band filters of average half-width of 12nm as described in chapter 5.

(ii) Sensitivity : The sensitivity of a sensor is a measure of the incremental change in output for a given change in the measurand.For a sensor having a linear response the sensitivity is the slope of the calibration curve in the linear region.
Because the caibration curve for this sensor is not linear over the complete range tested it was not possible to quote an overall sensitivity value. However, the sensitivity is approximately 0.04AFU/mg N l^{-1} (AFU =Absorption Factor Unit) in the 0 to 20mg N l^{-1} region. At higher concentrations the slope gradually decreases and as a result the

sensitivity decreases to approximately 0.02 AFU/mg N l^{-1} in the 34 to 40mg N l^{-1} region.

(iii) Resolution : The resolution is defined as the ability of a sensor to distinguish between closely adjacent values of the measurand.This can be calculated as 2 standard errors from the indicated value.Taking the worst case standard error from Table 6.1 as 0.0049 AFU and using the sensitivity value 0.04 AFU/mg N $l^{-1}$the resolution is approximately 0.25mg N l^{-1} in the 0 to 20mg N l^{-1} region. For the non linear region the sensitivity value is approximately 0.02 AFU/mg N l^{-1} and the worst case standard error is 0.0053 AFU. Consequently, the resolution is only 0.53 mg Nl^{-1} or just under 1.5% of the range.

(iv) Repeatability : Repeatability is a measure of the agreement between a number of consecutive measurements of a chosen value of the measurand.Repeatability is expressed in terms of a parameter called the confidence interval. The confidence interval is a range within which one may reasonably assume that the true value of a quantity being measured will be found. The 95% confidence interval is given by the equation :

$$\mu = \bar{x} \pm t \text{ (s.e.)}$$

where μ is the true value, $\bar{x}$ is the average value, t is a constant obtained from statistical tables [32] and s.e is the standard error. The value of t compensates for the uncertainty introduced by using a sample size less than infinity. The UK Water Industry [10] gives a definition of repeatability in terms of the random uncertainty in a measurement at the 95% confidence level. Their specification for a nitrate monitoring instrument states that the random uncertainty should be within 2.5% of the reading. The average value for the absorbance factor was calculated from 250 individual measurements (i.e. 250 filter wheel rotations).Tables indicate that the appropriate t value for this number of measurements was 1.96. Fig 6.3 shows the spread of values obtained for 3 consecutive measurements for the 12mg N l^{-1} reference solution.The standard error on each of the measurements was multiplied by t =1.96 to obtain the error bars shown. Thus, the measurement uncertainty falls well within the ± 2.5% specification set down by the UK Water Industry.

(v) Limit of Detection : The limit of detection is defined as the analyte concentration

giving a signal equal to the blank signal plus three standard errors of the blank [32].Using this criteria the limit of detection over a round trip distance of 2m was found to be 0.5mg N l^{-1}.

(vi) Accuracy : Accuracy describes the closeness of a measured value to the actual value of a measurand. The accuracy of a sensor is usually quoted in terms of the maximum error between the actual and measured values of a quantity. The overall accuracy of a sensor can be affected by a combination of random and systematic errors. Random errors cause individual results to fall on both sides of the average value and they affect the precision of a measurement. Systematic errors cause the results of a set of measurements to be in error in the same direction (e.g. all readings too high or too low).[32].
A measure of the random errors was obtained by calculating the combined standard error in the absorbance values.This was performed by the computer program FILT6F and the errors are listed in Table 6.1 above. The random errors were just under 1% of the absorbance factor values.
The systematic error was taken to be the sum of the resolution error (approx.1.5%) and the repeatability error (approx.2%).
To arrive at an overall figure for system accuracy the systematic errors are combined with the random errors in quadrature [32]. The accuracy of the nitrate sensor was calculated to be just under $\pm$4% of the indicated reading.

(vii) Response Time : The response time can be defined formally as the time interval from the instant a step change occurs in the measurand to the instant when the change in the indicated value passes and remains beyond 90% of its steady state amplitude difference.
For this sensor the response time depends on the speed of rotation of the filter wheel since 250 rotations of the filter wheel are used to provide individual measurements of the absorbance factor. The rotation speed was set to 250 rpm so that an absorbance factor is calculated once every minute. If a step change occurs during any set of 250 measurements then the new steady state value will not be displayed until the next set of 250 measurements is complete.Therefore, the longest response time is two minutes at the current filter wheel setting of 250 rpm. This is not an important parameter for the particular application of groundwater monitoring as concentration changes occur very slowly.

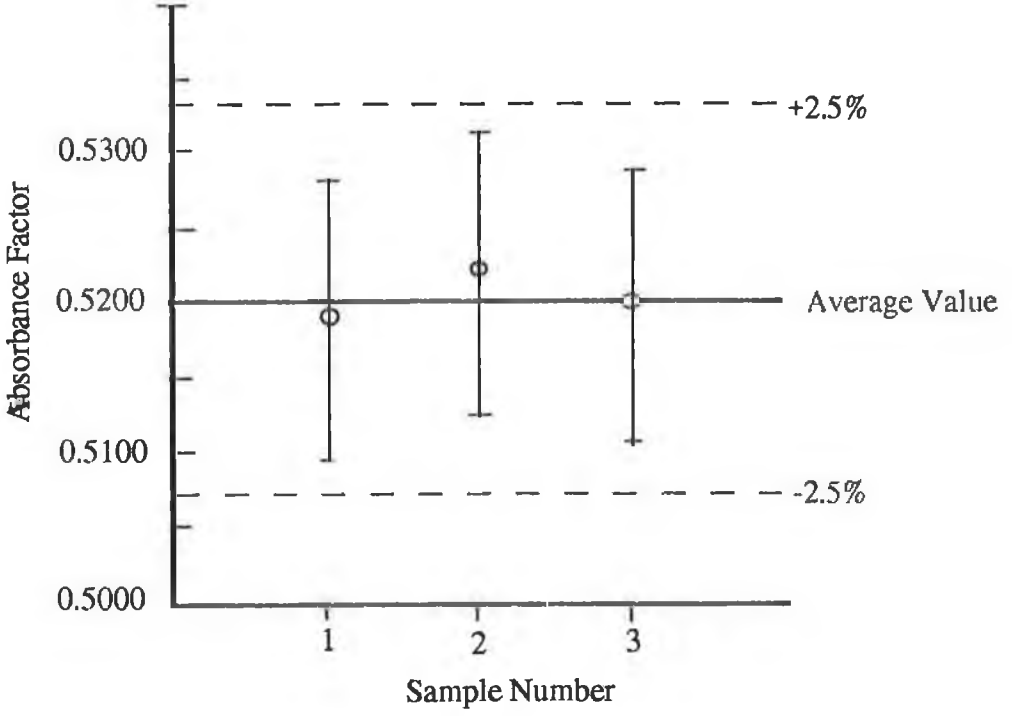

Fig. 6.2 Repeatability Test for 12mg N l^{-1} Standard Nitrate Solution

6.4 Comparison with standard techniques

The prototype nitrate sensor was used to measure nitrate concentrations in four water samples supplied by Teagasc from nitrate contaminated boreholes in Ballyderown, Co. Cork. The results obtained by the sensor were compared with results obtained using two standard methods. The methods used were (1) Ion Selective Electrode analysis and (2) a direct UV absorption method using a Shimadzu Spectrophotometer. The results from the three methods are tabulated in Table 6.3.

Table 6.3 Comparison of Sensor Results with Two Standard Methods

Sample	UV Spectrophotometer	Ion Selective Electrode	Optical Fibre Sensor
(1)	33.0 mg N l^{-1}	33.0 mg N l^{-1}	32.5 mg N l^{-1}
(2)	25.5 mg N l^{-1}	23.5 mg N l^{-1}	24.5 mg N l^{-1}
(3)	36.5 mg N l^{-1}	38.0 mg N l^{-1}	36.5 mg N l^{-1}
(4)	11.0 mg N l^{-1}	7.8 mg N l^{-1}	8.0 mg N l^{-1}

In most cases the table shows close agreement between the three methods used. However the result obtained for sample 4 using the UV spectrophotometer differs greatly from the results obtained using the other two methods.This may have been due to inaccurate standard solutions being used to calibrate the instrument in the low concentration range.

6.5 Remote Operation

In order to be viable as a groundwater nitrate monitor the sensor must be capable of detecting nitrates at considerable distances from the optical detector and signal processing electronics. With this in mind the optical fibre sensor was tested using a total length of 40m of optical fibre i.e.20m between the source and absorption cell and 20m between the absorption cell and the detector. This length is sufficient to monitor nitrate concentrations down most boreholes in Ireland.The sensor was calibrated with the same standard nitrate solutions as used for the 2m fibre work descibed earlier.The 25mm focal length lens was replaced in the best focus position to launch light into the fibre.The calibration curve is shown in fig.6.3 and the data points are tabulated in Table 6.4. In this case the absorbance factor values are much higher than in the case of the 2m length of fibre.This is a result of the greater total attenuation at 210nm compared to 275nm.When the signals at these two wavelengths are ratioed the lower transmission at 210nm results in a larger absorbance

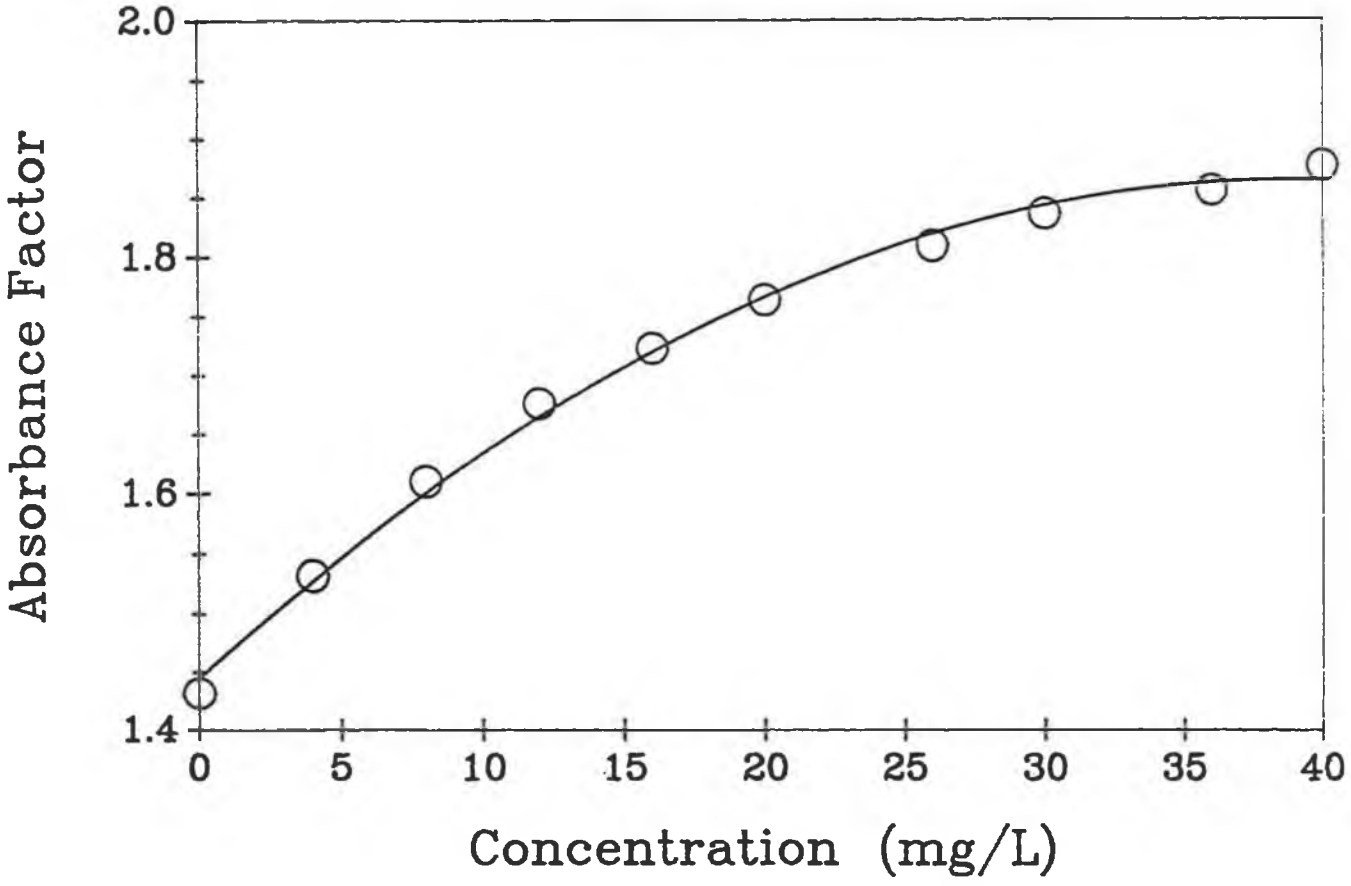

Fig. 6.3 Calibration Curve for 40 m Optical Fibre Sensor

Concentration mg N / l	Absorbance Factor	Standard Error
0	1.4324	0.0051
4	1.5321	0.0053
8	1.6104	0.0056
12	1.6767	0.0056
16	1.7230	0.0063
20	1.7646	0.0061
26	1.8094	0.0063
30	1.8365	0.0067
36	1.8564	0.0064
40	1.8777	0.0069

Table 6.4 Remote Operation Calibration Curve Data

factor value.The calibration curve shows a marked levelling off of the slope for concentrations greater than 30mg N l^{-1} and hence a great reduction in sensitivity in this region. Consequently the range of the sensor over a total distance of 40m is limited to around 30mg N l^{-1}. Because of the non linear shape of the calibration curve the sensitivity could only be approximated as 0.017 AFU/mg N l^{-1} in the 0 to 12mg N l^{-1} region and 0.008 AFU/mg N l^{-1} in the 12 to 30mg N l^{-1} region. Resolution was also reduced compared to the short fibre system. The resolution was calculated as 0.74mg N l^{-1} in the 0 to 12 mg N l^{-1} region and approximately 1.75mg N l^{-1} in the 12 to 30mg n l^{-1} region. This compares to 0.25mg N l^{-1} in the 0 to 20mg N l^{-1} region for the short fibre version.

6.6 Effect of Interfering Species

A number of chemical species other than nitrates absorb in the 210nm region.These include nitrites, iron III, carbonates, and humic acids which are products of decaying organic material. If any of these are present in water being analysed for nitrate concentration they could give rise to erroneous results.A number of these interferents were examined in the presence of nitrate to assess their effect, if any, on the operation of the nitrate sensor. When assessing the impact of interferents on the sensor a concentration of 4mg N l^{-1} was used for comparison purposes.This was chosen as it was easily detected by the system and was below the EC guide level concentration of 5.6mg N l^{-1}.

The effects of interferents were examined at various stages of the project and, accordingly, different versions of the sensor were used in the assessment.

(1) Nitrites :Nitrite was examined using the experimental system described in chapter 2. A 100mg N l^{-1} nitrite stock solution was prepared by dissolving 0.493g of sodium nitrite in one litre of pure deionised water.This was then diluted to form a 1mg N l^{-1} working solution.By referencing to pure water this solution gave an absorbance of 0.08 AU at 210 nm. Therefore, nitrite does have an interfering effect on the determination of nitrate by absorbance measurements at 210nm but the levels found in natural waters are extremely low, less than 0.01 mg N l^{-1}.Consequently the error introduced by nitrite in natural waters is negligible. In addition, since nitrite is the actual toxicant of interest, any elevated nitrate results due to excess nitrite levels present in the water may be very useful as a pollution

indicator.

(2) Carbonates : Most groundwaters contain appreciable amounts of carbonates particularly in limestone areas.The effect of carbonates on the accuracy of the prototype nitrate sensor was investigated by using a solution containing 4mg N l^{-1} and 300mg CO_3 l^{-1} in the form of Calcium Carbonate.This carbonate concentration is typical of that found in natural waters. The absorbance factor obtained was compared to the absorbance factor for the 4mg N l^{-1} standard reference solution.The results were as follows :

Solution (mg l^{-1})	Absorbance factor
4mg Nitrate+300mg Carbonate	0.1593 $\pm$ 0.0045
4mg Nitrate	0.1527 $\pm$0.0045

The carbonate results in a 4.5% error in the absorbance factor value compared to the pure nitrate standard solution. Clearly,this relative error would be smaller at larger nitrate concentrations where the operation of the device is more relevant. Given that the nature of groundwater monitoring does not require information on very low nitrate levels, the error introduced by carbonate is acceptable.

(3) Humic Acids : Humic acids are naturally occurring, long chain organic compounds which are formed by decaying organic material. Groundwater normally contains very little organic material, whereas surface water may contain large amounts.The effect of humic acid on nitrate determination using the prototype sensor was investigated using a solution containing 40mg l^{-1} of humic acid and 4mg N l^{-1}. This humic acid concentration is much greater than would be expected in most waters and considerably more than that found in groundwater where organic material concentrations are negligible. The results obtained with this solution were compared with the readings obtained with the 4mg N l^{-1} reference solution as shown in on the next page.

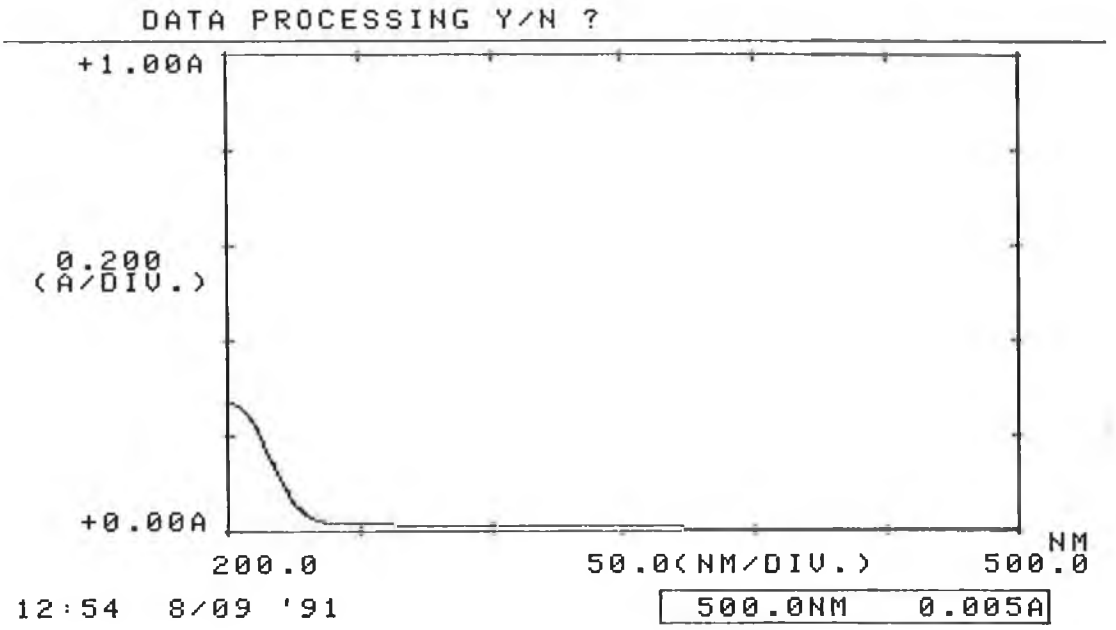

Fig.6.4 Absorbance Spectrum for 4mg N l^{-1} Standard Solution

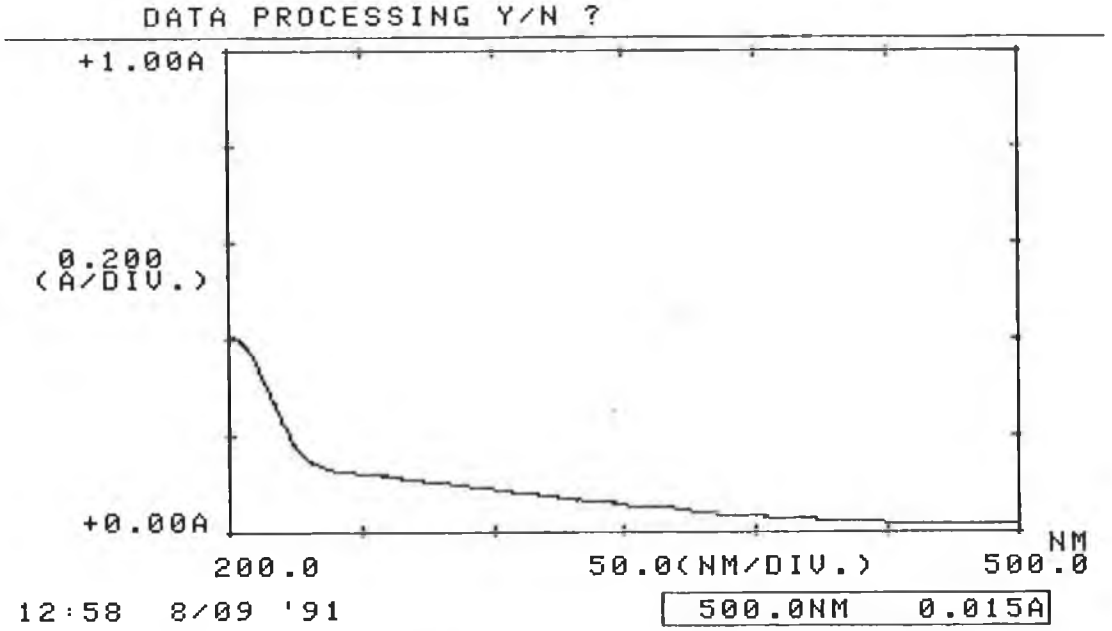

Fig.6.5 Absorbance Spectrum for 4mg N l^{-1} + 40mg l^{-1} Humic Acid Solution

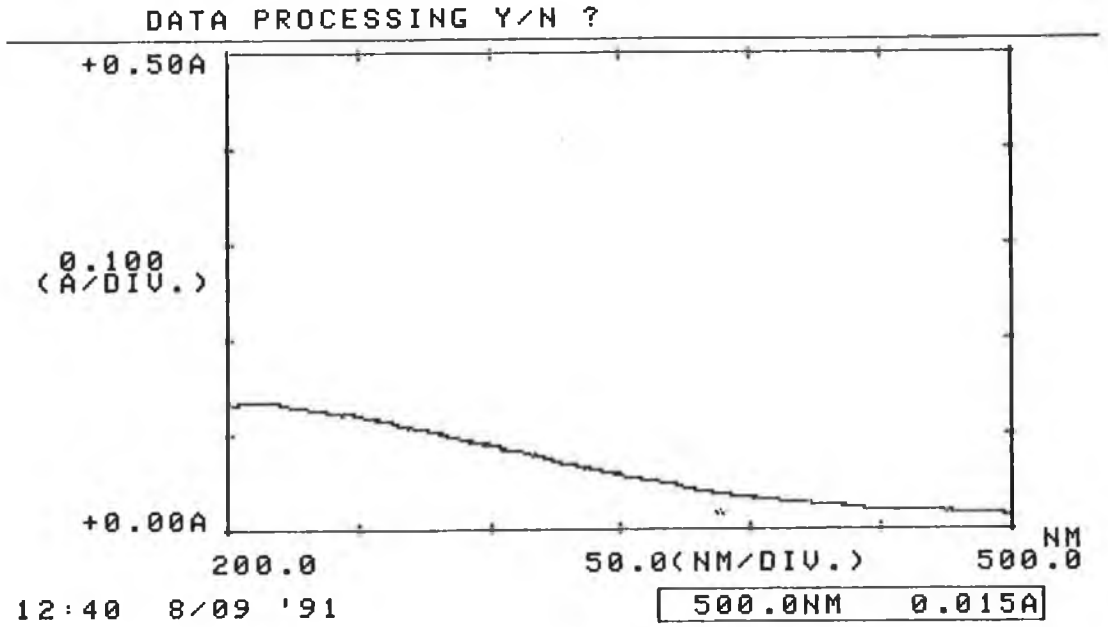

Fig.6.6 Absorbance Spectrum for 40mg l^{-1} Humic Acid Solution

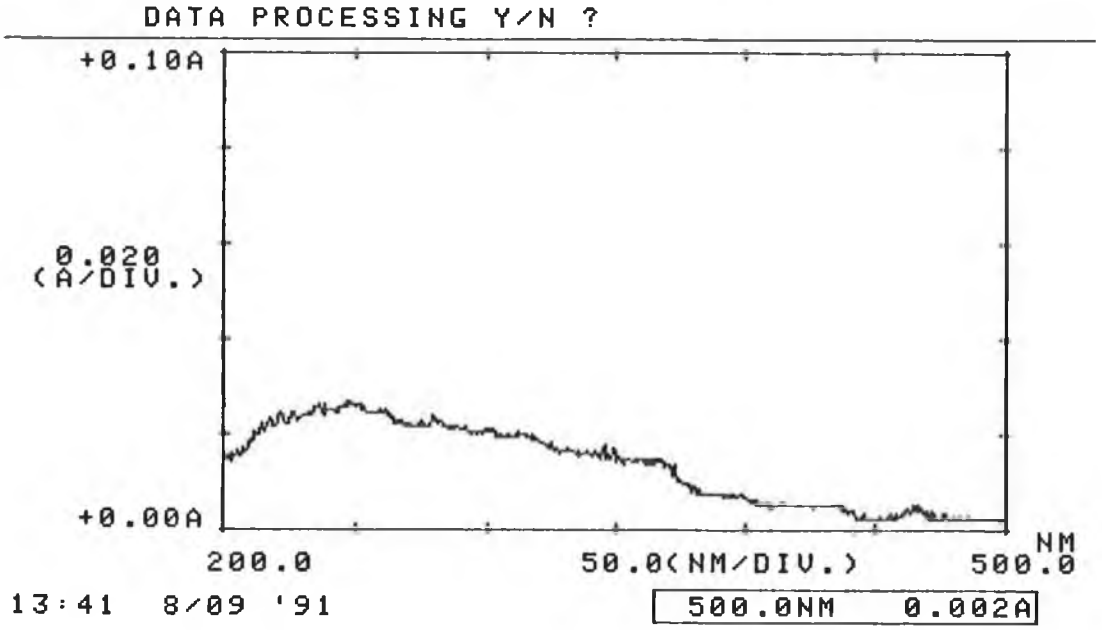

Fig.6.7 Absorbance Spectrum for 4mg l^{-1} Iron(III) Solution

Solution (mg l^{-1})	Absorbance Factor
4mg N +40mg Humic Acid	0.1867 $\pm$ 0.0043
4mg N	0.1670 $\pm$ 0.0043

The presence of 40mg l^{-1} humic acid produced a 10% error in the absorbance factor reading compared to the nitrate reference solution reading. A Shimadzu UV-160A spectrophotometer was used to investigate the absorbance spectrum of the samples used in the prototype system above.The results are shown in fig. 6.4 and fig.6.5 respectively. These can be compared with an absorbance spectrum for the 40mg l^{-1} humic acid solution shown in fig. 6.6. The 4mg **N** l^{-1} solution has a peak absorbance of 0.24 absorbance units ,whereas the solution containing both 4mg N l^{-1} and 40mg l^{-1} humic acid has a peak absorbance of 0.4 absorbance units. The humic acid also exhibits an absorbance value of 0.15 AU at 275nm where nitrate has negligible absorbance. Clearly, the prototype system which uses 275nm as reference wavelength is partly compensating for the absorbance due to humic acid in the mixed solution.

(4) IronIII : Iron compounds are found in varying concentrations in groundwater and surface water. The absorbance spectrum of a 4mg l^{-1} ironIII solution in the form of ammonium ironIII sulphate was found using the spectrophotometer.
This solution exhibited a relatively flat absorption spectrum from 210nm to 260nm with a maximum absorbance value of 0.03 AU as shown in fig. 6.7. If ironIII is found in high concentrations in waters under investigation for nitrate content, then it may have an interfering effect. Some techniques for avoiding ironIII and other interferences are discussed below.

6.7 Discussion

From the results described above it can be seen that some naturally occuring species can have an interfering effect on the determination of nitrate by UV absorption

measurements.In all cases described here the interfering species tend to cause an over estimation of the nitrate content of the water. One method of elliminating these interferents is to prevent them entering the absorption cell. Thompson and Blankley [22] used a conventional dialysis membrane as a filter to prevent iron and large molecules such as humic acids from entering the absorption cell. This technique could be readily applied to the absorption cell in the system described in this work. The effect of other inteferents such as carbonates depends upon their concentration and this in turn depends upon the geographical location of the water source.The interfering effect of nitrite can be discounted as it occurs in extremely small concentrations and any excessive amounts are a clear indication of serious pollution.

6.8 Conclusion

The perfomance of the prototype optical fibre nitrate sensor described in chapter 5 was evaluated and the effects of some possible intefering species were investigated. Using a short length of fibre the sensor was shown to perform satisfactorily over the range 0 to 40mg N l^{-1} at least. The sensor showed excellent repeatability and good agreement with other standard laboratory methods. In the absence of interfering species overall accuracy was estimated to be approximately 4%. In addition, the sensor was capable of analysing samples over 20m (i.e. 40m round trip) from the optical detector and associated electronics. Remote operation such as this , however, results in reduced range and resolution.

Chapter 7

Conclusions / Future Work

The remote determination of nitrate concentration by a simple UV absorbance measurement scheme using optical fibres has been demonstrated. A prototype optical fibre nitrate sensor mainly intended for groundwater monitoring was designed, constructed and tested.The sensor demonstrated high sensitivity, resolution and repeatability over the range of nitrate concentrations covered by EC directives when short lengths of fibre were used. Sensor performance was degraded when nitrate determinations were carried out over relatively long distances but was still more than adequate to cover the EC guide and maximum admissible concentrations for nitrate in water.

Characterisation of sensor performance revealed areas where the design needs modification before a more portable, versatile and perhaps commercial sensor is obtained.The sensor system requires modification to the following areas :

(1) The absorption cell is not well suited to being lowered down a borehole at present because the minimum bend radius of the fibre (50mm) tends to make the effective size of the sensor head quite large. This can be overcome by designing an absorption cell where the fibres enter side by side rather than opposite each other as in the current design. The light could be turned through 90 degrees using mirrors or 45 degree prisms and the collection efficiency of the fibres could be improved if miniature quartz lenses are used in the absorption cell.The effects of some interferents could be eliminated by incorporating a nitrate ion permeable membrane into the absorption cell.

(2) In order to provide a compact, portable instrument as originally envisaged the detection and signal processing electronics should be miniaturised.This is readily achievable as all the data acquisition and processing functions could be accommodated on a single board computer running on a battery supply. The photomultiplier tube could be replaced by a UV sensitive photodiode to provide a more rugged instrument with some loss in sensitivity. This could be offset by increasing the light throughput through the

system by using UV anti-reflection coated optics to focus light from the lamp into the fibre and by using miniature fused silica lenses in the absorption cell to improve collection efficiency.

While the sensor was designed as a portable nitrate detection system for in-situ spot checking of borehole sites it could also be used for continuous monitoring if left in place. However, field trials would have to be carried out to investigate surface fouling of any fibre or other immersed optical surfaces.

The nitrate sensor described in this work could be configured as a simple optical comparator to compare the absorption values for two different solutions i.e. one solution being a reference solution containing,for example, the maximum admissable concentration of 11.6mg N l^{-1}. This would require a sealed absorption cell containing the reference solution to be lowered down the borehole beside the active absorption cell in order to cancel out differences due to fibre attenuation, bend loss etc. A rotating mirror would alternately switch the light signals from the reference and absorption cells into the detector circuitry and the output of the system would simply be an alarm if the water under test had an absorbance factor greater than the reference solution.

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APPENDIX A

Step Index Optical Fibre

A diagram showing a cross section of a step index optical fibre is shown in fig. A1. The fibre consists of a central light guiding portion called the core surrounded by a cylindrical region called the cladding. This in turn is surrounded by an outer protective coating called the jacket.

The refractive index of the cladding, n_{cl} is less than that for the core, n_{core}.Light striking the cladding from the core will be totally internally reflected if the angle of incidence is greater than the critical angle ,θ_c.The critical angle occurs at the angle of incidence at which the transmitted ray is refracted along the surface of the core/cladding interface. The critical angle is found from :

$$\sin(\theta_c)= n_{cl}/n_{core} \qquad \text{Eq.A1}$$

Because the refractive index of the core is a constant and the index changes abruptly at the core/cladding interface, the type of fibre shown in fig. A1 is known as a step index fibre.

Equation A1 can be used to find the size of the cone of light that will be accepted by an optical fibre.In fig. A1 a ray is drawn that is incident on the core/cladding interface at the critical angle. If the cone angle is θmax then by Snells law :

$$n_i\sin\theta_{max} = n_{core}\sin\theta_t$$

$$= n_{core}\sin(90^0-\theta_c)$$

$$= n_{core}\cos(\theta_c)$$

$$= n_{core}[1-\sin^2(\theta_c)]^{1/2}$$

From Eq. A1 $\sin(\theta_c) = n_{cl}/n_{core}$, therefore

$$n_i\sin\theta_{max} = (n_{core}^2 - n_{cl}^2)^{1/2} \qquad \text{Eq. A2}$$

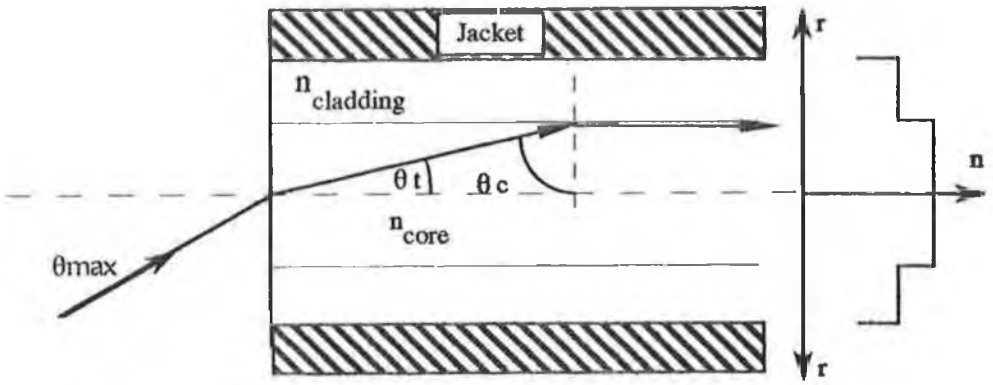


Fig. A1 Step Index fibre

The numerical aperture,NA, is a measure of how much light can be collected by an optical system, whether it is an optical fibre or photographic lens. It is the product of the refractive index of the incident medium and the sine of the maximum ray angle.
In most cases, the light is incident from air and $n_i = 1$. In this case the numerical aperture of a step index fibre from Eq. A2 and Eq.A3 is given by :

$$NA = (n_{core}^2 - n_{cl}^2)^{1/2} \qquad \text{Eq. A3}$$

In the general case where the incident medium may be a substance other than air the numerical aperture is given by :

$$NA = n_i \sin(\theta_{max}) \qquad \text{Eq. A4}$$

Appendix B

The Beer-Lambert Law

The radiant power of a beam of radiation is proportional to the number of photons per unit time. Absorption occurs when a photon collides with a molecule and raises that molecule to an excited state. Each molecule can be thought of as having a cross sectional area for photon capture, and photons must pass within this area to interact with the molecule. The cross-sectional area varies with wavelength and represents in effect, a probability that photons will be captured by any given molecule. The rate of absorption as a beam of photons passes through a medium depends on the number of photon collisions with absorbing atoms or molecules per unit time. If the number of absorbing molecules is doubled, by doubling either the path length of the radiation through the medium or the concentration of the absorbing species, the rate of absorption doubles. Likewise, doubling the beam power doubles the number of photons that pass through the medium in unit time. This doubles the number of collisions with absorbing molecules per unit time when the number of absorbing molecules remains constant.

If a parallel beam of monochromatic radiation of radiant power P_o, traverses an infinitesimally small distance dx, of an absorber, the decrease in power, -dP, is given by equation B1 since the number of absorbing species is proportional to the thickness but independent of P :

$$-dP = k' P\, dx \qquad \text{Eq. B1}$$

The proportionality constant, k', depends on the wavelength of the radiation. The concentration , C, is assumed to remain constant. Separating the variables in Eq. B1 gives:

$$\frac{-dP}{P} = -d(\ln P) = k'\, dx \qquad \text{Eq. B2}$$

which is a mathematical statement of the fact that the radiant power absorbed is proportional to the thickness traversed. Now if P_o is the radiant power at x = 0 and P represents the radiant power of the transmitted (unabsorbed) radiation that emerges from

the medium at x = b, then Eq. B2 can be integrated along the entire path :

$$-\int_{Po}^{P} d\ln P = k' \int_{0}^{b} dx \qquad \text{Eq. B3}$$

$$\ln P_0 - \ln P = \ln \left(\frac{P_0}{P}\right) = k'b \qquad \text{Eq.B4}$$

Equation B4 is known as Lambert's law and it states that for parallel, monochromatic radiation that passes through an absorber of constant concentration, the radiant power decreases logarithmically as the path length increases arithmetically.

The dependence of radiant power on the concentration of absorbing species can be developed in a similar manner if the wavelength and path length of the beam through the sample remain constant. In this case the number of absorbing molecules that collide with photons is proportional to the concentration, C. This can be expressed as :

$$-dP = k''P\, dC \qquad \text{Eq. B5}$$

Separation of variables followed by integration from C = 0 to C = C yields

$$\ln \frac{P_0}{P} = k'' C \qquad \text{Eq.B6}$$

This relationship is known as Beer's law. If both concentration and thickness are variable, the combined Beer - Lambert law is given by :

$$\ln \frac{P_0}{P} = k\, b\, C \qquad \text{Eq. B7}$$

Replacing natural logarithms by base 10 logarithms and calling the new constant a gives :

$$\log \frac{P_0}{P} = a\, b\, C \qquad \text{Eq. B8}$$

Absorbance A is defined as :

$$A = \log \frac{P_0}{P} = a\ b\ C \qquad \text{Eq. B9}$$

Transmittance, T is given by :

$$T = \frac{P}{P_0} \qquad \text{Eq. B10}$$

therefore

$$A = \log \frac{1}{T} = -\log T \qquad \text{Eq. B11}$$

The proportionality constant (a) in equations B8 and B9 is known as the absorptivity if C is given in grams of absorbing material per litre and b is in centimeters. Therefore (a) has the units litre g^{-1} cm^{-1}. If concentration is expressed in molar concentration and b in centimeters the proportionality constant is called the molar absorptivity and is designated as ε. Thus the Beer - Lambert law can also be written as :

$$A = \log \frac{P_0}{P} = \varepsilon b\ C \qquad \text{Eq. B12}$$

where ε is in units of litre $mole^{-1}$ cm^{-1}.

A plot of absorbance versus concentration should be a straight line passing through the origin. For this reason, absorption of radiation by molecules at specific wavelengths is frequently used for quantitative analysis.

The bulk of the derivation given above is taken from Fundamentals of Analytical Chemistry by Skoog, West and Holler 1986.

APPENDIX C

Circuit Diagrams

The following pages contain circuit diagrams of:

Fig. C1 The mains wiring diagram used in the experimental and prototype systems.

Fig. C2 The dual 12v power supply used in the experimental and prototype systems.

Fig. C3 The +5v supply used in the experimental and prototype systems.

Fig. C4 The -High Voltage supply used to bias the photomultiplier tube.

Fig. C5 The Bytronic Associates Ltd. interface card for the BBC microcomputer.

Fig. C6 The stepper motor driver circuit.

Fig. C7 The 24 bit counter circuit.

Fig. C8 The shutter circuit.

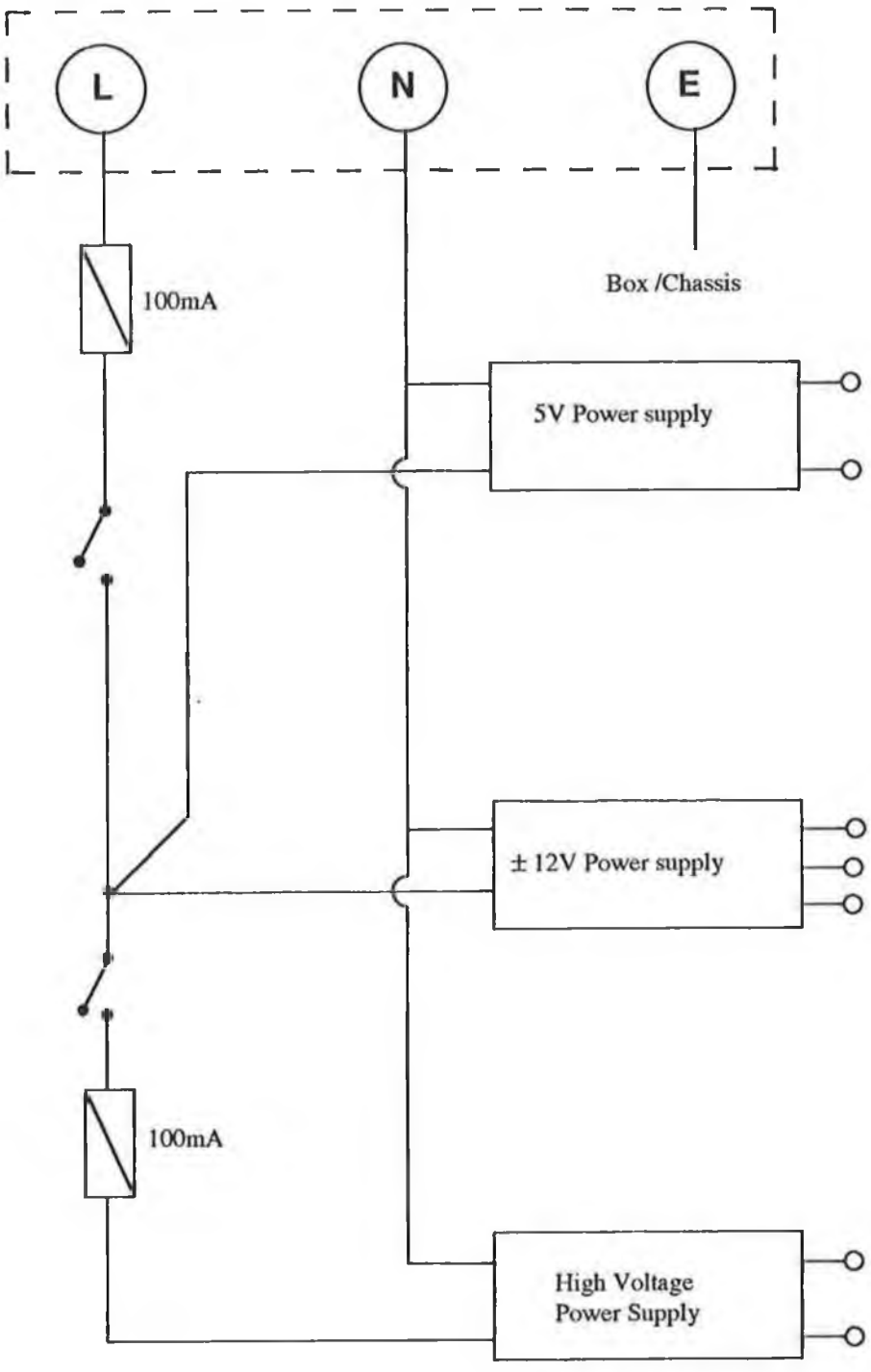


Fig. C.1 Mains Wiring Diagram for Photon Counter

Fig. C2 ±12V POWER SUPPLY

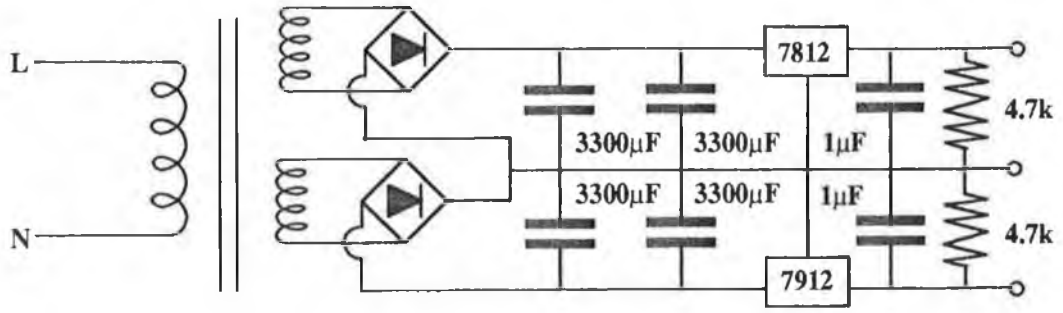


Fig. C3 5V POWER SUPPLY

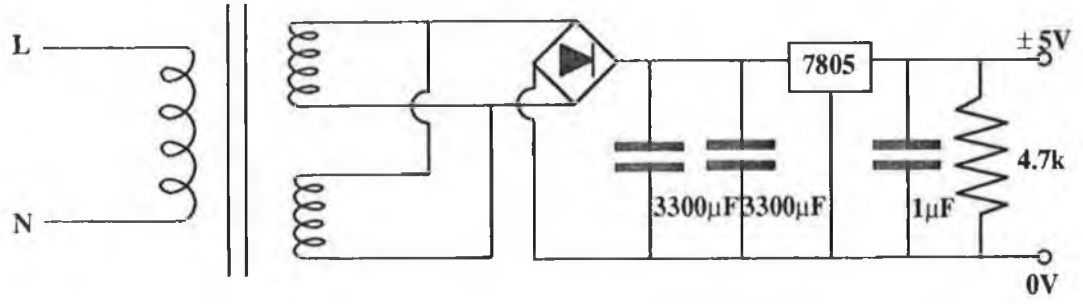
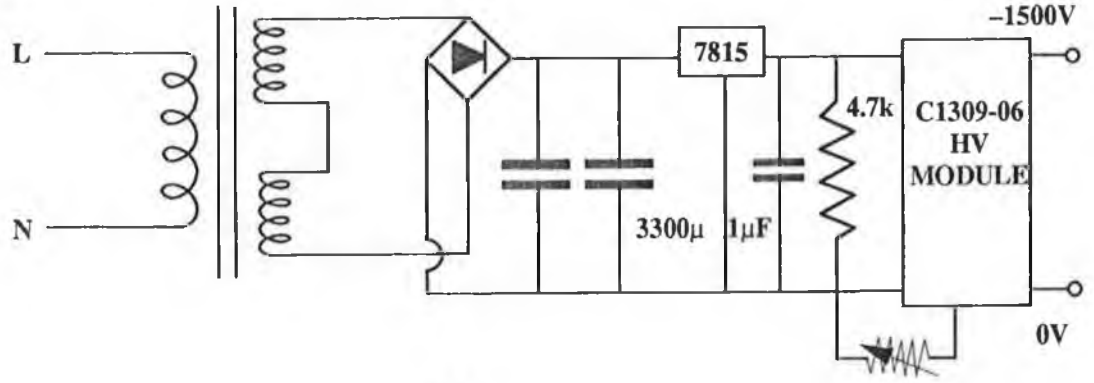


Fig. C4 HIGH VOLTAGE POWER SUPPLY



C4

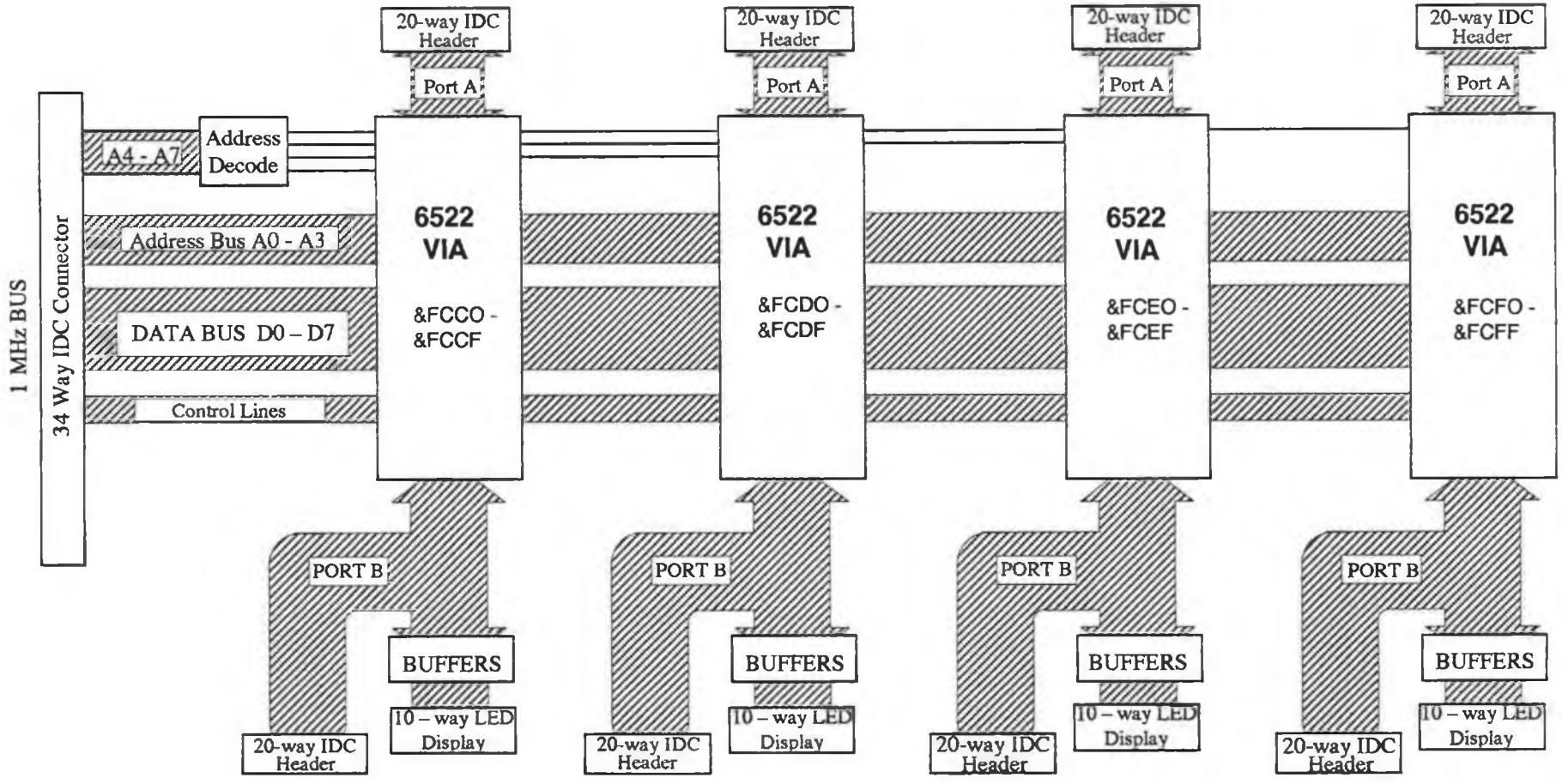


Fig. C5 BYTRONIC BBC MICRO DIGITAL I/O BOARD

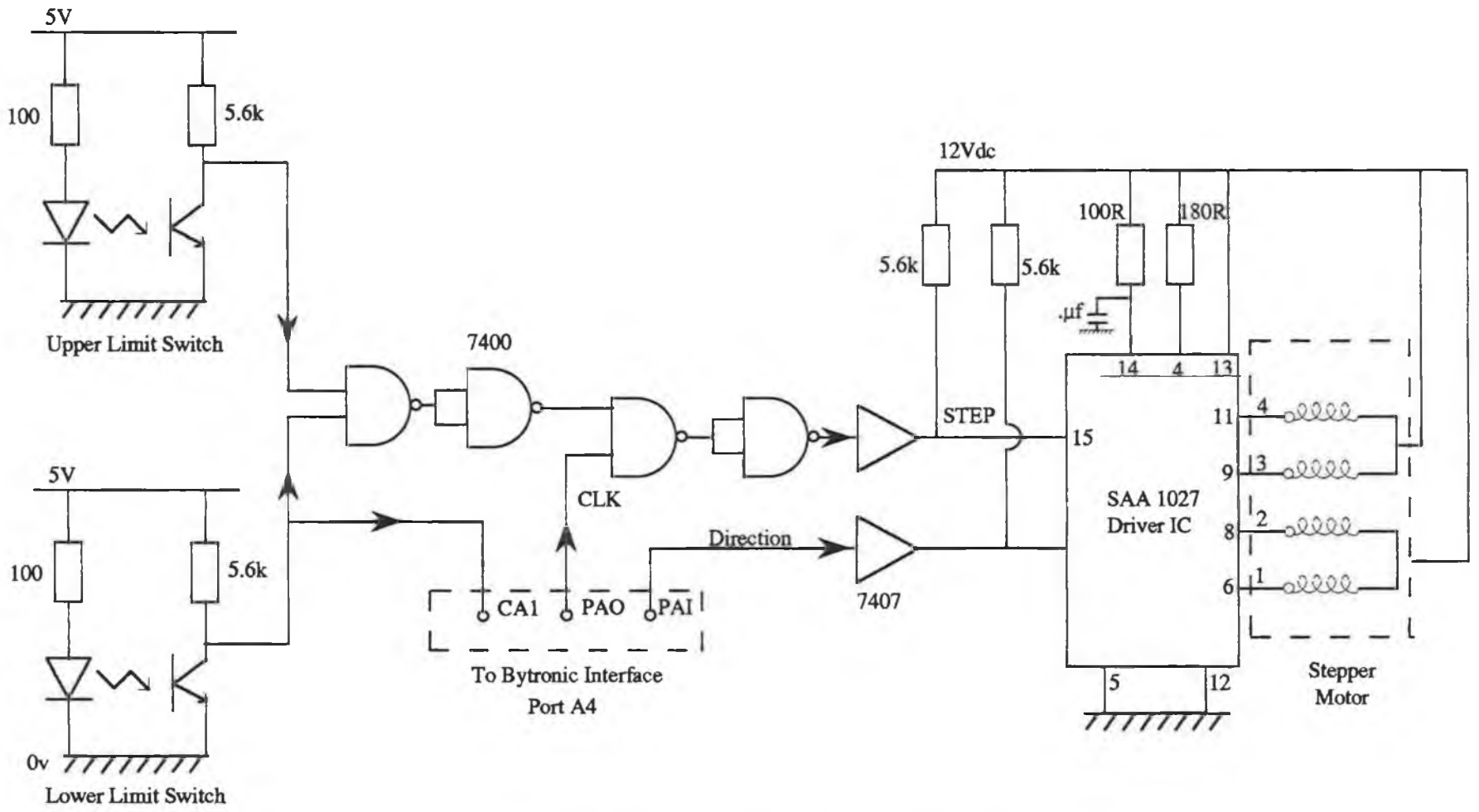


Fig. C6 Stepper Motor Driver Circuit

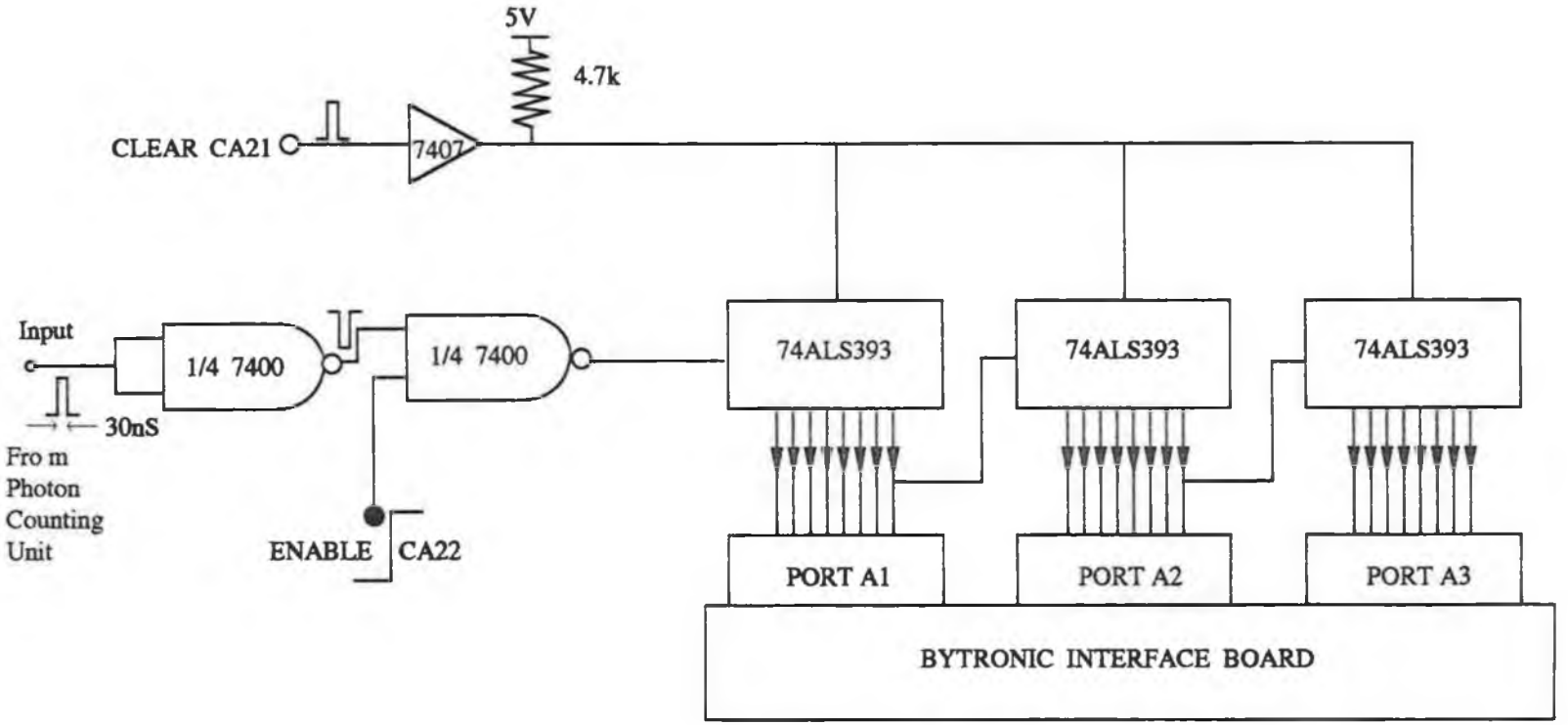


Fig. C7 Counter Circuit (24 BIT)

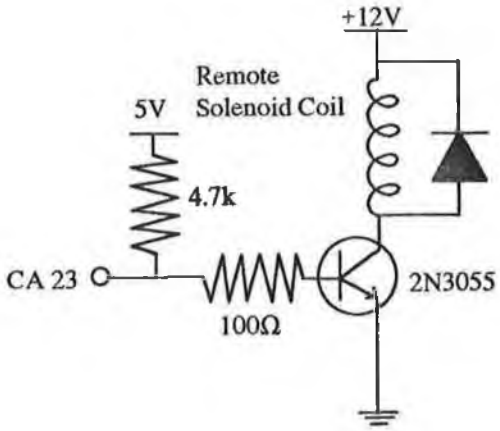


Fig. C8 SHUTTER CIRCUIT

APPENDIX D

Software Programs

The following pages contain all the software written for this project in BASIC.
The programs Count1J, Absorb and Graphm were used with the experimental system to investigate absorbance dependency on wavelength. The programs Getdat2 and Analyse6 were also used with the experimental system to investigate dual wavelength referencing.Finally, the program Filt6F was used with the prototype system to provide data acquisition and analysis functions.

```
>L.
  10 PROCSETUP
  20 PROCCALIBRATE
  30 PROCSTARTWAVE
  40 PROCNANOMETRE
  50 PROCSCAN
  60 PROCRESULT
  70 PROCFILE
  80 END
  90 REM PHOTON COUNTING PROGRAM WITH ADC VERSION 1J
 100 REM INITIAL SET UP PORTB1,PORTB2 AS O/P  PORTB3,PORTB4 AS
INPUTS
 110 *********************************
 120 DEF PROCSETUP
 130 ?&FCC2=&FF :?&FCD2=&FF :?&FCE2=&00 :?&FCF2=&00
 140 ?&FCC0=0:?&FCD0=0
 150 REM SET UP PORT A'S AS INPUTS EXCEPT A4
 160 ?&FCC3=0:?&FCD3=0:?&FCE3=0:?&FCF3=255
 170 REM SET UP PORT A4 AS ZERO INITIALLY
 180 ?&FCF1=0
 190  INPUT "TIME YOU WISH TO COUNT FOR IN SECONDS", T
 200  T=T*100
 210 INPUT "NO. OF COUNTING SAMPLES",N
 220 DIM A(N)
 230 REM ARRAY FOR STORING SIGNAL S
 240 DIM B(N)
 250 REM ARRAY FOR STORING ADC VALUES
 260 INPUT "START WAVELENGTH SW",SW
 270 INPUT "FINISH WAVELENGTH FW",FW
 280 INPUT "WAVELENGTH INCREMENT WI",WI
 290 LET DP = (FW-SW)/WI
 300 REM STORE AVERAGE VALUES
 310 DIM C(DP)
 320 REM STORE ERRORS
 330 DIM D(DP)
 340 DIM N(DP)
 350 REM STORE FOR NANOMETRE SCALE
 360 ENDPROC
 370 ********************************************************
 380 DEF PROCLOOP
 390 REM TO SAMPLE N TIMES AT EACH WAVELENGTH
 400 FOR J=1 TO N
 410 PROCSB
 420 PROCBACKGROUND
 430 PROCADC
 440 PROCSIGNAL
 450 NEXT J
 460 ENDPROC
 470 ********************************************************
*************
 480 DEF PROCSB
 490 REM SAMPLING SIGNAL PLUS BACKGROUND
 500 REM START WITH ALL CONTROL LINES LOW EXCEPT CB24
 510 ?&FCCC=&CC:?&FCDC=&CC:?&FCEC=&CC
 520 REM OPEN APERTURE TO ADMIT SIGNAL (CA23)
 530 ?&FCEC=&CC
 540 REM CLEAR COUNTER BRING CA21 HIGH THEN LOW AGAIN
 550 ?&FCCC=&EE:?&FCCC=&CC
 560 REM TO ENABLE INPUT TO COUNTER SET CA22 HIGH
 570 ?&FCDC=&EE
 580 REM COUNT FOR A PRESET TIME  DELAY1
 590 TIME =0
 600 REPEAT : UNTIL TIME=T
 610 REM TO DISABLE INPUT TO COUNTER SET CA22 LOW
 620  ?&FCDC=&CC
```

D1 Program Count1J

```
  630 REM CLOSE APERTURE (CA23)
  640 ?&FCEC=&EE
  650 REM READ COUNTERS
  660 SB=?&FCC1+(?&FCD1*256)+(?&FCE1*65536)
  670 PRINT
  680 PRINT "SIGNAL+BACKGROUND",SB
  690 ENDPROC
  700*************************************************************
*
  710 DEF PROCBACKGROUND
  720 REM SAMPLING BACKGROUND ONLY
  730 REM CLEAR COUNTERS
  740 ?&FCCC=&EE:?&FCCC=&CC
  750 REM ENABLE COUNTERS
  760 ?&FCDC=&EE
  770 REM COUNTING BACKGROUND ONLY DELAY1
  780 TIME =0
  790 REPEAT:UNTIL TIME =T
  800 REM DISABLE COUNTERS
  810 ?&FCDC=&CC
  820 REM READ COUNTERS
  830 B=?&FCC1+(?&FCD1*256)+(?&FCE1*65536)
  840 PRINT "BACKGROUND COUNT",B
  850 ENDPROC
  860*************************************************************
************
  870 DEF PROCSIGNAL
  880 REM CALCULATING AND STORING SIGNAL COUNT
  890 S=(SB-B)
  900 PRINT "SIGNAL COUNT",S
  910 PRINT "ADC VALUE    ",ADV
  920  A(J)=S
  930 ENDPROC
  940*************************************************************
**********
  950 DEF PROCPROCESS
  960 REM SIGNAL PROCESSING
  970 REM CALCULATING AVERAGE SIGNAL VALUE
  980 SUM=0
  990 FOR I=1 TO N
 1000 SUM=SUM+A(I)
 1010 NEXT I
 1020 AV=SUM/N
 1030 REM STORE AVERAGE SIGNALS IN ARRAY
 1040 C(X)=AV
 1050 REM CALCULATING COUNT ERROR
 1060 E=SQR(AV/N)
 1070 D(X)=E
 1080 PRINT
 1090 PRINT
 1100 PRINT"AVERAGE SIGNAL COUNT AT "NM "NM",AV, "+-" E
 1110 REM TO CALCULATE AVERAGE ADC VALUE
 1120  SUMA=0
 1130 FOR I=1 TO N
 1140 SUMA=SUMA+B(I)
 1150 NEXT I
 1160 MEAN=SUMA/N
 1170 PRINT "MEAN ADC VALUE",MEAN
 1180 ENDPROC
 1190*************************************************************
********
 1200 DEF PROCSTP
 1210 REM 8 STEPS PER NANOMETER
 1220 STP=WI*8
 1230 FOR I=1 TO STP
 1240 ?&FCF1=3
```

```
 1250 PROCDELAY
 1260 ?&FCF1=2
 1270 PROCDELAY
 1280 NEXT I
 1290 ENDPROC
 1300 ************************************************************
************
 1310 DEF PROCDELAY
 1320 FOR Q=1 TO 50
 1330 NEXT Q
 1340 ENDPROC
 1350 ************************************************************
************
 1360 DEF PROCSCAN
 1370 NM=SW-WI
 1380 FOR X=1 TO DP
 1390 NM=NM+WI
 1400 PRINT "NM" NM
 1410 PROCLOOP
 1420 PROCPROCESS
 1430 PROCSTP
 1440 NEXT X
 1450 ENDPROC
 1460************************************************************
************
 1470 DEF PROCRESULT
 1480 NM=SW-WI
 1490 FOR R=1 TO DP
 1500 NM=NM+WI
 1510 PRINT NM "NM" C(R),"+-"D(R)
 1520 NEXT R
 1530 ENDPROC
 1540 REM************************************************************
************
 1550 DEF PROCFILE
 1560 PRINT "DO YOU WISH TO SAVE DATA?"
 1570 A$=GET$
 1580 IF A$= "Y" THEN 1590 ELSE 1660
 1590 PRINT
 1600 INPUT "FILENAME" B$
 1610 V=OPENOUT B$
 1620 FOR X=1 TO DP
 1630 PRINT £V,N(X) ,C(X)
 1640 NEXT X
 1650 CLOSE £V
 1660 PRINT "DO YOU WISH TO READ A FILE?"
 1670 F$=GET$
 1680 IF F$= "Y" THEN 1690 ELSE END
 1690 INPUT "FILENAME" Z$
 1700 U=OPENIN Z$
 1710 REPEAT
 1720 INPUT £U,N,Z
 1730 PRINT N,Z
 1740 UNTIL EOF£U
 1750 CLOSE £U
 1760 ENDPROC
 1770 REM ************************************************************
***************
 1780 DEF PROCNANOMETRE
 1790 NM=SW-WI
 1800 FOR G=1 TO DP
 1810 NM=NM+WI
 1820 N(G)=NM
 1830 NEXT G
 1840 ENDPROC
 1850************************************************************
```

```
*
 1860DEF PROCCALIBRATE
 1870 REM GO TO LOW END STOP
 1880 ?&FCF1=1
 1890 PROCDELAY
 19007&FCF1=0
 1910 PROCDELAY
 1920 PRINT ?&FCFD,"?&FCFD"
 1930 IF ( ?&FCFD AND 2)=0 THEN 1870 ELSE 1940
 1940 LET STPCOUNT=0
 1950 LET MAXSTEPCOUNT=0
 1960 LET MAXSB=0
 1970 REM MOVE IN POSITIVE DIRECTION TO FIND ZERO ORDER
 1980 FOR L=1 TO 200
 1990 ?&FCF1=3
 2000 PROCDELAY
 2010 ?&FCF1=2
 2020 PROCDELAY
 2030 PROCSB
 2040 REM COMPARE SIGNAL VALUES
 2050 STPCOUNT=STPCOUNT+1
 2060 IF SB > MAXSB THEN MAXSTEPCOUNT=STPCOUNT
 2070 IF SB > MAXSB THEN MAXSB=SB
 2080 PRINT "SB",SB
 2090 PRINT "STEPCOUNT",STPCOUNT
 2100 PRINT "MAXSTEPCOUNT",MAXSTEPCOUNT
 2110 PRINT "MAXSB",MAXSB
 2120 FOR I=1 TO 200:NEXT I
 2130 NEXT L
 2140 ZER=200-MAXSTEPCOUNT
 2150 PRINT "ZER", ZER
 2160 ENDPROC
 2170 ************************************************************
*********
 2180 DEF PROCPOSDIR
 2190 ?&FCF1=3
 2200 PROCDELAY
 2210 ?&FCF1=2
 2220 PROCDELAY
 2230 ENDPROC
 2240 ************************************************************
********
 2250 DEF PROCNEGDIR
 2260 ?&FCF1=1
 2270 PROCDELAY
 2280 ?&FCF1=0
 2290 PROCDELAY
 2300 ENDPROC
 2310************************************************************
********
 2320 DEF PROCSTARTWAVE
 2330 PRINT "PAUSE"
 2340 FOR JJ=1 TO 2000:NEXT JJ
 2350 REM TO MOVE FROM ZERO ORDER TO START WAVELENGTH
 2360 LET NOS=(SW*8)-ZER
 2370 FOR XX=1 TO NOS
 2380 PROCPOSDIR
 2390 NEXT XX
 2400 ENDPROC
 2410 ************************************************************
***********
>
```

```
 >VDU3
L.
   10 DIMY(200)
   20 DIM H(200)
   30 DIM R(200)
   40 DIM SC(200)
   50 DIM W(200)
   60 DIM L(200)
   70 CLS
   80 PROCGETFILE
   90 PROCGETANOTHERFILE
  100 PROCCHECK
  110 PROCDIVIDE
  120 PROCSCALE
  130 PROCADJUST
  140 PROCABSORB
  150 PROCNEWFILE
  160 PROCREADFILE
  170 END
  180 REM THIS PROGRAM READS A DATAFILE AND STORES CONTENTS IN
ARRAY FOR FURTHER PROCESSING
  190 DEF PROCGETFILE
  200 P=0
  210 PRINT "DO YOU WISH TO READ A FILE?"
  220 F$=GET$
  230IF F$= "Y" THEN 240 ELSE END
  240 *CAT
  250 INPUT "FILENAME" Z$
  260 U=OPENIN Z$
  270 REPEAT
  280 INPUT £U,N,Z
  290 Y(P)=Z
  300 P=P+1
  310 UNTIL EOF£U
  320 CLOSE £U
  330 PRINT P
  340 ENDPROC
  350 DEF PROCGETANOTHERFILE
  360  Q=0
  370 PRINT "DO YOU WISH TO READ ANOTHER FILE?"
  380 F$=GET$
  390 IF F$= "Y" THEN 400 ELSE END
  400 INPUT "FILENAME" Z$
  410 T=OPENIN Z$
  420 REPEAT
  430 INPUT £T,N,Z
  440 H(Q)=Z
  450 SC(Q)=N
  460  Q=Q+1
  470 UNTIL EOF£T
  480 CLOSE £T
  490 PRINT Q
  500 ENDPROC
  510 DEF PROCCHECK
  520 IF P=Q THEN  PRINT "OK" ELSE PRINT "FILES ARE NOT SAME SI
ZE"
  530 ENDPROC
  540 DEF PROCDIVIDE
  550 FOR I=0 TO Q-1
  560 LET RES=Y(I)/H(I)
  570 R(I)=RES
  580 PRINT SC(I), R(I)
  590 NEXT I
  600 ENDPROC
  610 DEF PROCNEWFILE
```

D2 Program Absorb

```
 620 INPUT "NEW FILE NAME" B$
 630 V=OPENOUT B$
 640 FOR X=1 TO Q-1
 650 PRINT £V,SC(X),L(X)
 660 NEXT X
 670 CLOSE£V
 680 ENDPROC
 690 DEF PROCREADFILE
 700 PRINT "DO YOU WISH TO READ A FILE?"
 710 F$=GET$
 720 IF F$="Y" THEN 730 ELSE END
 730 INPUT "FILENAME" Z$
 740 U=OPENIN Z$
 750 REPEAT
 760 INPUT£U,SC,L
 770 PRINT SC,L
 780 UNTIL EOF £U
 790 CLOSE£U
 800 ENDPROC
 810 DEF PROCSCALE
 820 MAX =0
 830 MAX1=0
 840 FOR I=1 TO (Q-1)
 850 PRINT "I",I
 860 IF R(I)>R(I-1) THEN MAX=R(I) ELSE MAX=MAX
 870 IF MAX>MAX1 THEN MAX1=MAX
 880 NEXT I
 890 PRINT "MAX NO.", MAX1
 900 ENDPROC
 910 DEF PROCADJUST
 920 FOR J=1 TO (Q-1)
 930 LET SVAL= R(J)/MAX1
 940 PRINT SVAL
 950 W(J)=SVAL
 960 NEXT J
 970 ENDPROC
 980 DEF PROCABSORB
 990 FOR J=1 TO (Q-1)
1000 LET AB=LOG(1/W(J))
1010 PRINT AB
1020 L(J)=AB
1030 NEXT J
1040 ENDPROC
>
```

```
 LEscape
>>L.
   10CLS
   20MODE 0
   30PRINT TAB(24,4),"General graph ploting program"
   40PRINT TAB(28,6),"Jules Braddell AP4"
   45 PRINT TAB(28,8) "MODIFICATIONS BY JOE MAXWELL 1989"
   50PRINT:*CAT
   60INPUT TAB(24,30),"Name of input data file";NAME$
   70CLS
   90Blank$="
"
  100PRINT TAB(0,15),Blank$
  110PRINT TAB(24,15),"Select scaling option for axes"
  120PRINT TAB(14,17),"1  - User selection of scaling"
  130PRINT TAB(14,18),"2  - Autoscaling 0 -> X max , 0 -> Y max
 "
  140PRINT TAB(14,19),"3  - Autoscaling X min -> X max , Y min
-> Y max "
  145PRINT TAB(14,20),"4  - Autoscaling X min -> X max , Y=-4.6
 -> 4.6"
  150PRINT TAB(40,22),"          "
  160INPUT TAB(30,22)," 1 ,2 ,3 or 4",Select1
  170IF Select1=1 OR Select1=2 OR Select1=3 OR Select1=4 ELSE G
OTO 150
  180ON Select1 GOTO 190,330,370,361
  190REM Selection 1, User selects axes scaling
  200PRINT TAB(24,15),Blank$
  210PRINT TAB(14,17),Blank$
  220 PRINT TAB(14,18),Blank$
  230PRINT TAB(14,19),Blank$
  240PRINT TAB(30,22),Blank$
  245 PRINT TAB(14,20),Blank$
  250INPUT TAB(30,15),"Minimum X value",Xmin
  260PRINT TAB(46,15),"          "
  270INPUT TAB(30,15),"Maximum X value",Xmax
  280PRINT TAB(46,15),"          "
  290INPUT TAB(30,15),"Minimum Y value",Ymin
  300PRINT TAB(46,15),"          "
  310INPUT TAB(30,15),"Maximum Y value",Ymax
  320GOTO 380
  330
  340PROCAutoscaling
  350Ymin=0:Xmin=0
  360GOTO 380
  361 PROCAutoscaling
  362 Ymin=-4.6
  363 Ymax=4.6
  365 GOTO 380
  370PROCAutoscaling
  380
  390PRINT TAB(24,15),Blank$
  400PRINT TAB(14,17),Blank$
  410 PRINT TAB(14,18),Blank$
  420PRINT TAB(14,19),Blank$
  430PRINT TAB(30,21),Blank$
  435PRINT TAB(14,20),Blank$
  436PRINT TAB(30,22),Blank$
  440PRINT TAB(24,15),"Select display format"
  450PRINT TAB(14,17),"1  - Line from the previous data point"
  460PRINT TAB(14,18),"2  - Pixel at point position"
  470PRINT TAB(35,20),"          "
  480INPUT TAB(30,20)," 1 or 2",Select2
  490IF Select2=1 OR Select2=2 ELSE GOTO 470
  500 IF Select2=1 THEN Type=5 ELSE Type=69
  510 CLS
```

D3 Program Graphm

```
 520 B = OPENIN(NAME$)
 700 MOVE 150,123
 710 DRAW 150,923
 720 MOVE 150,123
 730 DRAW 1250,123
 740 FOR X=150 TO 1250 STEP 220
 750   MOVE X,123
 760   DRAW X,108
 770 NEXT X
 780 FOR Y=123 TO 923 STEP 160
 790   MOVE 150,Y
 800   DRAW 135,Y
 810 NEXT Y
 820 @%=&0200
 830 StepSize = (Xmax-Xmin)/5
 840 X=6
 850 FOR I=0 TO 4
 860   Lable = Xmin + (StepSize*I)
 870   PRINT TAB(X,29),Lable
 880   X = X+14
 890 NEXT I
 900 PRINT TAB(75,29),Xmax
 910 Y=28
 920 StepSize = (Ymax-Ymin)/5
 930 FOR I=0 TO 5
 940   Lable = Ymin + (StepSize*I)
 950   PRINT TAB(2,Y),Lable
 960   Y = Y-5
 970 NEXT I
 980 @%=&10
 990 VDU 24,150;123;1250;923;
1000 LenX = Xmax-Xmin
1010 LenY = Ymax-Ymin
1020 INPUT £B,X
1030 XPos = (((X-Xmin)/LenX)*1100) + 150
1040 INPUT £B,Y
1050 YPos = (((Y-Ymin)/LenY)*800) + 123
1060 MOVE XPos,YPos
1070 REPEAT
1080   INPUT £B,X
1090   XPos = (((X-Xmin)/LenX)*1100) + 150
1100   INPUT £B,Y
1110   YPos = (((Y-Ymin)/LenY)*800) + 123
1120   PLOT Type,XPos,YPos
1130 UNTIL EOF £B
1140 VDU 26
1150 INPUT TAB(4,30),"Rikadenki (Y/N)",Ans2$
1160 IF Ans2$="Y" THEN PROCRikadenki
1170 INPUT TAB(4,30),"Screendump (Y/N)",Ans2$
1180PRINT TAB(4,30),"                    "
1190 REMCLS
1205 *DRIVE0
1210 IF Ans2$="Y" THEN CHAIN"VERTIGO"
1220 INPUT TAB(4,30),"Plot another (Y/N)",Ans2$
1230 IF Ans2$="Y" THEN GOTO 10
1240 INPUT TAB(4,30),"Main program  (Y/N)",Ans2$
1250 IF Ans2$="Y" THEN CHAIN"FRENZY"
1260 END
1270 DEF PROCAutoscaling
1280 A=OPENIN(NAME$)
1290 FOR I%=1 TO 4  : REM Overide title and other strings
1310 NEXT I%
1320 INPUT £A,X
1330 INPUT £A,Y
1340 Xmax=X:Xmin=X
1350 Ymax=Y:Ymin=Y
```

```
1360 REPEAT
1370  INPUT £A,X
1380  INPUT £A,Y
1390  IF X>Xmax THEN Xmax=X
1400  IF X<Xmin THEN Xmin=X
1410  IF Y>Ymax THEN Ymax=Y
1420  IF Y<Ymin THEN Ymin=Y
1430 UNTIL EOF£A
1440 CLOSE £A
1450 ENDPROC
1460 DEF PROCRikadenki
1470 C = OPENIN (NAME$)
1480 CLS
1490 VDU 2,21
1500 PRINT "HS5QO"
1510 INPUT £C, Title$
1520 X = INT((83-LEN(Title$))*15)
1530 PRINT "M",X,",1755"
1540 PRINT "P" + Title$
1550 PRINT "M",X,",1754"
1560 X1=LEN(Title$)*30
1570 PRINT "M",X+2,",1755"
1580 PRINT "P" + Title$
1590 INPUT £C, Info$
1600 X = INT((104-LEN(Info$))*12)
1610 PRINT "M",X,",1685"
1620 PRINT "S4"
1630 PRINT "P" + Info$
1640 PRINT "S5"
1650 INPUT £C, XAxis$
1660 X = INT((83-LEN(XAxis$))*15)
1670 PRINT "M",X,",0"
1680 PRINT "P" + XAxis$
1690 INPUT £C, YAxis$
1700 Y = INT((60-LEN(YAxis$))*15)
1710 PRINT "Q1"
1720 PRINT "M50,",Y
1730 PRINT "P" + YAxis$
1740 PRINT "HQO"
1750 PRINT "M130,130"
1760 PRINT "X1,464,5"
1770 PRINT "M130,130"
1780 PRINT "X0,310,5"
1790 PRINT "S3"
1800 PRINT "M130,88"
1810 @%=&0200
1820 PRINT "P",Xmin
1830 @%=&10
1840 Stepsize = (Xmax-Xmin)/5
1850 X=85
1860 FOR I%=1 TO 5
1870   X = X+464
1880   Lable = Xmin +  (Stepsize*I%)
1890   PRINT "M",X,",88"
1900   @%=&0200
1910   PRINT "P",Lable
1920   @%=&10
1930 NEXT I%
1940 PRINT "M112,130"
1950 PRINT "Q1"
1960 @%=&0200
1970 PRINT "P",Ymin
1980 @%=&10
1990Stepsize=(Ymax-Ymin)/5
2000 Y=85
2010 FOR I%=1 TO 5
```

```
 2020    Y = Y+310
 2030    Lable = Ymin +  (Stepsize*I%)
 2040    PRINT "M112,",Y
 2050    @%=&0200
 2060    PRINT "P",Lable
 2070    @%=&10
 2080 NEXT I%
 2090 REPEAT
 2100INPUT £C,X
 2110INPUT £C,Y
 2120UNTIL X>=Xmin OR X<=Xmax OR Y>=Ymin OR Y<=Ymax
 2130XPos=INT((((X-Xmin)/LenX)*2320) + 130)
 2140YPos=INT((((Y-Ymin)/LenY)*1550) + 123)
 2150PRINT "M",XPos,",",YPos
 2160IF Select2=2 THEN PRINT "H1"
 2170 REPEAT
 2180IF X<Xmin OR X>Xmax OR Y<Ymin OR Y>Ymax THEN GOTO 2240
 2190INPUT £C,X
 2200XPos=INT((((X-Xmin)/LenX)*2320) + 13C)
 2210INPUT £C,Y
 2220YPos = INT((((Y-Ymin)/LenY)*1550) + 130)
 2230IF Select2=1 THEN PRINT "D",XPos,",",YPos ELSE PRINT "M",X
Pos,","YPos:PRINT "N1"
 2240UNTIL EOF£C
 2250PRINT "H"
 2260VDU 6,3
 2270CLOSE £C
 2280ENDPROC
>
```

```
Escape
>>L.
   10 MODE7
   20 PROCSETUP
   30 PROCCALIBRATE
   40 PROCSTARTWAVE
   50 PROCSETARRAY
   60 PROCBIGLOOP1
   70 PROCFILE2
   80 END
   90 **********************************
  100 REM INITIAL SET UP PORTB1,PORTB2 AS O/P  PORTB3,PORTB4 AS
 INPUTS
  110 DEF PROCSETUP
  120 ?&FCC2=&FF :?&FCD2=&FF :?&FCE2=&00 :?&FCF2=&00
  130 ?&FCC0=0:?&FCD0=0
  140 REM SET UP PORT A'S AS INPUTS EXCEPT A4
  150 ?&FCC3=0:?&FCD3=0:?&FCE3=0:?&FCF3=255
  160 REM SET UP PORT A4 AS ZERO INITIALLY
  170 ?&FCF1=0
  180 INPUT" NO. OF TIMES YOU WISH TO CALCULATE ABSORBANCE", BB

  190  INPUT "TIME YOU WISH TO COUNT FOR IN SECONDS", T
  200  T=T*100
  210 INPUT "ABSORBING WAVELENGTH SW",SW
  220 INPUT "REFERENCE WAVELENGTH FW",FW
  230 ENDPROC
  240 ************************************************************
  250 DEF PROCLOOPA
  260 PROCSB
  270 PROCBACKGROUND
  280 PROCSIGNAL
  290 SBA(AA)=SB
  300 BA(AA)=B
  310 SA(AA)=S
  320 ENDPROC
  330 ************************************************************
*************
  340 DEF PROCSB
  350 REM SAMPLING SIGNAL PLUS BACKGROUND
  360 REM START WITH ALL CONTROL LINES LOW EXCEPT CB24
  370 ?&FCCC=&CC:?&FCDC=&CC:?&FCEC=&CC
  380 REM OPEN APERTURE TO ADMIT SIGNAL (CA23)
  390 ?&FCEC=&CC
  400 REM CLEAR COUNTER BRING CA21 HIGH THEN LOW AGAIN
  410 ?&FCCC=&EE:?&FCCC=&CC
  420 REM TO ENABLE INPUT TO COUNTER SET CA22 HIGH
  430 ?&FCDC=&EE
  440 REM COUNT FOR A PRESET TIME  DELAY1
  450 TIME =0
  460 REPEAT : UNTIL TIME=T
  470 REM TO DISABLE INPUT TO COUNTER SET CA22 LOW
  480  ?&FCDC=&CC
  490 REM CLOSE APERTURE (CA23)
  500 ?&FCEC=&EE
  510 REM READ COUNTERS
  520 SB=?&FCC1+(?&FCD1*256)+(?&FCE1*65536)
  530 PRINT
  540 PRINT "SIGNAL+BACKGROUND",SB
  550 ENDPROC
  560************************************************************
*
  570 DEF PROCBACKGROUND
  580 REM SAMPLING BACKGROUND ONLY
```

D4 Program Getdat2

```
  590 REM CLEAR COUNTERS
  600 ?&FCCC=&EE:?&FCCC=&CC
  610 REM ENABLE COUNTERS
  620 ?&FCDC=&EE
  630 REM COUNTING BACKGROUND ONLY DELAY1
  640 TIME =0
  650 REPEAT:UNTIL TIME =T
  660 REM DISABLE COUNTERS
  670 ?&FCDC=&CC
  680 REM READ COUNTERS
  690 B=?&FCC1+(?&FCD1*256)+(?&FCE1*65536)
  700 PRINT "BACKGROUND COUNT",B
  710 ENDPROC
  720 ************************************************************
*******
  730 DEF PROCSIGNAL
  740 REM CALCULATING AND STORING SIGNAL COUNT
  750 S=(SB-B)
  760 PRINT "SIGNAL COUNT",S
  770 ENDPROC
  780************************************************************
**********
  790 DEF PROCSTP
  800 REM 8 STEPS PER NANOMETER
  810 STP=WI*8
  820 FOR I=1 TO STP
  830 ?&FCF1=3
  840 PROCDELAY
  850 ?&FCF1=2
  860 PROCDELAY
  870 NEXT I
  880 ENDPROC
  890 ************************************************************
***********
  900 DEF PROCDELAY
  910 FOR Q=1 TO 50
  920 NEXT Q
  930 ENDPROC
  940 ************************************************************
***********
  950 DEF PROCFILE2
  960 PRINT
  970 INPUT "TO SAVE DATA INPUT FILENAME" B$
  980 V=OPENOUT B$
  990 FOR X=1 TO BB
 1000 PRINT £V,SBA(X),BA(X),SA(X),SBR(X),BR(X),SR(X)
 1010 NEXT X
 1020 CLOSE £V
 1030 PRINT "DO YOU WISH TO READ A FILE?"
 1040 F$=GET$
 1050 IF F$= "Y" THEN 1060 ELSE END
 1060 INPUT "FILENAME" Z$
 1070 U=OPENIN Z$
 1080 REPEAT
 1090 INPUT £U,SBA,BA,SA,SBR,BR,SR
 1100 PRINT SBA,BA,SA,SBR,BR,SR
 1110 UNTIL EOF£U
 1120 CLOSE £U
 1130 CLOSE£0
 1140 ENDPROC
 1150 REM ************************************************************
**************
 1160DEF PROCCALIBRATE
 1170 REM GO TO LOW END STOP
 1180 ?&FCF1=1
 1190 PROCDELAY
```

```
 1200?&FCF1=0
 1210 PROCDELAY
 1220 PRINT ?&FCFD,"?&FCFD"
 1230 IF ( ?&FCFD AND 2)=0 THEN 1170 ELSE 1240
 1240 LET STPCOUNT=0
 1250 LET MAXSTEPCOUNT=0
 1260 LET MAXSB=0
 1270 REM MOVE IN POSITIVE DIRECTION TO FIND ZERO ORDER
 1280 FOR L=1 TO 200
 1290 ?&FCF1=3
 1300 PROCDELAY
 1310 ?&FCF1=2
 1320 PROCDELAY
 1330 PROCSB
 1340 REM COMPARE SIGNAL VALUES
 1350 STPCOUNT=STPCOUNT+1
 1360 IF SB > MAXSB THEN MAXSTEPCOUNT=STPCOUNT
 1370 IF SB > MAXSB THEN MAXSB=SB
 1380 PRINT "SB",SB
 1390 PRINT "STEPCOUNT",STPCOUNT
 1400 PRINT "MAXSTEPCOUNT",MAXSTEPCOUNT
 1410 PRINT "MAXSB",MAXSB
 1420 FOR I=1 TO 200:NEXT I
 1430 NEXT L
 1440 ZER=200-MAXSTEPCOUNT
 1450 PRINT "ZER", ZER
 1460 ENDPROC
 1470 ************************************************************
*********
 1480 DEF PROCPOSDIR
 1490 ?&FCF1=3
 1500 PROCDELAY
 1510 ?&FCF1=2
 1520 PROCDELAY
 1530 ENDPROC
 1540 ************************************************************
********
 1550 DEF PROCNEGDIR
 1560 ?&FCF1=1
 1570 PROCDELAY
 1580 ?&FCF1=0
 1590 PROCDELAY
 1600 ENDPROC
 1610************************************************************
********
 1620 DEF PROCSTARTWAVE
 1630 PRINT "PAUSE"
 1640 FOR JJ=1 TO 2000:NEXT JJ
 1650 REM TO MOVE FROM ZERO ORDER TO START WAVELENGTH
 1660 LET NOS=(SW*8)-ZER
 1670 FOR XX=1 TO NOS
 1680 PROCPOSDIR
 1690 NEXT XX
 1700 ENDPROC
 1710 ************************************************************
***********
 1720 DEF PROCPOSJUMP
 1730 LET NOSTP=FW-SW
 1740 FOR QQ=1 TO ( NOSTP*8)
 1750 PROCPOSDIR
 1760 NEXT QQ
 1770 ENDPROC
 1780 **********************************
 1790 DEF PROCNEGJUMP
 1800 LET NOSTP=FW-SW
 1810 FOR WW=1 TO (NOSTP*8)
```

```
1820 PROCNEGDIR
1830 NEXT WW
1840 ENDPROC
1850 ********************************
1860 DEF PROCBIGLOOP1
1870 FOR AA=1 TO BB
1880 PRINT AA
1890 PROCLOOPA
1900 PROCPOSJUMP
1910 PROCLOOPR
1920 PROCNEGJUMP
1930 NEXTAA
1940 ENDPROC
1950************************************
1960 DEF PROCSETARRAY
1970 DIM SBA(BB)
1980 DIM BA(BB)
1990 DIM SA(BB)
2000 DIM SBR(BB)
2010 DIM BR(BB)
2020 DIM SR(BB)
2030 ENDPROC
2040************************************
2050 DEF PROCLOOPR
2060 PROCSB
2070 PROCBACKGROUND
2080 PROCSIGNAL
2090 SBR(AA)=SB
2100 BR(AA)=B
2110 SR(AA)=S
2120 ENDPROC
2130 ************************************************************
>
```

```
>L.
   10 MODE3
   20 DIM SBA(100)
   30 DIM BA(100)
   40 DIM SA(100)
   50  DIM SBR(100)
   60  DIM BR(100)
   70  DIM SR(100)
   80 @%=&0000040A
   90 PROCREADFILE
  100 PROCAVERAGESBA
  110 PROCAVERAGEBA
  120 PROCAVERAGESA
  130 PROCAVERAGESBR
  140 PROCAVERAGEBR
  150 PROCAVERAGESR
  160 PROCAVERAGEERROR
  170 PROCABSORBANCE
  180 VDU 15
  190 END
  200 *********************************
  210 DEF PROCREADFILE
  220 DP=0
  230 PRINT "THIS PROGRAM ANALYSES DATA FROM PHOTON COUNTER"
  240 *CAT
  250 INPUT "FILENAME" Z$
  260 U=OPENIN Z$
  265 VDU2
  266 PRINT "     SBA         ,BA       ,SA       ,SBR         ,BR
     ,SR "
  267 PRINT
  270 REPEAT
  280 INPUT £U,SBA,BA,SA,SBR,BR,SR
  290 PRINT SBA,BA,SA,SBR,BR,SR
  295 PRINT
  300 REM ENTER VALUES INTO ARRAY
  310 SBA(DP)=SBA
  320 BA(DP)=BA
  330 SA(DP)=SA
  340 SBR(DP)=SBR
  350 BR(DP)=BR
  360 SR(DP)=SR
  370 DP=DP+1
  380 UNTIL EOF£U
  390 CLOSE £U
  400 CLOSE£0
  410 ENDPROC
  420 *********************************
  430 DEF PROCAVERAGESBA
  440 SUM=0
  450 FOR I=0 TO (DP-1)
  460 SUM =SUM+SBA(I)
  470 NEXT I
  480 AVSBA=SUM/DP
  490 VDU2
  500 VDU1,27,1,108,1,15
  510 PRINT Z$
  520 PRINT
  530 PRINT "AVERAGE SIGNAL+BACKGROUND AT ABSORBING WAVELENGTH"
,AVSBA
  540 PRINT
  550 ENDPROC
  560 *********************************
  570 DEF PROCAVERAGEBA
```

D5 Program Analyse6

```
  580 SUM=0
  590 FOR I=0 TO (DP-1)
  600 SUM =SUM+BA(I)
  610 NEXT I
  620 AVBA=SUM/DP
  630 PRINT "AVERAGE BACKGROUND AT ABSORBING WAVELENGTH",AVBA
  640 PRINT
  650 ENDPROC
  660 ************************************
  670 DEF PROCAVERAGESA
  680 LET AVSA=AVSBA-AVBA
  690 PRINT "AVERAGE SIGNAL AT ABSORBING WAVELENGTH",AVSA
  700 PRINT
  710 ENDPROC
  720****************************************
  730 DEF PROCAVERAGESBR
  740 SUM=0
  750 FOR I=0 TO (DP-1)
  760 SUM =SUM+SBR(I)
  770  NEXT I
  780 AVSBR=SUM/DP
  790 PRINT
  800 PRINT "AVERAGE SIGNAL+BACKGROUND AT REFERENCE WAVELENGTH"
,AVSBR
  810 PRINT
  820 ENDPROC
  830 ****************************************
  840 DEF PROCAVERAGEBR
  850 SUM=0
  860 FOR I=0 TO (DP-1)
  870 SUM =SUM+BR(I)
  880 NEXT I
  890  AVBR=SUM/DP
  900 PRINT "AVERAGE BACKGROUND AT REFERENCE WAVELENGTH",AVBR
  910 PRINT
  920 ENDPROC
  930****************************************
  940  DEF PROCAVERAGESR
  950 LET AVSR=AVSBR-AVBR
  960 PRINT "AVERAGE SIGNAL AT REFERENCE WAVELENGTH",AVSR
  970 PRINT
  980 ENDPROC
  990****************************************
 1000 DEF PROCABSORBANCE
 1010 T=AVSA/AVSR
 1020 FRACTA=(ERA/AVSA)^2
 1030 FRACTR=(ERRF/AVSR)^2
 1040 SIGMAT=T*SQR(FRACTA+FRACTR)
 1050 LET AVAB=LOG(1/T)
 1060 UPAB=LOG(1/(T+SIGMAT))
 1070 LOAB=LOG(1/(T-SIGMAT))
 1080 DIFF=0
 1090 IF (UPAB-AVAB)>=(AVAB-LOAB) THEN DIFF =ABS(UPAB-AVAB) ELS
E DIFF=ABS(AVAB-LOAB)
 1100 PRINT
 1110 PRINT "TRANSMISSION",T,"+-",SIGMAT
 1120 PRINT
 1130 PRINT
 1140 PRINT "AVERAGE ABSORBANCE",,AVAB, "+-",DIFF
 1150 VDU3
 1160 ENDPROC
 1170 ************************************************
 1180 DEF PROCAVERAGEERROR
 1190 REM S.D. OF MULTIPLE COUNTS IS GIVEN BY ROOT AV/N
 1200 REM AVSA IS AVERAGE SIGNAL AT ABSORBING WAVELENGTH
 1210 LET ERA=SQR(AVSA/DP)
```

```
1220 PRINT
1230 PRINT "AVERAGE A COUNT",AVSA"+-"ERA
1240 LET ERRF=SQR(AVSR/DP)
1250 PRINT
1260 PRINT "AVERAGE R COUNT",AVSR"+-"ERRF
1270 ENDPROC
1280 ************************************************
>
```

```
 VDU3
>L.
   10 REM PROGRAM "FILT6F". THIS PROGRAM PERFORMS DATA ACQUISIT
ION AND ANALYSIS FOR LAB BASED FIBRE OPTIC NITRATE SENSOR
   20 OSASCI=&FFE3
   30 DIM PROG% 500
   40 P%=PROG%
   50 MODE3
   60 REM SETTING UP ARRAYS IN MEMORY FOR DATA FROM ADC
   70 STOREA=HIMEM-1200
   80 STORE1=HIMEM-1500
   90 STORE2=HIMEM-1800
  100 STORE3=HIMEM-2100
  110 STORE4=HIMEM-2400
  120 STORE5=HIMEM-2700
  130 STORE6=HIMEM-3000
  140 STORE7=HIMEM-3300
  150 STORE8=HIMEM-3600
  160 REM SETTING UP INTERFACE PORTS AS INPUTS AND OUTPUTS
  170 ?&FCE2=0:?&FCF2=0
  180 ?&FCC2=255 :?&FCC3=255:?&FCD2=255
  190 ?&FCC0=0
  200 ?&FCD0=0
  210 FOR PASS=0 TO 3 STEP 3
  220 REM MACHINE CODE ROUTINE FOR DATA ACQUISITION
  230 ********************************************************
  240 [OPT PASS
  250 .BEGIN NOP
  260 LDX £0
  270 .GO NOP
  280 JSR CLR1
  290 JSR START
  300 JSR BACK1
  310 JSR START
  320 JSR FILT2
  330 JSR START
  340 JSR BACK2
  350 JSR START
  360 JSR FILT1
  370 INX
  380  CPX £250
  390 BNE GO
  400 CLI
  410 CLC
  420 RTS
  430 .CLR1 LDA£255
  440 STA &FCED
  450 TXA
  460 STA STOREA
  470 .CHK1 LDA &FCED \LOOKING FOR INTERRUPT ON CB13 FOR START
  480 AND £16
  490 BEQ CHK1
  500 LDA STOREA
  510 TAX
  520 RTS
  530.START NOP
  540 .CLR LDA £255
  550 STA &FCFD      \CLEAR IFR
  560 TXA
  570 STA STOREA
  580 .CHK LDA &FCFD      \CHECKING FOR NEG TRANSITION ON CB1
  590 AND £&10
  600 BEQ CHK
  610 LDA STOREA
  620 TAX
```

D6 Program Filt6F

```
L. 630,1250
 630 RTS
 640 .FILT1 LDA &FCE0
 650 STA STORE1,X
 660 LDA &FCF0
 670 STA STORE2,X  \STORE CONTENTS OF PORTS
 680 RTS
 690 .BACK1 LDA &FCE0
 700 STA STORE3,X
 710 LDA &FCF0
 720 STA STORE4,X  \STORE CONTENTS OF PORTS
 730 RTS
 740 .FILT2 LDA &FCE0
 750 STA STORE5,X
 760 LDA &FCF0
 770 STA STORE6,X  \STORE CONTENTS OF PORTS
 780 RTS
 790 .BACK2 LDA &FCE0
 800  STA STORE7,X
 810 LDA &FCF0
 820  STA STORE8,X  \STORE CONTENTS OF PORTS
 830 RTS
 840 ]
 850 ************************************************************
 860 NEXT PASS
 870 CALL BEGIN
 880 REM SETTING UP DUMMY VARIABLES
 890 SUMS210=0
 900 SMS210D=0
 910 SUMS275=0
 920 SMS275D=0
 930 SUMBACK1=0
 940 SUMBACK2=0
 950 SMBACK1D=0
 960 SMBACK2D=0
 970 VDU14
 980 REM CALCULATING SIGNAL AT 210NM,SIGNAL AT275NM, BACKGROUND SIGNA
LS
 990 REM CALCULATING THE AVERAGE OF 250 FILTER WHEEL ROTATIONS.
1000 FOR N=0 TO 249
1010 BACK1=(?(STORE3+N)*16)+(?(STORE4+N)DIV16)
1020 BACK2=(?(STORE7+N)*16)+(?(STORE8+N)DIV16)
1030 S210=(?(STORE1+N)*16)+(?(STORE2+N)DIV16)
1040 S275=(?(STORE5+N)*16)+(?(STORE6+N)DIV16)
1050 REM THIS SECTION CALCULATES THE SQUARES OF THE SIGNAL AND BACKGR
OUND VALUES
1060 S210D=S210*S210
1070 S275D=S275*S275
1080 BACK1D=BACK1*BACK1
1090 BACK2D=BACK2*BACK2
1100 SUMS210=SUMS210+S210
1110 SUMS275=SUMS275+S275
1120SUMBACK1=SUMBACK1+BACK1
1130 SUMBACK2=SUMBACK2+BACK2
1140 SMS210D=SMS210D+S210D
1150 SMS275D=SMS275D+S275D
1160 SMBACK1D=SMBACK1D+BACK1D
1170 SMBACK2D=SMBACK2D+BACK2D
1180 NEXT N
1190 ************************************************************
1200 REM THIS SECTION CALCULATES AVERAGE SIGNALS
1210 AS275=SUMS275/N
1220 AS210=SUMS210/N
1230 REM THIS SECTION CALCULATES STANDARD DEVIATIONS
1240 ABACK2=SUMBACK2/N
1250 SD210=((SMS210D/N)-(AS210)^2)^.5
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1260 SD275=((SMS275D/N)-(AS275)^2)^.5
1270 SDBACK1=((SMBACK1D/N)-(ABACK1)^2)^.5
1280 SDBACK2=((SMBACK2D/N)-(ABACK2)^2)^.5
1290 REM CALCULATION OF STANDARD ERRORS
1300 SE210=SD210/((N^0.5))
1310 SE275=SD275/((N^0.5))
1320 SEBACK1=SDBACK1/((N^0.5))
1330 SEBACK2=SDBACK2/((N^0.5))
1340 REM CALCULATION OF TRUE SIGNALS
1350 TSIG=AS210-ABACK1
1360 TREF=AS275-ABACK2
1370 REM CALCULATION OF ERRORS ON TSIG AND TREF
1380 SETSIG=(((SE210)^2)+((SEBACK1)^2))^.5
1390 SETREF=(((SE275)^2)+((SEBACK2)^2))^.5
1400 REM CALCULATION OF OPTICAL TRANSMISSION
1410 TRANS=TSIG/TREF
1420 REM CALCULATION OF STANDARD DEV. ON OPTICAL TRANSMISSION
1430 SETRANS=TRANS*(((SETSIG/TSIG)^2)+((SETREF/TREF)^2))^0.5
1440 REM CALCULATE ABSORBANCE
1450 A=LOG(1/TRANS)
1460 REM CALCULATE S.D OF ABSORBANCE
1470 SEABS=ABS(SETRANS*(-0.434/TRANS))
1480 PRINT "ABSORBANCE",A,SEABS
1490 VDU2
1500 VDU1,27,1,108,1,17
1510 PRINT "FILENAME",NAME$
1520 PRINT "AVERAGE SIGNAL AT 210NM",AS210
1530 PRINT "AVERAGE SIGNAL AT 275NM",AS275
1540 PRINT "AVERAGE SIGNAL AT BACKGROUND1",ABACK1
1550 PRINT "AVERAGE SIGNAL AT BACKGROUND2",ABACK2
1560 PRINT "STANDARD DEVIATION OF S210", SD210
1570 PRINT "STANDARD DEVIATION OF S275", SD275
1580 PRINT "STANDARD DEVIATION OF BACK1",SDBACK1
1590 PRINT "STANDARD DEVIATION OF BACK2",SDBACK2
1600 PRINT "NO. OF SAMPLES N",N
1610 PRINT "STANDARD ERROR AT 210",SE210
1620 PRINT "STANDARD ERROR AT 275",SE275
1630 PRINT "STANDARD ERROR AT BACK1",SEBACK1
1640 PRINT "STANDARD ERROR AT BACK2",SEBACK2
1650 PRINT "BACKGROUND ADJUSTED SIGNAL AT 210NM",TSIG
1660 PRINT "BACKGROUND ADJUSTED SIGNAL AT 275NM",TREF
1670 PRINT "ERROR ON ADJUSTED SIGNAL AT 210NM",SETSIG
1680 PRINT "ERROR ON ADJUSTED SIGNAL AT 275NM",SETREF
1690 PRINT " TRANSMISSION",TRANS
1700 PRINT "ERROR ON TRANSMISSION",SETRANS
1710 PRINT "ABSORBANCE",A "+-",SEABS
1720 VDU3
1730 GOTO 870
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