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Remote Spectrophotometric Water Quality Monitoring

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by John Richard Clinch

on

Remote Spectrophotometric Water Quality Monitoring

The conventional approach to water quality monitoring is to combine periodic sampling with batch analysis in the laboratory. Such a procedure is both labour intensive and time consuming, there are likely to be sample stability and contamination problems, and the information provided is unlikely to be continuous or immediate.

This research focussed on the design and construction of fully automated and portable monitors based on flow injection analysis and incorporating solid state photometric detectors.

A novel solid state photometric detector was constructed, incorporating light emitting diodes as the light source, which could be used in conjunction with flow injection analysis.

Manifolds were studied for a range of species of interest (phosphate, nitrate, ammonia and aluminium) in the field of water quality monitoring and were optimised for their suitability for continuous use.

An automated monitor for nitrate was constructed and long term evaluation trials were carried out at several locations for water quality monitoring. Results are also presented for the use of a nitrate monitor in hydroponic cultivation.

An automated monitor was also built for the monitoring of ammonia levels in natural waters, which was field tested on the River Avon (Wiltshire).

A manifold was also evaluated for the monitoring of residual aluminium levels in drinking water and is currently being commissioned at a water treatment works in Somerset.

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To Mum and Dad for all their

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over the years

Abstract

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This research focussed on the design and construction of fully automated and portable monitors based on flow injection analysis and incorporating solid state photometric detectors.

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CHAPTER ONE

INTRODUCTION

1.1. Water

Water is the second most abundant and important resource used by man after air (1). Water is required for drinking, cooking, agriculture, transport, industry and recreation, and as such is vital to the quality of life. The average human has 65% of his total body weight in the form of water (2) and this must be maintained within rigid limits. Man is sensitive to variations as small as 1%, whilst a deficiency of 15% in water content is usually fatal (3).

In more than one third of the land area of the world, water is found to be the limiting factor on human activity. Some of the poorer countries in the world can be identified as those without adequate water resources whilst in the developed world water resources are generally greater than the demand and, thus are taken for granted. In developing third world countries as little as 12 l of fresh water suffices for each person per day, whereas in London, a person uses more than 150 l per day (4). In the United States as much as 250 l per person per day can be used. In the developed parts of the world, large quantities of water are also used in industry; estimated in 1977 to be as much as 300 l per person per day (5).

Without a supply of clean water, crops will not grow, animals become stunted and die, soil erosion takes place due to lack of vegetation and the land becomes barren, as

is the case in many parts of the world. The United Kingdom has a plentiful water supply which has often been taken for granted. Because of this, over the past two hundred years our watercourses have become polluted and it is only in the last thirty years that serious steps have been taken to control it. In many cases water is taken from the natural environment, and in the process of being used becomes polluted, and is then often discharged back into the aquatic system with little or no treatment, where it may then be either abstracted again for further use or, it flows out into the sea. In many areas (e.g. London) the water supplied to some customers by the water authorities has been recycled through the system many times. Public concern about water pollution was recently highlighted in a survey (6) which showed that more of the people questioned were worried about chemicals put into rivers and the sea (86%), than about any other environmental issue, including disposal of nuclear waste (82%), destruction of wildlife (82%) and acid rain (68%).

1.1.1. History of Water Pollution Control in the U.K.

The United Kingdom is fortunate that it s water supply and treatment system was pioneered over a century ago during the industrial revolution (7).

The turning point in the history of Britain´s water supply was the industrial revolution, prior to this, industry and population settlements were on a small scale. In most cases, adequate supplies of water were obtainable from the immediate vicinity of these places and, because there was little industry the only

requirement was for domestic supply. Thus prior to the industrial revolution springs and wells were the main sources of water and, wastewater was usually discharged into surface waters.

With the industrial revolution, towns grew in size and there was a massive increase in the demand for water in these population centres, both for domestic and industrial uses. Large quantities of potable water were required and were increasingly taken from further afield as the original sources dried up, or became unusable due to pollution. As industry developed rapidly, so did the amount of effluent and this was usually discharged directly into the nearest watercourse, usually downstream of any abstraction points used by that town, with no treatment. As a result of this the quality of the lowland rivers deteriorated rapidly, many of them resembling little more than open sewers, and aquatic pollution became accepted as an inevitable byproduct of the rapid industrialisation that was taking place. The pollution led to contamination of water supplies, especially of those towns situated on the lower parts of rivers and, water bo rne disease epidemics (cholera and typhoid especially) became commonplace (8).

The first attempts at limiting this dangerous pollution came with the Fishery Acts of 1861 (9) and 1865 (10) which sought to prevent the destruction of aquatic life in English rivers by outlawing the discharge of liquid and solid matter which could poison fish. These acts were followed by the establishment of a Rivers

Pollution Prevention Commission which sat between 1865 and 1874 and was responsible for the River Pollution Prevention Act of 1876 (11).

This act removed the right to pollute rivers freely and also banned the discharge of solid wastes altogether, but left a loophole whereby some industrial concerns could still discharge if there was no other option open to them. The main problem with this act was that the power of enforcement was placed with the local authorities who were often the major polluters, and who, in the absence of any recognised standards for water quality were reluctant to take the expensive step of cleaning up the discharges. Thus, despite mounting public protests, the quality of Britain´s waterways continued to decline.

In 1888 with the restructuring of local government, the powers of enforcing the 1876 River Pollution Prevention Act were passed to the newly formed county councils, but as before, with no standards laid down on the amount and quality of the discharges, little was done. In the early 1890´s, in recognition of the worsening situation, four river authorities were formed in the more heavily polluted areas of the north of England. They were financially and administratively independent and had wide ranging powers over the control of the water in their areas, and, by efficient inspection and use of these powers, they managed to control some of the worst abuses of the river systems.

During the late nineteenth century and early twentieth century the aquatic system became increasingly polluted with the result that towns were finding it increasingly difficult to obtain clean water supplies. This forced them to look for water sources at great distances from the towns and was the start of the integrated water supply system that is in existence now.

The Rivers Pollution Prevention Commission recommended that all the rivers in England should be placed under the control of a central authority, but it was not until the drought of 1937 that the government noticed how serious the situation was with regard to pollution and the public water supply. This led to the formation of the Central Advisory Water Committee which had a more realistic approach to the control of pollution, although it had no statutory powers.

In 1943 it published a report (12) which led to the 1944 white paper (13) entitled "A National Water Policy" which became The Water Act of 1945 (14). This empowered the Health Minister to be responsible for the conservation and proper use of water resources in England and Wales. In 1950 a system of 32 river boards was set up, based on river drainage basin boundaries, with overall responsibility for all the water conservation and pollution control in their areas.

The Rivers (Prevention of Pollution) Act (15) was passed in 1951 and required any person or company wishing to discharge into a watercourse to seek the consent of the appropriate river board and gave the river boards the

power to regulate the volume and quality of such discharges. This power was extended by further legislation in 1961 (16) to cover all discharges operating prior to the 1951 act.

The river boards were absorbed by 29 new river authorities in the 1963 Water Resources Act (17), which gave to the new authorities powers over all matters relating to land drainage, river pollution, fisheries, water abstraction and resource planning in their areas.

In 1973 an act was passed (18) forming the nine water authorities that exist today in England and Wales (figure 1.1.). These water authorities were empowered with the responsibility for all aspects of water management in their areas. They took over all the functions of the old river boards and took on powers previously controlled by the local authorities/county councils. The water authorities were responsible for the abstraction of water, be it from rivers, boreholes or upland catchment systems, and the treatment of that water so as to provide a 'wholesome' product to the consumer. They were also made responsible for sewage disposal and pollution control.

The Control of Pollution Act 1974 (19) set down the responsibilities of the water authorities with regard to water pollution more clearly. It gave them powers to control and license industrial discharges to both sewers and natural watercourses. They were also given powers to regulate certain types of land use (e.g. agriculture) if it was deemed to cause pollution problems.

Figure 1.1. Water Authorities of England and Wales

The quality of drinking water was governed by standards laid down by the National Water Council but, in 1975 the European Economic Community (E.E.C.) issued a directive (20) which set standards for drinking water in all member states.

1.1.2. Current Water Quality Standards Applying to the U.K.

Water quality, referring both to that supplied to the consumer and that which is abstracted for the drinking water supply, is governed by three E.E.C. directives (20-22). These directives have been summarised by the Water Research Council (23). They stipulate definitive standards for the quality of surface waters intended for abstraction into the drinking water supply (20), definitive standards for the quality of water intended for human consumption (21) and they also specify the methods to be used in the analysis, and the frequency of sampling of waters intended for abstraction into the drinking water supply (22).

The directives specify two levels for each parameter, the guide level which all member states should try to conform with and, the maximum admissible concentration which should not be exceeded without reference to the E.E.C.. Member states must fix their own quality standards based upon the guide levels given and these values must not exceed the values given in the directives as maximum admissible concentrations. Table 1.1. gives details of some of the parameters and their guide and maximum admissible concentrations.

Parameter	Guide Level ($mg\ l^{-1}$)	Maximum Admissible Concentration ($mg\ l^{-1}$)
Nitrate (as N)	5.65	11.3
Ammonia (as N)	0.038	0.38
Phosphate (as P)	0.087	1.091
Aluminium (as Al)	0.050	0.200
Iron (as Fe)	0.050	0.200

Table 1.1. E.E.C. Parameters for Drinking Water

The maximum admissible concentrations can only be exceeded by reference to the E.E.C., but only for parameters not classified as toxic (e.g. heavy metals, cyanide, fluoride, hydrogen sulphide, pesticides etc.) or microbiological (e.g. bacterial counts, viruses etc.). These derogations are allowed in situations arising from exceptional weather conditions or from the nature of the ground from which the supply emanates.

The implementation of the E.E.C. directives has led to an increasing demand for chemical analysis of water samples to be placed on water authorities. To ensure that this demand is met, increasing use of automated analytical methods is being seen, in order to cope with the large number of samples that have to be analysed.

1.2. Continuous Monitoring

The implementation of the E.E.C. directives has led to a large increase in the number of samples taken for analysis, both from treated and surface waters intended for abstraction into the drinking water supply system. Conventional methods of water analysis involve removing grab samples and then analysing them in a laboratory for the species of interest. The laboratory is often a large distance from the point of sampling and this can cause errors due to difficulties in providing a stable representative sample at the laboratory (24-26).

1.2.1. Applications of Continuous Monitoring

There are several applications of continuous monitoring, including,

(i) supply intake protection

(ii) water treatment plant control

(iii) river water quality monitoring

(iv) hydroponic cultivation

(v) effluent monitoring

(vi) process analysis.

1.2.1.1. Supply Intake Protection (27,28)

Under the E.E.C. directives, surface waters must comply with certain quality levels if they are to be used for abstraction into the public water supply. If any of these levels are exceeded then the supply intake should be shut down until measures have been taken to remedy the problem. Manual sampling means that large numbers of samples should be taken at regular intervals and analysed immediately, otherwise there is a risk of contaminated water entering the treatment process, where the pollutant may not be removed. With on-line continuous monitoring, as soon as the pollutant reaches a certain level the intake can be shut off, the problem can then be investigated and when remedied the supply can be reinstated.

1.2.1.2. Water Treatment Plant Control (29,30)

In water treatment plants many chemicals are added to the water in the course of it s treatment, including chlorine, sulphur dioxide, aluminium, iron, lime etc.. It is important to keep the residual levels of some of these chemicals low or they may cause the water to have a bad taste or even cause health problems for the consumer. Continuous monitors could measure the level of these chemicals and with feedback links to their dosing pumps

could ensure that the residual levels are kept low. This would also have the advantage that only the correct amount of the chemicals would be added and thus a cost saving could be made.

1.2.1.3. River Water Quality Monitoring (31-35)

Continuous records of levels of certain parameters in rivers are useful when designing computer models of the river system, so that when a pollution incident occurs it s immediate and subsequent effects can be predicted. This is helpful so that decisions can then be made about how to clean it up. Computer models are also useful in conditions of extreme weather, e.g. drought, where predictions can then be made about the effect of discharges on the river quality as the level falls, and decisions can then be made as to whether to shut down discharges in order to preserve the river quality. Continuous river water quality monitoring is also applicable to nutrient budget studies where data on several nutrients (e.g. nitrate, phosphate, silicate etc.) is obtained over a period of time for several locations within a river basin, which can then be used to provide a measure of the biological activity (e.g. the uptake by algae and other aquatic plants) of the river basin. By comparing the results obtained at different locations, estimates can be made of the input of nutrients to the river from the land and sewage effluents. Their removal by the aquatic environment can also be calculated.

1.2.1.4. Hydroponic Cultivation (36)

In hydroponic cultivation plants are grown in water to which all the nutrients that they require have been added. In current systems the liquid nutrient mixture is added to the water supply flowing into the top of the bed and, any nutrients that are not used by the plants flow to waste, usually into the nearest watercourse where they may cause problems by stimulating the growth of aquatic plants to the extent that other aquatic life may suffer. Continuous monitoring can be used to continuously check the level of nutrient at the exit to the bed and, by the use of a control loop connected to the nutrient supply valve, could regulate the supply of nutrients available to the plants so that the optimum use of nutrients is achieved. Also the possible recirculation of the water could be applied with a further saving in the amount of nutrient used.

1.2.1.5. Effluent Monitoring (37)

In industry close attention has to be paid to the quality of water discharged into both the sewage system and surface waters. Licenses have to be obtained from the relevant water authority which will give consent conditions for the discharge, i.e. the concentrations of pollutants and the volume of the discharge per day. The water authority will also charge a fee depending on the quality of the effluent discharged. If continuous monitoring is used to measure the effluent quality, the charges could be more closely related to the actual effluent composition instead of being based on an average

composition. Also the discharge could be automatically shut down if the effluent composition exceeded the consent conditions, thus preventing a possible pollution incident from occurring.

1.2.1.6. Process Analysis (38,39)

In an industrial process it is often necessary to continuously monitor the level of one or more parameters which are important to the process, as, a slight change in their levels could either increase the cost of the final product in terms of wasted raw materials or, adversely affect the nature of the finished product. Thus a continuous monitoring system coupled with feedback to the process control system would enable fine tuning of the process to be carried out.

1.2.2. Requirements for a Continuous Monitor

In the design of a continuous monitor system several important features must be considered (40),

(i) capability for unattended operation

(ii) low drift and autocalibration

(iii) size and weight

(iv) ease of maintenance

(v) data transmission.

1.2.2.1. Capability for Unattended Operation

A continuous monitor must be designed in such a way that it will operate unattended for a set period of time before requiring attention, in practice this means that all reagents and standards used must be stable over the unattended operating period and that any mechanical parts

that may need regular replacement (e.g. pump tubing, filters etc.) are reliable over the operating period.

1.2.2.2. Low Drift and Autocalibration

Most analytical systems suffer from drift to a greater or lesser degree and any monitor must be designed so as to minimise this drift, whether it be from temperature effects or, mechanical fouling of the sensor itself.

Autocalibration of the sensor system with known standards should ideally be carried out as frequently as possible so as to check the effects of drift and to correct them.

1.2.2.3. Size and Weight

The design of the monitor must take into account the fact that many monitoring stations are in inaccessible places and therefore the monitor should ideally be portable (i.e. capable of being carried easily) so that it can be easily installed. Also if the monitor size is such that it occupies a small space, then the buildings designed to house such systems will also be smaller and thus substantial cost savings can be achieved.

The weight of the monitor is important as the lighter the monitor, the easier it is to transport and install. Ideally it should weigh no more than 25 Kg.

1.2.2.4. Ease of Maintenance

The system must be designed in such a way that all parts are easily accessible for maintenance purposes. Routine maintenance schedules should be planned to be simple and, quickly carried out so that the instrument´s

down time is minimised. Maintenance should be easily carried out by a non-skilled technician.

1.2.2.5. Data Transmission

Current control/data aquisition systems have widely different input specifications e.g. voltage, current loop, binary coded decimal, serial data etc.. This means it would be necessary to have a microprocessor based system which then gives the flexibility to configure the output to one of several different options. The majority of systems are currently based on a 4-20 mA current loop, but with the increasing use of microcomputers this is likely to be replaced by RS232 serial links.

It is also necessary to provide a local output via a small display and a printer for a permanent record of the results in case of a failure in the data transmission system.

1.2.3. Current Continuous Monitoring Instrumentation

Current continuous monitoring instrumentation is based on electrochemical techniques (40-42). Apart from the simple parameters such as temperature, pH, conductivity and dissolved oxygen, continuous monitors also exist for ammonia, nitrate, fluoride and several other species for which there is a suitable ion-selective electrode.

The most widely used form of continuous water quality monitoring involves passing the sample stream through a series of flow cells which contain pH, temperature, dissolved oxygen and conductivity probes. These simple electrochemical probes have the advantage that they are relatively stable and usually only need calibrating once

a week. Systems involving these four parameters are widespread, with various forms of data transmission (e.g. via telephone line, UHF radio or satellite transmission) back to a central control where the data can be used in the assessment of the quality of the particular watercourse under study. One example of this system is that developed by the Sunderland and South Shields Water Company as part of it s River Wear scheme (43,44), for the protection of a water supply intake. This system consists of electrochemical sensors continuously monitoring dissolved oxygen, temperature, pH, conductivity, dissolved organic carbon, turbidity and ammonia.

The sensors are all duplicated, with the second set being run in a mains water supply. When an alarm condition is indicated by one of the river water sensors, the sensors automatically switch over so that the river water one is in tap water and the tap water one is in river water, so that the alarm condition can be verified. If it is found to be correct then an alarm signal is transmitted to the control system. If the sensors disagree then a sensor fault signal is sent to the control system.

Continuous monitors based on ion-selective electrodes e.g. for ammonia, suffer from drift and require careful temperature regulation and periodic recalibration with one or more standards. Also because of their relatively poor selectivity, other reagents have to be added to the sample stream in order to remove some of the interfering

species, therefore a more complex flow system is required.

Figure 1.2. shows a block diagram of a typical ammonia ion-selective electrode based monitor; the sample is merged with E.D.T.A. (ethylene diamine tetraacetic acid) which removes interfering metal ions e.g. calcium, magnesium etc. by complexation and then the sample is made strongly alkaline. This converts any ammonia present as ammonium ions to dissolved ammonia which the electrode can then respond to. All of these operations have to be carried out in a water bath so that the electrode is maintained at a constant temperature. Two point recalibration of the instrument is carried out, normally at six or twelve hourly intervals.

Most ion-selective electrodes suffer from two disadvantages:

(i) If the electrode incorporates a membrane, as in the nitrate and ammonia electrodes, it can be prone to fouling by organic matter present in the sample which will ultimately lead to the electrode output drifting with time. Also, abrasive particles in the sample (such as sand and grit) can damage the membrane, thus shortening it´s lifetime. This means that care has to be taken to supply the monitor with a sample free from suspended solids.

(ii) Ion-selective electrodes are prone to interference effects, e.g. the nitrate ion-

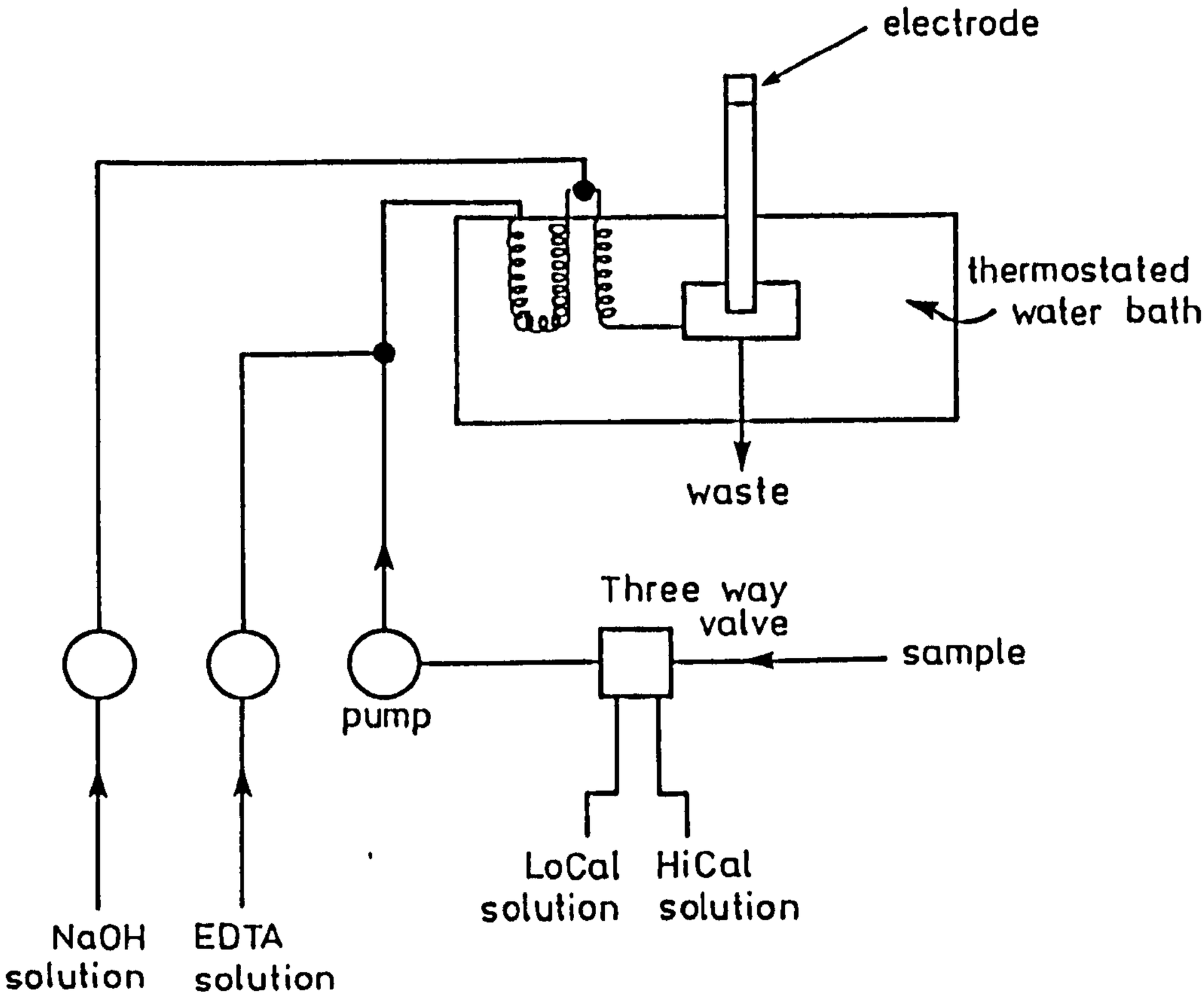


Figure 1.2. Diagram of Ammonia Ion-selective Electrode Based Monitor

selective electrode is based on an ion exchange membrane which is sensitive to nitrate but, it is also sensitive to sulphate, fluoride, acetate, phosphate, chloride and carbonate, all of which may be present in natural waters to an extent that would interfere with the electrode. In an effort to remove these interfering ions, sample pretreatment is carried out which either removes the interfering ions or changes them into a form which is less likely to interfere. Even though these suppressor solutions are added to the sample, one can never be totally sure that the response from the electrode is due solely to the species of interest.

The advantage of using these monitors is that they have been available commercially for 10-15 years and have been extensively tested in the water analysis field. The major limitation on their use is the small number of species for which ion-selective electrodes are available (45).

1.2.4. Spectrophotometric Based Monitors

Several commercially available monitors are based on spectrophotometric techniques. They can be divided into two distinct types, based on continuous flow and discrete techniques.

(i) Continuous flow techniques :- most of the currently used monitors are based on AutoAnalyser methods which have been modified for continuous monitoring. These monitors

suffer from the disadvantage that they use large volumes of reagents and standards, and require frequent operator attention.

(ii) Discrete techniques :- in this method a fixed amount of sample is removed from the sample line, usually by means of an automatic syringe, and is mixed in a vessel with the chromogenic reagents specific to the analyte being determined. The mixture is then forced into a cuvette where it s optical absorbance is then measured. The cell is cleaned and the process is repeated. The instrument has the facility for recalibration at regular intervals and the time between each determination can be preset. All the operations, addition of reagents, mixing etc. are carried out automatically. This type of analyser has only recently been developed and is applicable to many species of interest in water analysis, e.g. aluminium, nitrate, total hardness etc..

Spectrophotometric based monitors offer several advantages over ion-selective electrode based monitors;

(i) The chromogenic reagents used are generally specific for the species of interest.

(ii) Methods are available for almost all species of interest in water analysis.

(iii) Many spectrophotometric methods are as sensitive as the corresponding ion-selective electrode method.

The disadvantage of spectrophotometric methods is that they require relatively large volumes of reagents and standards and, that relatively expensive spectrophotometric detection systems are used.

Flow injection analysis coupled with inexpensive solid state photometric detectors can solve both of these problems.

1.3. Flow Injection Analysis

Flow injection analysis is a simple, precise and rapid technique (46,47). It is based on the injection of a liquid sample into a moving non-segmented stream of reagent or carrier. The reagent reacts with the sample in a controlled way to give a detectable product.

A simple flow injection manifold and the corresponding recorder output is shown in figure 1.3.. A pump (P) propels the carrier stream (R), into which a sample (S) is introduced via a six port rotary injection valve. The sample initially forms a well defined zone which disperses into the carrier stream during it s passage to the detector, where it s concentration is measured using a suitable flow through detector (D). A typical detector response signal is seen as a well defined peak, the height (H) of which is proportional to the concentration of the species being determined. The residence time (T) is the time between sample injection (S) and the time of the peak maximum. This residence time can be altered by varying system parameters (e.g. flow rate, length of tubing between injection valve and detector etc.) but is typically in the range 5 to 60 seconds.

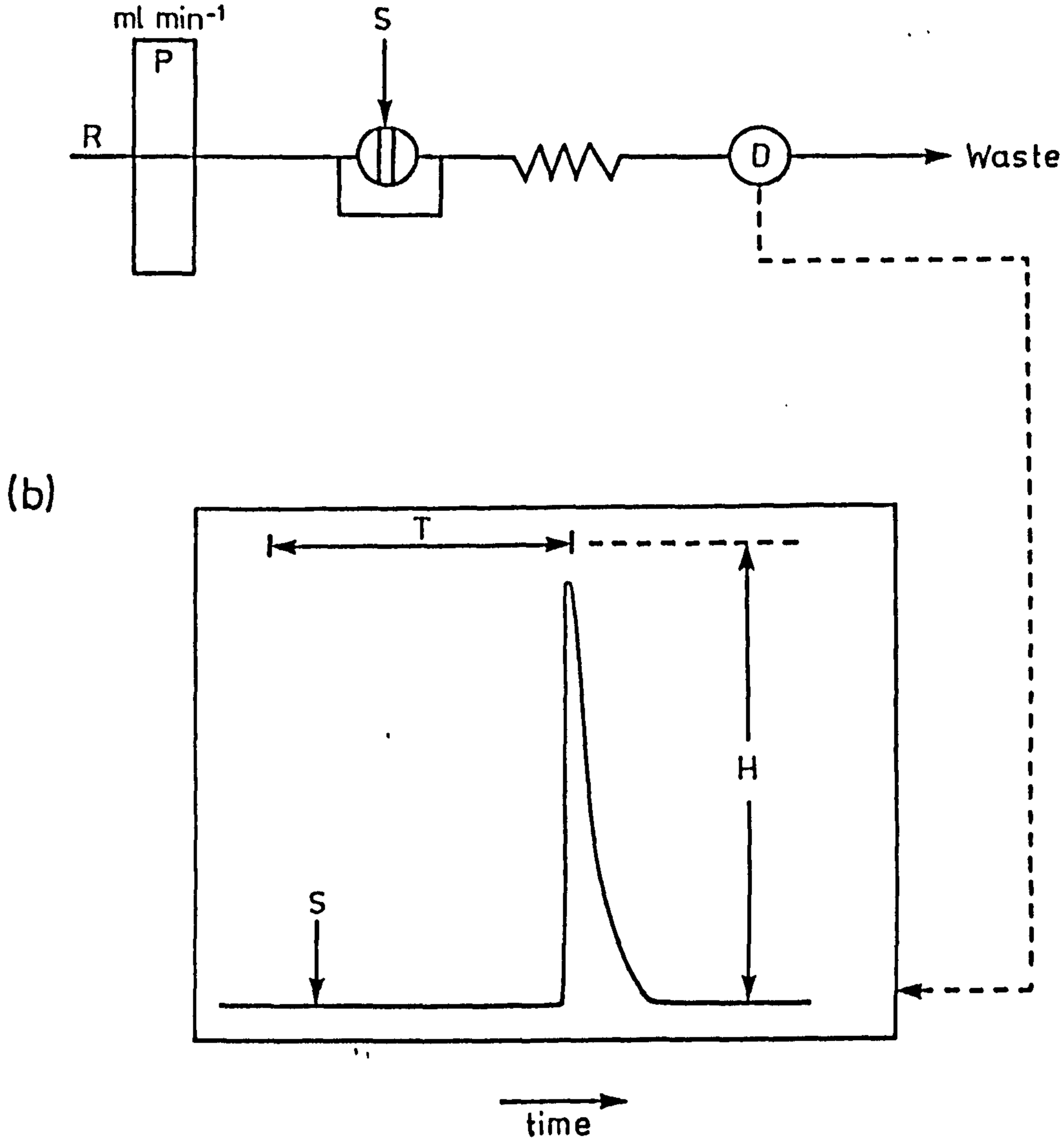


Figure 1.3. (A) Simple Flow Injection Manifold, (B) Corresponding Recorder Output

Flow injection analysis is normally a small scale technique, typical injection volumes are in the range 5 to 300 μl, flow rates are usually 1-3 ml min^{-1} and the tubing used has an internal diameter of 0.5-0.8 mm. High sample throughputs can be achieved of up to 300 samples per hour depending on the chemistry involved. Flow injection analysis can be used with a wide variety of detectors including spectrophotometric, electrochemical and atomic absorption spectrometry.

One possible drawback of using flow injection analysis in continuous monitors is that the reagent stream is flowing all of the time, and this is wasteful of reagents (although it would use much less than other continuous flow methods) if it was used continuously for long periods. A solution to this problem is to carry out a determination at a fixed time interval, thereby saving on reagents as, between each determination the system can be shut down. The system can thus be adapted to suit the rate of change in concentration within a preset time.

A modified form of flow injection analysis offers another solution to this problem, as it relies on the injection of the reagent into the flowing sample stream. This technique, sometimes called 'reverse' flow injection analysis (47,48), but more accurately named 'reagent injection' flow injection analysis, saves on reagent consumption because only a small volume of reagent is used each time and because the sample is the carrier stream, there can be no deposition of reagent onto the flow cell walls. This technique has been used in the

construction of a submersible seawater monitor for several analytical parameters (50).

1.4 Research Objectives

1. Design and construction of a solid state photometric detector incorporating light emitting diodes as the light source and photodiodes as the detector.

2. Evaluate the solid state detector on a simple chemistry e.g. phosphate, for it s suitability for incorporation into an automated monitor.

3. Design and construct an automated monitor for nitrate including an examination of current flow injection manifolds available for their suitability in an automated monitor, and exhaustively test it in the field.

4. Design and construct automated monitors for other parameters of interest e.g. ammonia and aluminium.

1.5. References

1. W.C.Walton, "The World of Water", Weidenfield and Nicolson, London, 1970, chapter 1.

2. K.Smith, "Water in Britain", Macmillan, London, 1972, chapter 1.

3. L.Leopold and K.Davis, "Water", Life Science Library, New York, 1966.

4. M.Overman, "Water : Solutions to a Problem of Supply and Demand", The Open University Press, Milton Keynes, 1976.

5. G.Best and S.Ross, "River Pollution Studies", Liverpool University Press, Liverpool, 1977.

6. Department of the Environment, "Digest of Environmental Protection and Water Statistics", HMSO, London, 1986.

7. Ibid 2, chapter 2.

8. C.Warren, "Biology and Water Pollution Control", W.B.Saunders Company, Philadelphia, 1971.

9. "Salmon Fishery Act 1981", The Statutes of the United Kingdom, 24 to 26 VICT, London, Chapter 109, pages 281-290.

10. "Salmon Fishery Act 1865", The Statutes of the United Kingdom, 28 and 29 VICT, London, chapter 121, pages 192-202.

11. "River Pollution Prevention Act 1876", The Law Reports : The Public General Statutes, 1876, London, chapter 75, pages 448-456.

12. "Third Report of the Central Advisory Water Committee : River Boards, August 1943", Cmd. 645, Accounts and Papers 1942-3, VI, HMSO, London.

13. "A National Water Policy", Cmd. 6515, Accounts and Papers 1943-44, VIII, HMSO, London.

14. "Water Act 1945", The Statutes of the United Kingdom, 8 and 9 GEOG, London, chapter 42, pages 558-669.

15. "Rivers (Prevention of Pollution) Act 1951", The Public General Acts of 1951, HMSO, London, 1951, chapter 64, pages 514-536.

16. "Rivers (Prevention of Pollution) Act 1961", The Public General Acts of 1961, HMSO, London, 1961, chapter 50, pages 581-594.

17. "Water Resources Act 1963", The Public General Acts of 1963, HMSO, London, 1963, chapter 38, pages 945-1131.

18. "Water Act 1973", The Statutes of the United Kingdom, 19 and 20 ELIZABETH 2, London, chapter 36, pages 615-734.

19. "Control of Pollution Act 1974",The Statutes of the United Kingdom, 22 and 23 ELIZABETH 2, London, chapter 40, pages 961-1096.

20. Official J. European Community, L194(1975)26.

21. Official J. European Community, L229(1980)11.

22. Official J. European Community, L271(1979)44.

23. J.Gardinier and G.Mance, "United Kingdom Water Quality Standards Arising from European Community Directives", Technical Report TR204, WRC Environment, Stevenage, 1984.

24. H.Casey and S.Smith, Trends Anal. Chem., 4(1985)256.

25. M.Comber and P.Nicholson, Anal. Proc., 21(1984)474.

26. P.Stuyfzand, Trends Anal. Chem., 6(1987)50.

27. Report of the Direct River Abstraction Group, Appendix H, Second Biennial Report (Feb 1977 - Jan 1979) of the Standing Technical Committee on Water Quality, Department of the Environment / National Water Council, 1979.

28. J.Wallwork and J.Schofield in "River Pollution Control", Ed. M.J.Stiff, Ellis Horwood, Chichester, 1980, chapter 12, pages 175-193.

29. R.Briggs, ISA Transactions, 21(1982)59.

30. W.Meredith and C.Rushton, Int. Environ. Safety, December 1981, pages 10-16.

31. D.Hinge, J. Inst. Water Eng. Sci., 34(1980)546.

32. J.Fenton and D.Young, Wat. Pollut. Control (London), 81(1982)343.

33. D.Caddy and P.Whitehead, Effluent and Water Treatment Journal, 21(1981)407.

34. G.Best, Public Health Engineer, 11(1983)26.

35. "Monitoring to Detect Changes in Water Quality Series", IAHS Publication n°. 157, Ed. D.Lerner, Wallingford, 1986.

36. J.R.Clinch, P.J.Worsfold, H.Casey and S.Smith, Anal. Proc., 25(1988)71.

37. W.D.Meredith, Prog. Wat. Tech., 9(1977)71.

38. P.E.Mix,"The design and Application of Process Analyser Systems", Wiley Interscience Series, Volume 70, New York, 1984.

39. J.Callis, D.Illman and B.Kowalski, Anal. Chim. Acta, 59(1987)624.

40. R.Briggs and K.V.Melbourne, Water Treatment Exam., 17(1968)107.

41. R.Briggs, H. Page and J.Schofield, Prog. wat. Tech., 9(1977)43.

42. K.Maney and H.E.Allen, "Automated Monitoring and Analysis", in "Examination of Water for Pollution Control - Volume 1 : Sampling, Data Analysis and Laboratory Equipment", Ed. M.J.Suess, Ellis Horwood, Chichester, 1982, chapter 3.

43. Ibid. 28

44. J.F.Wallwork, R.T.Heslop and N.L.Redshaw, Prog. Wat. Tech., 9(1978)215.

45. A.M.C.Edwards, A.T.McDonald and J.R.Petch, "The Use of Electrode Instrumentation for Water Analysis", British Geomorphological Research Group, Technical Bulletin N°. 15, Norwich, 1975.

46. J.Ruzicka and E.H.Hansen, "Flow Injection Analysis", Wiley Interscience Series, Volume 62, New York, 1977.

47. M.Valcarcel and M.D.Luque de Castro, "Flow Injection Analysis - Principles and Applications", Ellis Horwood, Chichester, 1987.

48. K.Johnson and R.Petty, Anal. Chem., 54(1982)1185.

49. K.Johnson and R.Petty, Limnol. Oceanogr., 28(1983)1260.

50. K.Johnson, C.Beehler and C.Sakamoto-Arnold, Anal. Chim. Acta, 179(1986)245.

CHAPTER TWO

SOLID-STATE PHOTOMETRIC DETECTORS

2.1. Introduction

Solid state photometric detectors incorporate light emitting diodes (LED's) as light sources and, either photodiodes or phototransistors as the light detecting element. These solid state devices offer several advantages over conventional spectrophotometric detectors when used in the design of photometric water quality monitors:

(i) Size - a solid-state detector system can be constructed to fit in a small housing, typically 170x120x55 mm for both the flowcell and the associated detector electronics.

(ii) Cost - simple filter photometers cost between £1000 and £3000 whereas a solid state detector can be constructed for about £50.

(iii) Power consumption - conventional spectrophotometers use large quantities of power (typically 200 W at 240 V), whereas a solid-state detector uses 2 W at 12 V.

(iv) Temperature - tungsten or deuterium lamps are used in conventional

spectrophotometers as light sources, but as they are not very efficient at converting electrical energy into light, they generate large quantities of heat. In the confined space of a water quality monitor this could cause problems due to overheating. Solid-state detectors employing LED´s as their light source run at ambient temperature, as LED´s are very efficient at producing light.

The use of LED´s and phototransistors in photometric modules was first reported by Flaschka et al. (1) in 1973 for the determination of copper (II) in aqueous solution. In 1976 a photometric probe was described by Anfalt et al. (2) which incorporated an LED and two photodiodes for the determination of total alkalinity in seawater. Since then the use of solid-state detectors has been successfully applied to flow injection analysis (3-12).

Solid-state detectors have also been used for more specialised applications, which include the use of a green LED in an ion chromatography detector (15) for the determination of cations following their derivatization with PAR (4-(2-pyridylazo)resorcinol), and the use of a blue LED as part of a fluorescence detector for carbon dioxide (16).

2.1.1. Light Emitting Diodes

The LED possesses a special type of p-n junction which can emit radiation under a forward bias (17,18). Excited electrons in the donor level below the conduction band of the junction, return to the acceptor level above the valence band and lose energy in the process. This energy is emitted as either infrared or visible radiation. Green LED´s are made from gallium phosphide and have an emission intensity maximum at 565 nm. Red and yellow LED´s use a layer of gallium arsenide phosphide on a gallium arsenide substrate and have emission intensity maxima at 635 nm and 583 nm respectively. The use of a layer of gallium arsenide phosphide on a gallium arsenide substrate yields an emission intensity maximum at 655 nm.

The emission spectra of three commonly available LED´s (Radio Spares), red, amber, and green were obtained (figure 2.1) using a silicon-intensified-target vidicon detector coupled to a polychromator. The spectra obtained show that the spectral bandwidth of an LED is typically 20-30 nm and this would appear to limit the number of photometric determinations which could be carried out using LED´s as light sources. Most spectrophotometric methods are based on the formation of a chromophore which has a spectral absorbtion bandwidth in excess of 30 nm and thus, by suitable choice of the LED used as the light source, most species can be determined, although with some loss of sensitivity. With the currently available LED´s (green, amber and red) most spectrophotometric methods between 500 and 700 nm can be used. Infrared

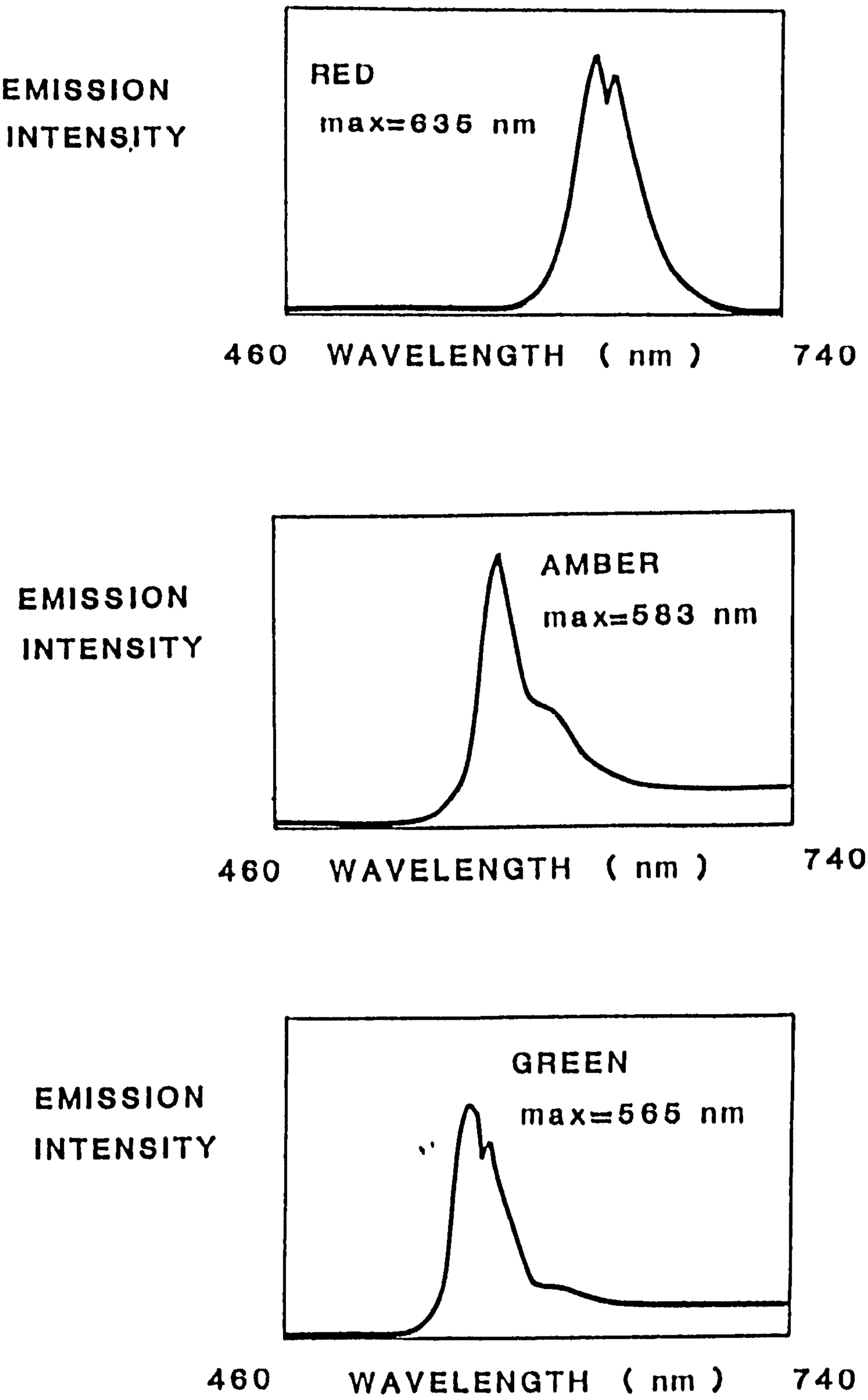


Fig 2.1. EMISSION SPECTRA OF LED'S

LED´s are also available with an emission maxima at 940 nm and, a blue LED is reported to be available (16) although attempts to aquire one have been unsuccessful.

LED´s consume small amounts of power (typically 20 mA at 2 volts) and, because they are very efficient at producing light, they run at ambient temperatures and thus have very long lifetimes (typically 20000-100000 hours (3)) compared to tungsten and deuterium lamps (1000-5000 hours). They are small, typically 4 mm in diameter and 8 mm in length, and most of this is packaging material to protect the p-n junction.

2.1.2. Integrating Photodiode

An integrating photodiode (Radio Spares No. 308-067) consists of a silicon PIN photodiode coupled with a high gain, low noise amplifier in a single package.

Silicon photodiodes (19) are sensitive to visible and infrared radiation, and allow a small current proportional to the intensity of the incident radiation to flow across a diode junction. They are held under a reverse bias potential and, when radiation falls on the light sensitive p-n junction of the diode, current flows across the junction. This current, called the photocurrent, is very small (in the order of nanoamps) and has to be amplified and converted to a voltage in the detector electronics. The connections between the photodiode and the amplifier are thus highly susceptible to environmental noise, and therefore require special design considerations.

The integrating photodiode overcomes this problem by feeding the output from the photodiode into a high gain, low noise amplifier contained within the same casing. The output obtained from such a device is large (from a dark to a light room, a response of 15 volts is obtained) and as such is less susceptible to environmental noise. The spectral response of an integrating photodiode (Radio Spares) is shown in figure 2.2..

2.2. Experimental

2.2.1. Design and Construction of a Solid-State Detector

Photodiodes, like all semiconductors, are temperature sensitive devices and so a ´dual beam´ system was used to compensate for any drift that could be caused by temperature changes. Two channels, a reference channel and a sample channel were used, with the output from the reference channel being subtracted from the output from the sample channel. This removed any temperature dependence of the system assuming that any change in temperature affected each integrating photodiode equally. This system also allowed for changes in the transmittance of the flow stream as any change would affect both sample and reference channels.

2.2.1.1. Detector Electronics

The detector circuit (figure 2.3)can be described in two parts, the source power supply and the integrating photodiode circuitry.

In the integrating photodiode circuitry, the outputs from the two photodiodes were fed into a low noise operational amplifier (IC1 - 741N) configured in a

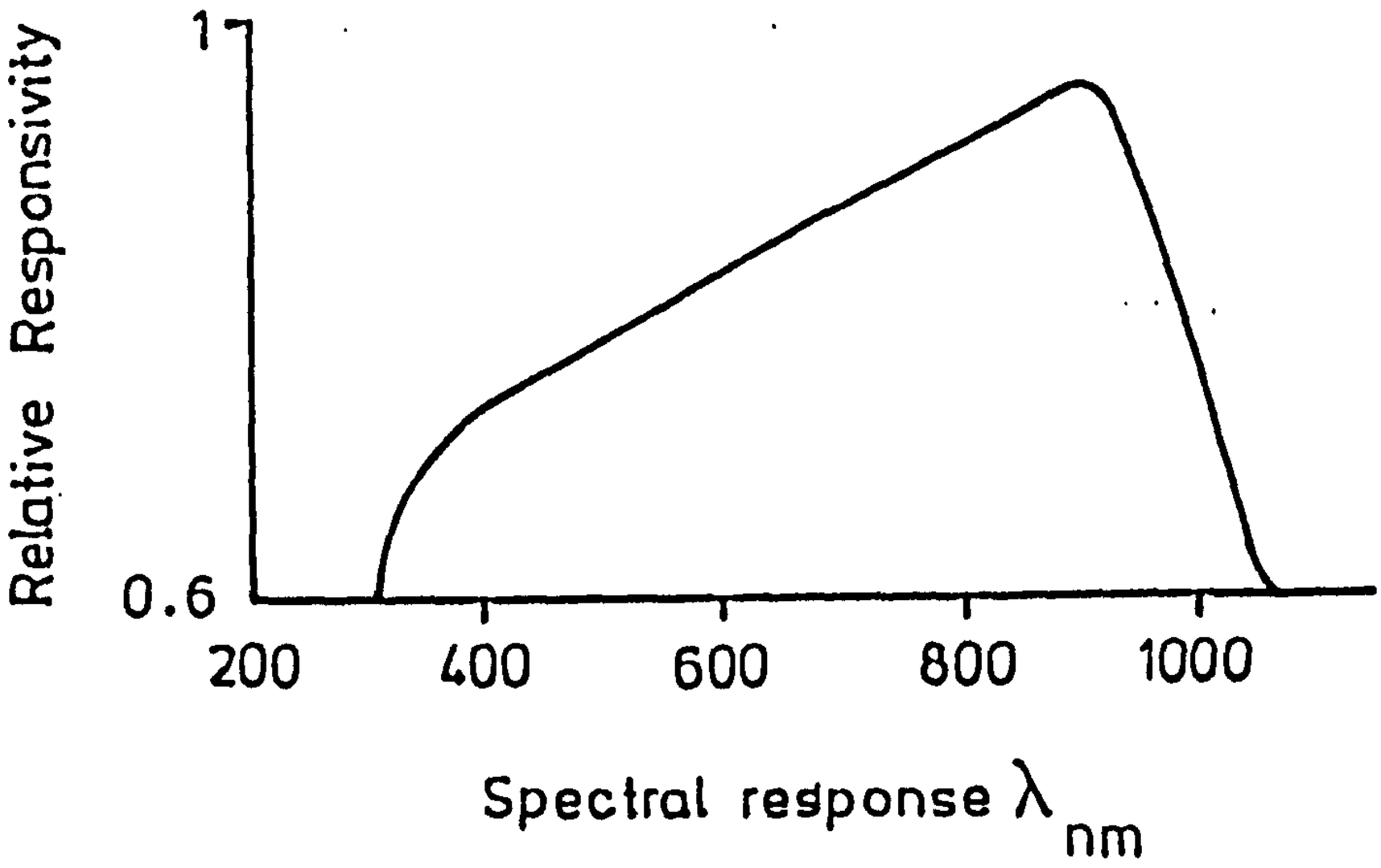
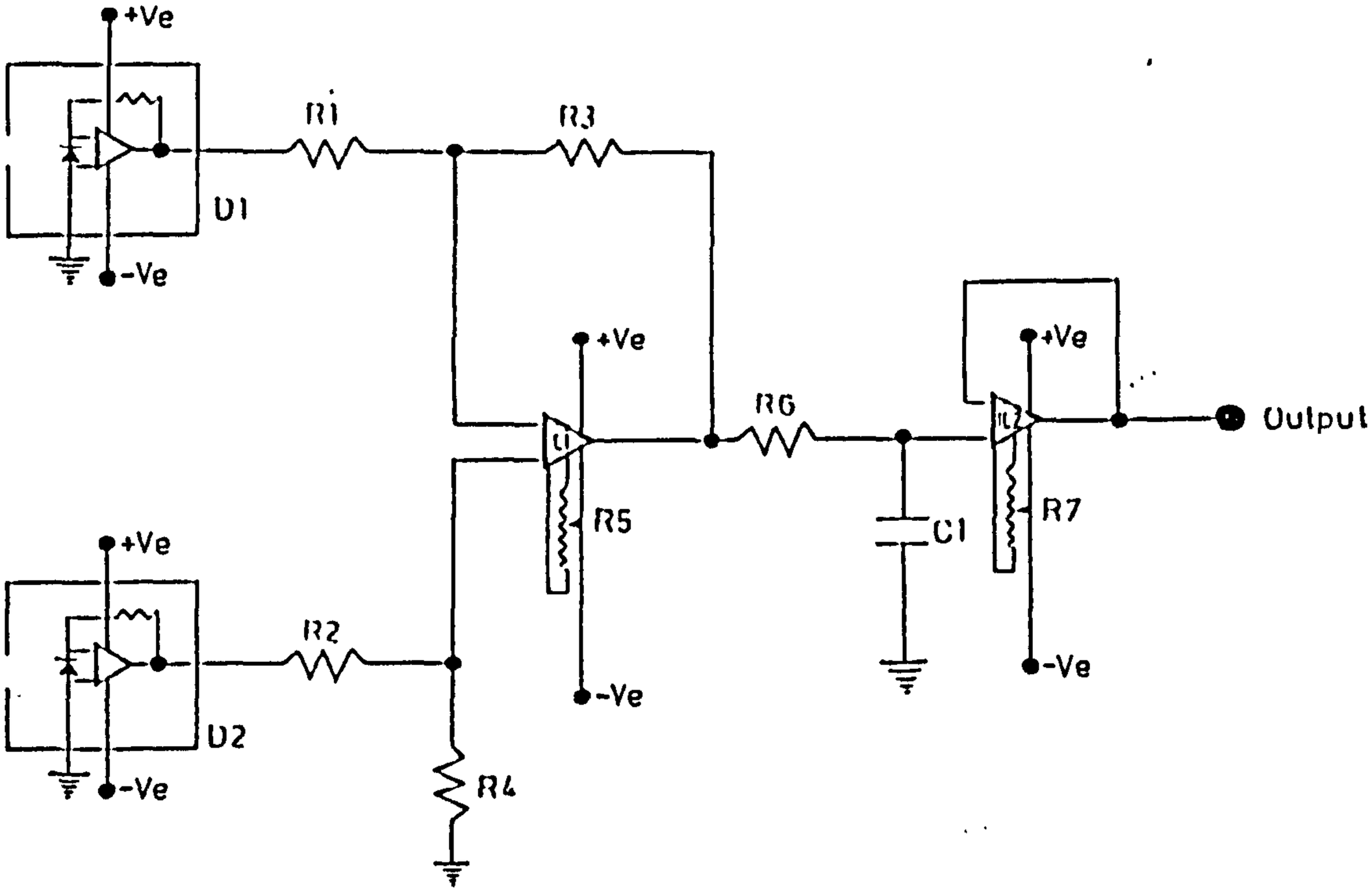
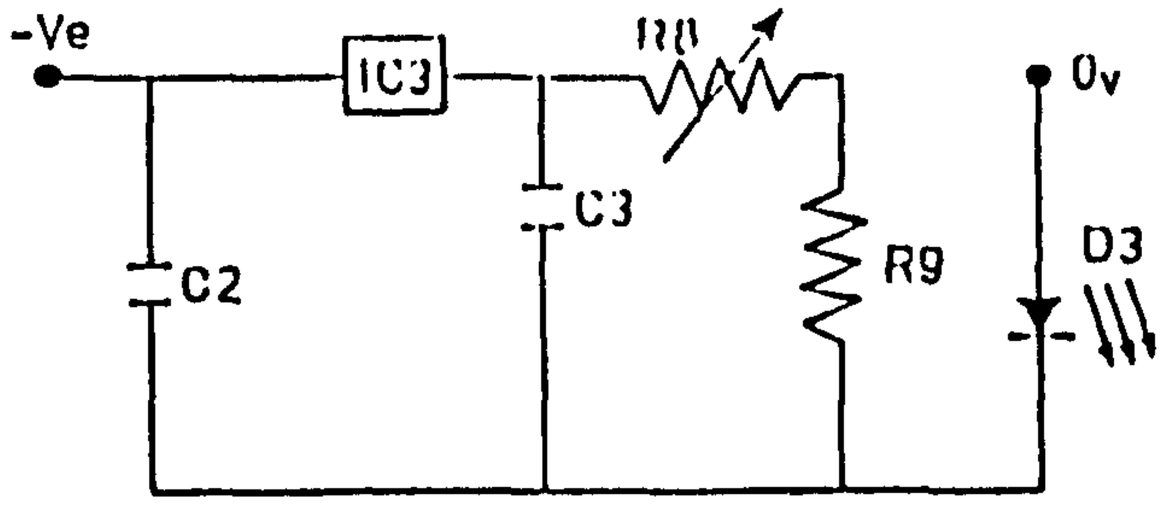


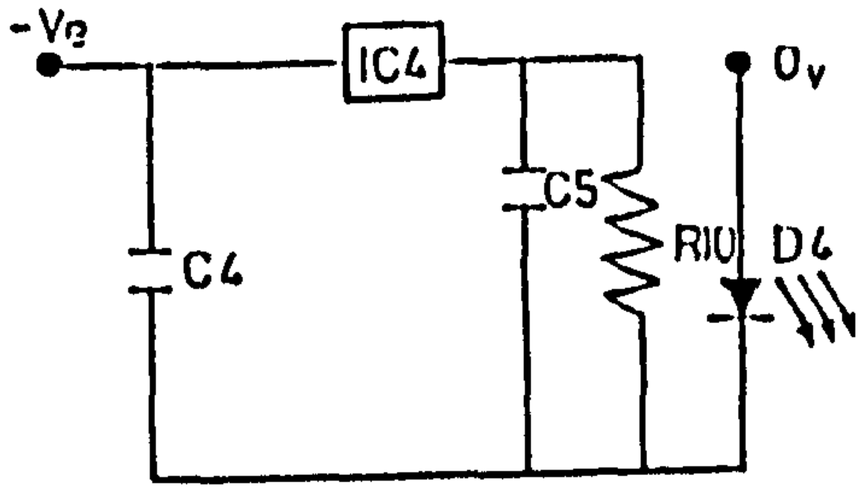
Figure 2.2. Spectral Response of Integrating Photodiode

DETECTOR CIRCUIT



SOURCE CIRCUITS





Reference

Sample

Key to Components

Resistors:	R1	4K7	Capacitor:	C1	1·0 µF
	R2	4K7		C2	220nF
	R3	100K		C3	470nF
	R4	100K		C4	220nF
	R5	10K		C5	470nF
	R6	470K			
	R7	25K	Diodes:	D1 & D2 – RS Cat no. 308-067	
	R8	1K		D3 & D4 – RS Cat no. 588-263	
	R9	1K2			
	R10	1K5	integrated circuits	IC1 & IC2 – 741N	
				IC3 & IC4 – 79L05	

Figure 2.3. Circuit Diagram of the Solid State Detector

differential mode, which subtracted the reference signal from the sample signal, giving an output which was proportional to the transmittance of the sample stream. This output was then fed into a low pass filter (R6 and C1, time constant=1.03 seconds) which removed any high frequency noise, including that derived from the 50 Hz mains supply. The signal was then fed into another operational amplifier (IC2 - 741N), configured as a unity gain voltage follower, which provided a low impedance output signal that was capable of driving a strip chart recorder.

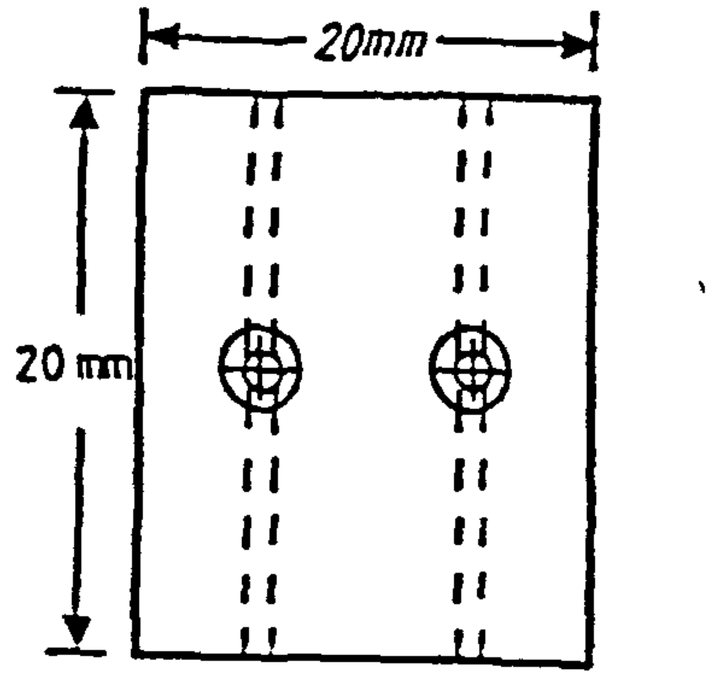
The LED´s were driven from constant current sources (IC3 and IC4 - 79L05 and associated passive components) which kept the light output of the LED´s constant. One of the constant current sources was variable so that the reference channel signal could be adjusted in order to zero the detector output.

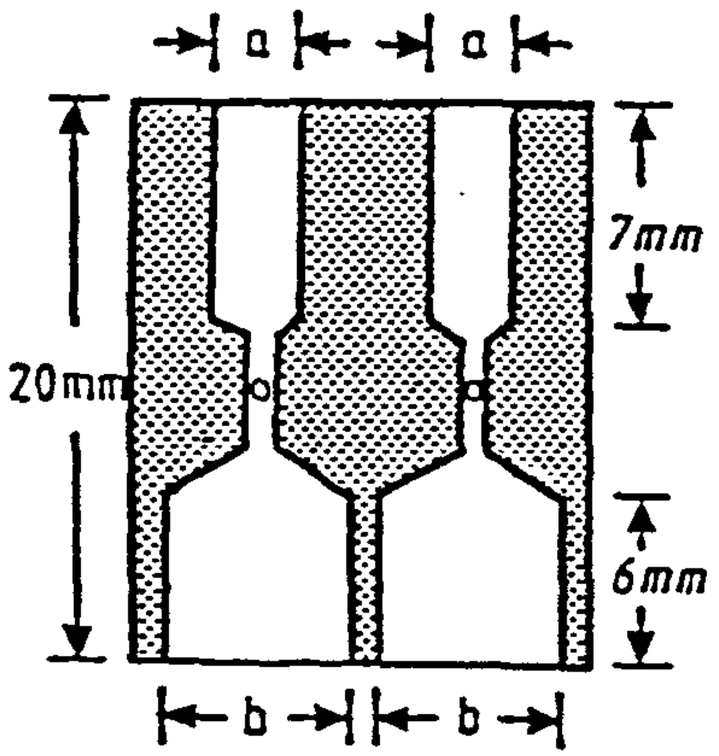
The circuit was constructed on a purpose designed printed circuit board which helps in the elimination of noise and simplifies the construction of the detector electronics. The circuit in use had low noise (1 mV peak to peak) and negligible baseline drift was observed over a one month period.

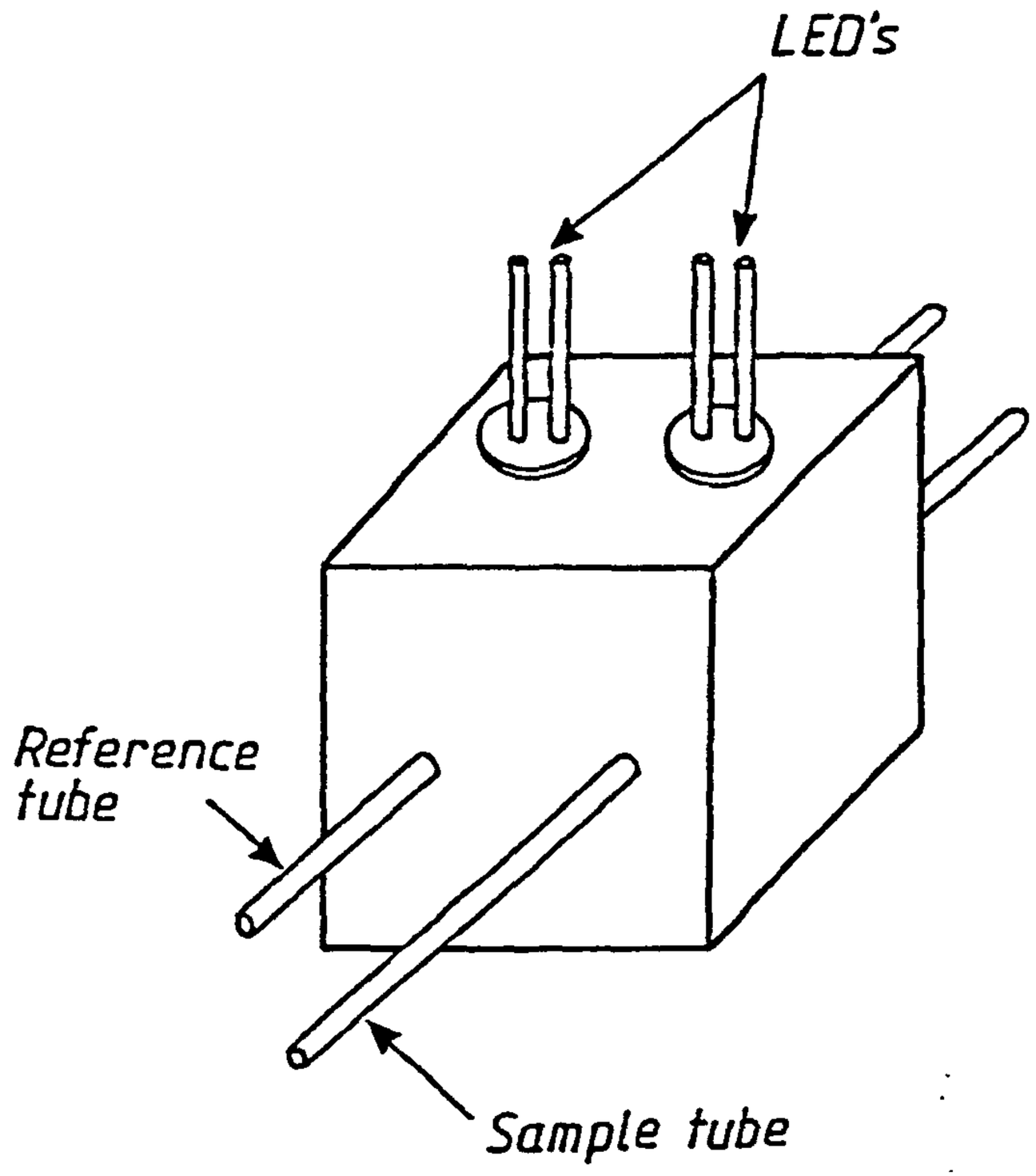
2.2.1.2. Flowcell Design

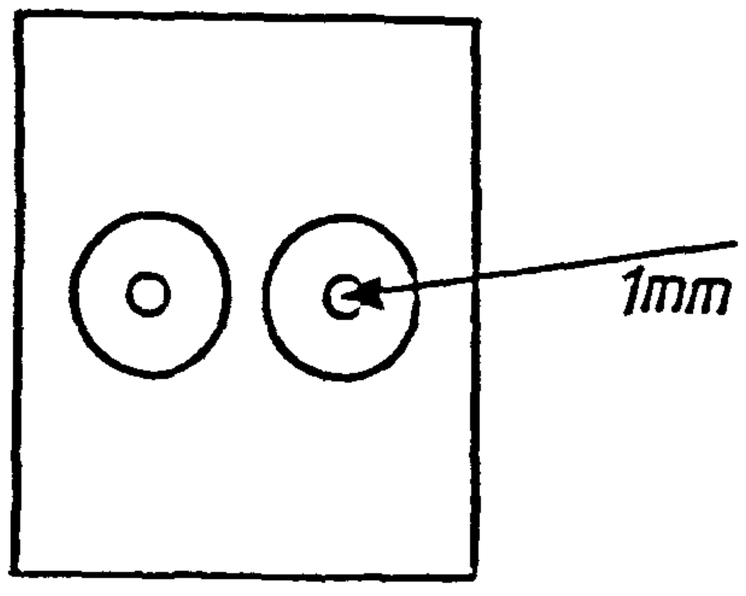
The flowcell block (figure 2.4) was machined out of aluminium, and in it, the light from the LED source passed through the teflon tubing carrying the flow stream and impinged on the integrating photodiode detector. This gave an effective pathlength of 0.8 mm if 0.8 mm

Figure 2.4. Flowcell Block









1. Dimensions 'a' nom. 5mm to fit LED.
2. Dimensions 'b' nom. 8.5mm to fit photodiode
3. Material : Aluminium.

internal diameter teflon tubing was used. Glass capillaries were evaluated for possible use in the detector block but they were found to make the detector sensitive to small changes in the refractive index of the flowing stream.

The completed flowcell block and the associated detector electronics were fitted inside a light tight box (figure 2.5.).

2.2.2. Comparison of the Solid-state Photometric Detector with a Conventional UV/Visible Detector

The 'home made' solid-state detector was compared with an LKB Ultrospec II in a simple flow injection manifold (figure 2.6.).

The LKB Ultrospec II is a single beam uv/visible spectrophotometer (20) with a Czerny Turner monochromator incorporating a 1200 line per mm holographic grating. The instrument has a wavelength range of 200 to 900 nm with an accuracy of 1 nm and a reproducibility of 0.5 nm. The bandwidth of the instrument is 5 nm and it has a stability of 0.002 absorbance units and a noise level of 0.001 absorbance units. The LKB was equipped with a 10 mm path length quartz flow cell (Hellma) and was set at 635 nm. The solid-state photometric detector had a path length of 0.8 mm and used red LED's as the light source (emission maxima=635 nm).

2.2.2.1. Experimental

A stock solution of copper (II) sulphate (AnalaR - BDH) was prepared by dissolving 100 g of $CuSO_4.5H_2O$ in 1

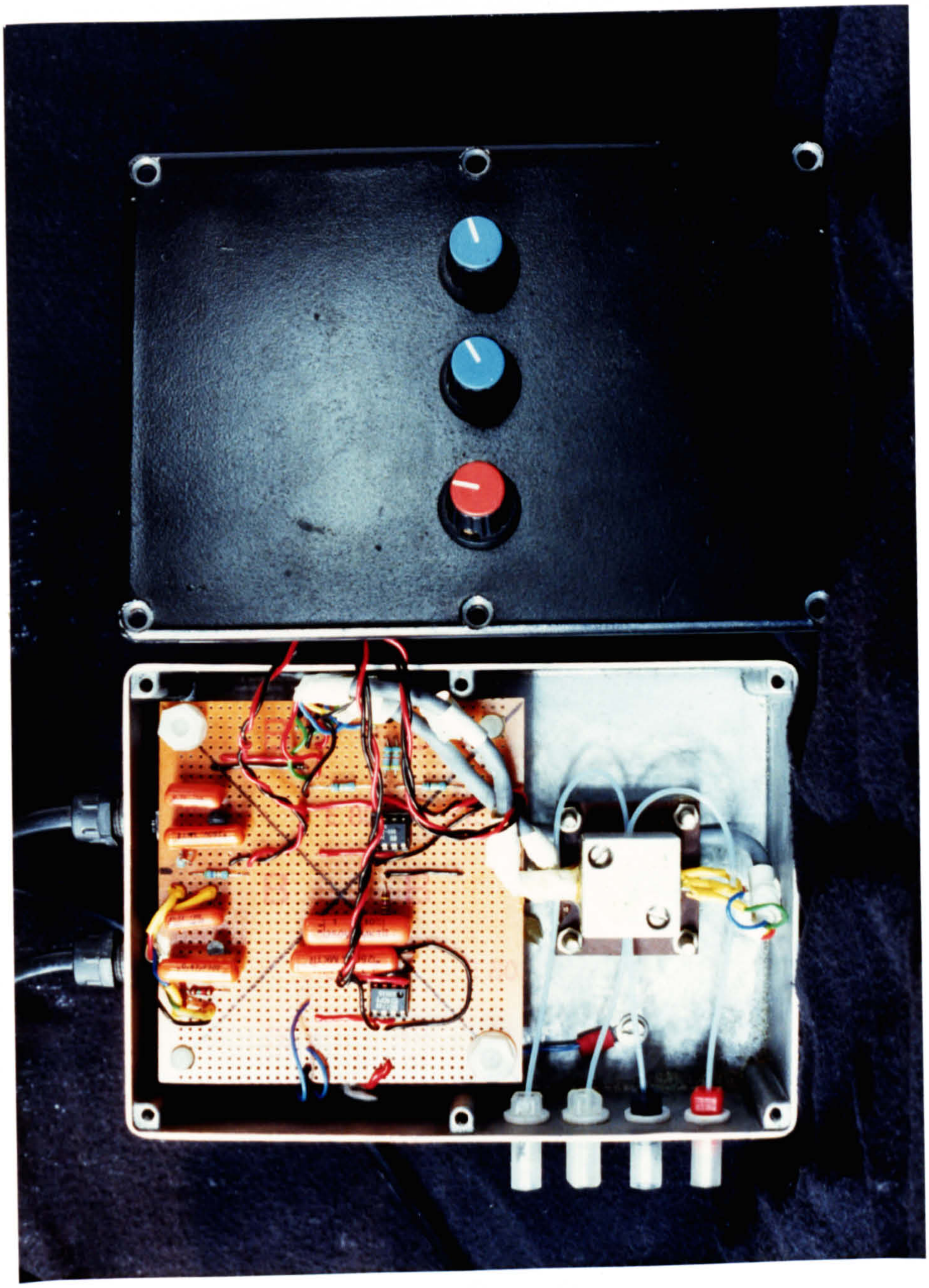


Figure 2.5. Solid State Photometric Detector

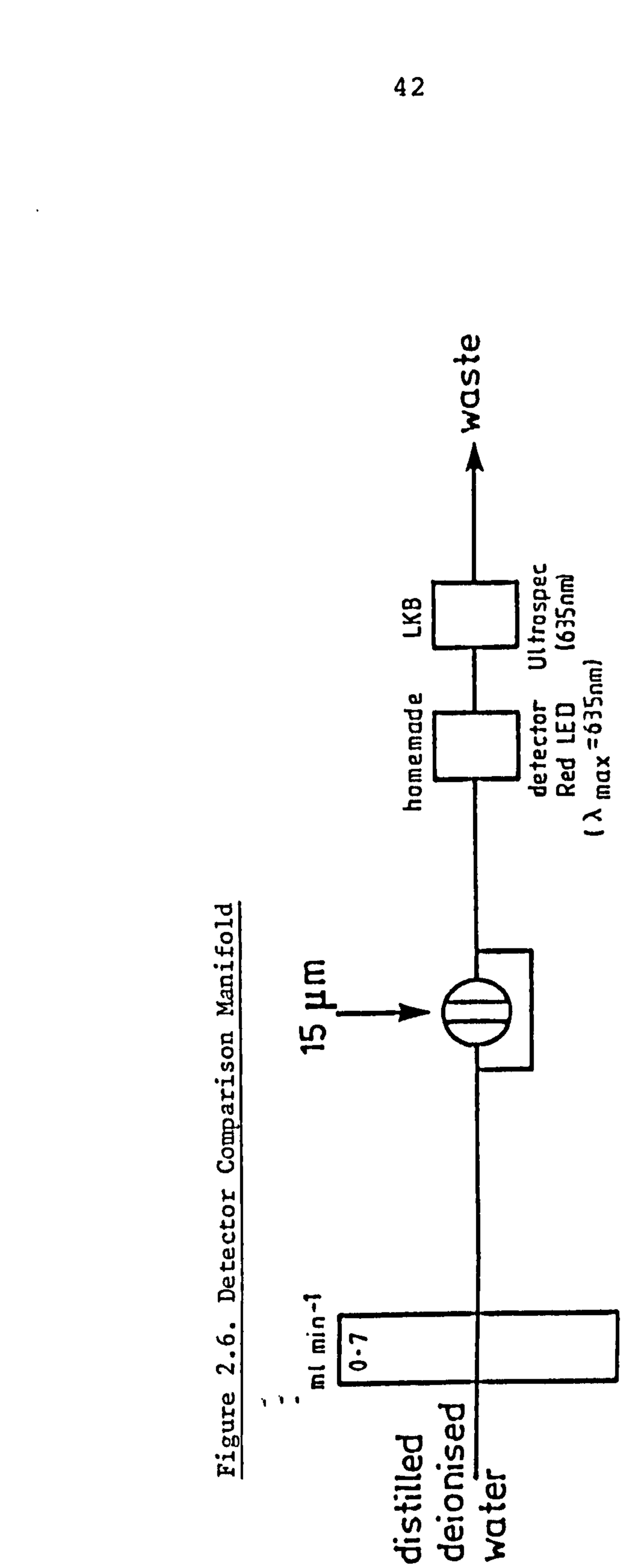


Figure 2.6. Detector Comparison Manifold

litre of water. Working solutions were prepared by serial dilution of this stock solution.

A stock solution of methylene blue (Hopkin and Williams) was prepared by dissolving 1.0 g in 1 litre of water. Working solutions were prepared by serial dilution of this stock solution.

For the comparison of linear range, working solutions of methylene blue were prepared in the range 0 to 0.5 g l^{-1} and were injected into the simple manifold (figure 2.6.). Both detectors showed the same linear range, 0 to 0.1 g l^{-1} methylene blue, above which, the signal levelled off due to saturation of the detectors by the methylene blue.

In the calculation of the limit of detection (X_{blank} + 2σ) for both detectors, working copper (II) sulphate solutions in the range 0 to 100 g l^{-1} were prepared. Calibrations were obtained for both the LKB and homemade detectors which showed that the solid-state detector was capable of determining lower concentrations of copper (II) sulphate. The solid state detector had a L.O.D. of 0.8 g l^{-1} $CuSO_4.5H_2O$ whilst the Ultrospec had a L.O.D. of 5.6 g l^{-1} $CuSO_4.5H_2O$. This was expected as the copper (II) sulphate absorption band is broad and, the LKB only has a bandwidth of 5 nm, whereas the solid-state detector has a bandwidth of 30 nm.

The drift of the solid-state detector was found to be negligible over a period of one month (2 mV) which compares favourably with the quoted figures for the LKB (0.002 absorbance units per hour). The measured noise of

the solid-state detector was 1 mV peak to peak compared to 0.001 absorbance units on the LKB.

2.3. References

1. H.Flaschka, C.McKeithan and R.Barnes, Anal. Lett., 6(1973)585.

2. T.Anfalt, A.Graneli and M.Strandberg, Anal. Chem., 48(1976)357.

3. D.Betteridge, E.L.Dagless, B.Fields and N.F.Graves, Analyst, 103(1978)897.

4. D.Betteridge, Z. Anal. Chem., 312(1982)441.

5. D.J.Hooley and R.E.Dessy, Anal. Chem., 55(1983)313.

6. K.S.Johnson and R.L.Petty, Limnol. Oceanogr., 28(1983)1260.

7. M.Trojanowicz, W.Augustyniak and A.Hulanicki, Mikrochim. Acta, II(1984)17.

8. K.S.Johnson, C.L.Beehler and C.M.Sakamoto-Arnold, Anal. Chim. Acta, 179(1986)245.

9. P.J.Worsfold, J.R.Clinch and H.Casey, Anal. Chim. Acta, 197(1987)43.

10. M.Trojanowicz, J.Szpunar and E.Pobozy, in press.

11. D.Betteridge, W.C.Cheng, E.L.Dagless, P.David, T.G.Goad, D.R.Deans, D.A.Newton and T.B.Pierce, Analyst, 108(1983)1.

12. T.Sly, D.Betteridge, D.Wibberley and D.G.Porter, J. Autom. Chem., 4(1982)186.

13. J.R.Clinch, P.J.Worsfold and F.Sweeting, Anal. Chim. Acta, (in press).

14. J.R.Clinch, P.J.Worsfold and H.Casey, Anal. Chim. Acta, 200(1987)523.

15. G.J.Schmidt and R.P.W.Scott, Analyst, 109(1984)997.

16. N.Opitz and D.W.Lubers, in "Oxygen Transport to Tissue", Vol.6, Plenum, New York, 1984, Ed. D.Bruley, H.Bicher and D.Renan.

17. M.Grayson, "Encyclopedia of Semiconductor Technology", Wiley Interscience Publications, New York, 1984, pages 410-435.

18. G.Elion and H.Elion, "Electro-optics Handbook", Marcel Dekker Inc., 1979, pages 103-117.

19. Ibid. 18, pages 557-608.

20. LKB Ultrospec II Service Manual, LKB Biochrom, Cambridge, 1985.

CHAPTER THREE

DETERMINATION OF PHOSPHATE

3.1. Introduction

3.1.1. Environmental Aspects

Phosphor us may be present in natural waters in a wide variety of physical and chemical forms (1-3). It may be present in solution, in suspension or adsorbed onto particulate matter, as orthophosphate, condensed pyrophosphates (e.g pyro- and polyphosphates) or as organophosphorus compounds.

Orthophosphate is the most important form as it is readily available for uptake in biological processes. The orthophosphate concentration of natural waters can vary widely depending on the influence of man. The major source of orthophosphate is sewage effluent where it is considered that half the phosphate load is attributable to bodily discharges and the other half to detergents and washing powders (4). In detergents and washing powders, phosphorus is present as tri-poly-phosphates which give sequestering properties to the washing powder. These tri-poly-phosphates undergo a slow reversion to orthophosphate in natural waters where they are subject to attack by enzymes and microorganisms. In industrial boilers orthophosphate is added to precipitate calcium and thus prevent boiler scale which can be difficult to remove. Ortho- and other forms of phosphate are applied in large quantities to agricultural land as fertiliser and, although most of this is not actually used by the

plants (5), its limited solubility means that it is only carried into the aquatic system during heavy rainfall. Organophosphorus compounds are used as insecticides and may also find their way into surface waters by misuse or by natural processes, although the quantities used are small compared to other sources.

Phosphorus is an essential nutrient and is often the limiting factor in the biological activity of a body of water (6). In such cases the discharge of large amounts of phosphate, either from sewage or land run-off, will stimulate the growth in nuisance quantities of photosynthetic aquatic microorganisms. This can lead to phenomena such as 'algal' bloom occurring, where previously nutrient starved (oligotrophic) rivers and lakes become nutrient rich (eutrophic) and the dissolved oxygen content of the water may then be lowered to such an extent that other aquatic lifeforms may suffer.

The prevention of eutrophication in waters is usually achieved by careful monitoring of the phosphate inputs to the body of water as these tend to be point sources such as effluent outflows. Conventional biological treatment of sewage is not very good at removing phosphate, typically only as little as 25% is removed.

As half the phosphate load of sewage is attributable to detergent use, considerable research effort is being undertaken to find alternatives for use as sequestering agents. One suggested alternative is nitrilotriacetic acid which has been found to act as an effective substitute for phosphate but, its widespread use would

lead to a 2% increase in the nitrate content of sewage effluents.

There are several treatments that can be used to remove orthophosphate from sewage effluent (7), the most common of which is lime precipitation (8). This makes use of the very low solubility of calcium hydroxyapetite ($Ca_5OH(PO_4)_3$) by adding lime to the raw sewage effluent. Up to 95% of the orthophosphate can be removed by this process. Alum ($Al_2(SO_4)_3.18H_2O$) is also used to remove orthophosphate by a combination of coprecipitation and adsorption. Removal of greater than 90% has been reported after subsequent multimedia and charcoal filtration (9). Careful control is required with this process otherwise aluminium will be released into the aquatic environment with subsequent detrimental effects.

Orthophosphate has no known toxic effect in its aqueous form and the E.E.C. has set a guideline level for phosphate in drinking water as 87 $\mu g\ l^{-1}$ phosphorus as phosphate (PO_4-P) and a maximum admissible concentration of 1091 $\mu g\ l^{-1}$ (10). This is primarily to prevent problems associated with the water when it finally discharged back into the aquatic environment.

The levels of orthophosphate found naturally in waters are low, typically in the range 10-200 $\mu g\ l^{-1}$, and so it is important that a sensitive assay is available to meet this demand.

3.1.2. Methods for the Determination of Orthophosphate

Many methods have been reported for the determination of orthophosphate including,

(i) Molybdenum blue method :- this is based on the work of Murphy and Riley (11,12) in which phosphate reacts with an acidic molybdate solution containing antimony and the resulting phosphomolybdic acid is then reduced to a blue coloured complex, molybdenum blue. This method is the most widely used and has been adapted to flow injection analysis (13-15).

(ii) Malachite green method :- phosphate reacts with molybdate and malachite green under acid conditions to form a green coloured complex which can then be determined spectrophotometrically.

(iii) Voltammetric method :- phosphate reacts with molybdate in acidic medium and the resultant molybdophosphoric acid is determined by its reduction at a glassy carbon electrode (17-19).

(iv) Ion chromatography :- phosphate is separated from other ions on an anion-exchange column, and is either selectively determined using a post-column reaction specific for orthophosphate (20) or by using an electrochemical detector (21) which has the added advantage of determining other anions as well.

The molybdenum blue method was chosen for further investigation into its suitability for incorporation into a water monitor as it seemed to offer the best

sensitivity and selectivity for the determination of orthophosphate.

3.1.3. Molybdenum Blue Method

The general reaction scheme is,

$$H_3PO_4 + 12H_2MoO_4 \longrightarrow H_3P(M_{12}O_{40}) + 12H_2O$$

$$Mo(VI) \longrightarrow Mo(V)$$

Phosphate reacts with an acidic solution of ammonium molybdate and the resultant yellow phosphomolybdic acid can be determined directly by uv/visible spectroscopy (22). Reduction of the yellow heteropoly acid produces an intensely blue coloured complex, molybdenum blue, which can be determined spectrophotometrically at wavelengths between 660 and 880 nm (figure 3.1.).

The reduction step is usually carried out using ascorbic acid, other reductants, notably stannous chloride (15) have been reported, but these suffer from poor reproducibility possibly due to the unstable nature of the reductants used.

The usual method of determining phosphate spectrophotometrically has been the manual assay, but this has now been superseded by automated methods including flow injection analysis which has speeded up the number of analyses that can be carried out in a given time. Three flow injection manifolds were studied for their suitability for incorporation into a water monitor.

3.2. Experimental

3.2.1. Reagents

All reagents were prepared in distilled deionised water.

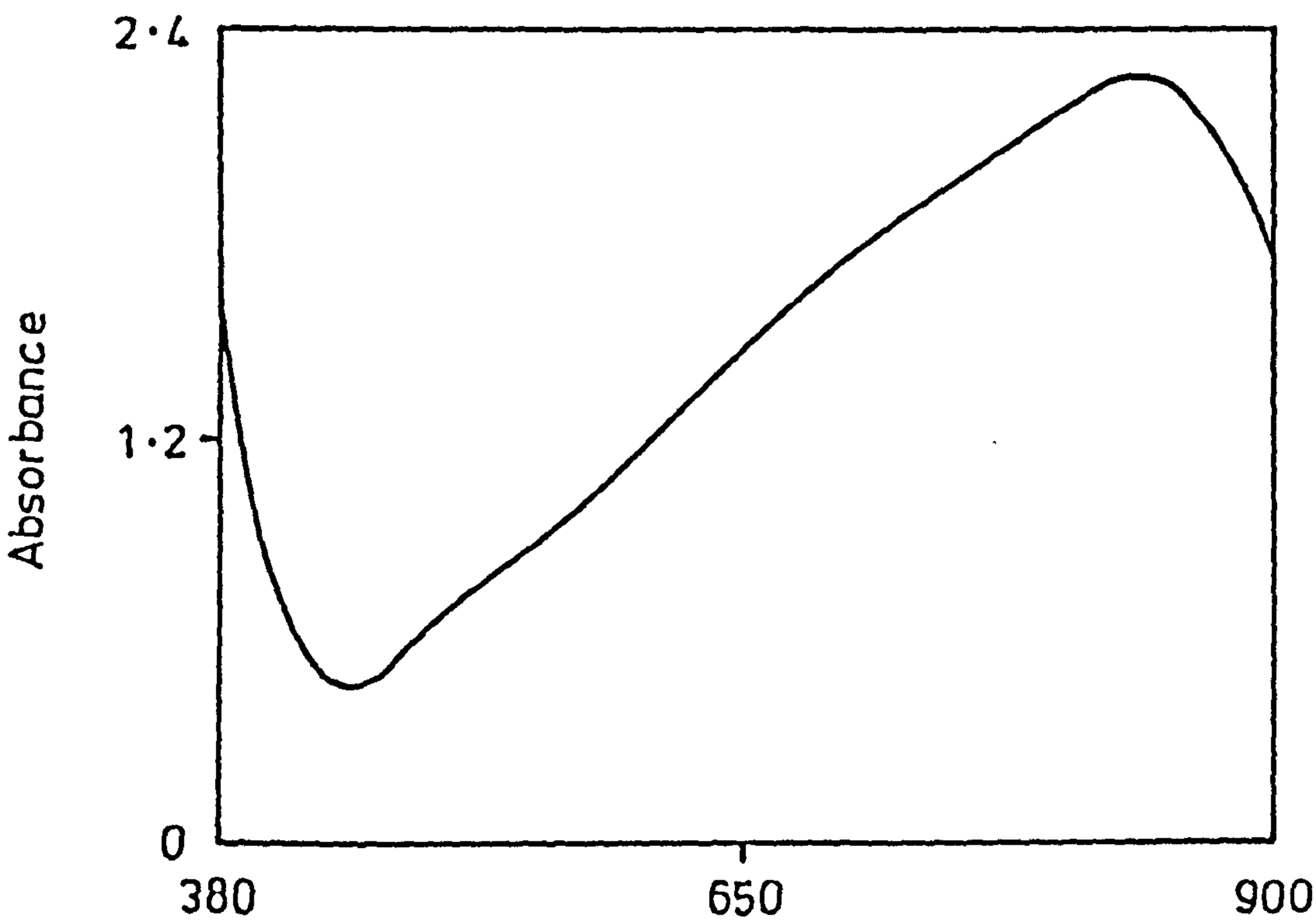


Figure 3.1. Absorption spectrum of Molybdenum Blue

Phosphate standard solution (1000 mg l^{-1}), 0.4390 g of potassium dihydrogen orthophosphate 'AnalaR' (BDH), previously dried at 105 ^{o}C for 2 hours, was dissolved in 1 litre of water which contained 400 mg l^{-1} of sodium azide (BDH) as a preservative.

Ammonium heptamolybdate 'AnalaR' (BDH).

Ascorbic acid 'AnalaR' (BDH).

Antimony potassium tartrate 'AnalaR' (H&W).

Stannous chloride 'AnalaR' (BDH).

Glycerol 'GPR' (BDH).

EIL stock silicate solution (1000 mg l^{-1} SiO_2-Si).

Nitric acid sp. gr. 1.42 'AnalaR' (BDH).

Sulphuric acid 'AR' (Avondale laboratories).

3.2.2. Equipment

Detectors : A single beam uv/visible spectrophotometer (LKB Ultrospec set at 660 nm) was used for the two and three stream manifolds, whilst a solid state detector was used for the reagent injection manifold (using red LED's as the light sources).

Manifold : The manifold was constructed using teflon tubing of 0.5 mm i.d. (Anachem) with an Ismatec mini-S-820 peristaltic pump (Labdata services) and a Rheodyne six port rotary injection valve (Anachem).

3.2.3. Two Stream Manifold

This manifold (figure 3.2.) was based on that described by Ruzicka and Hansen (23). Acid molybdate and ascorbic acid streams were merged, mixed in a coil and sample was introduced into the merged stream. The

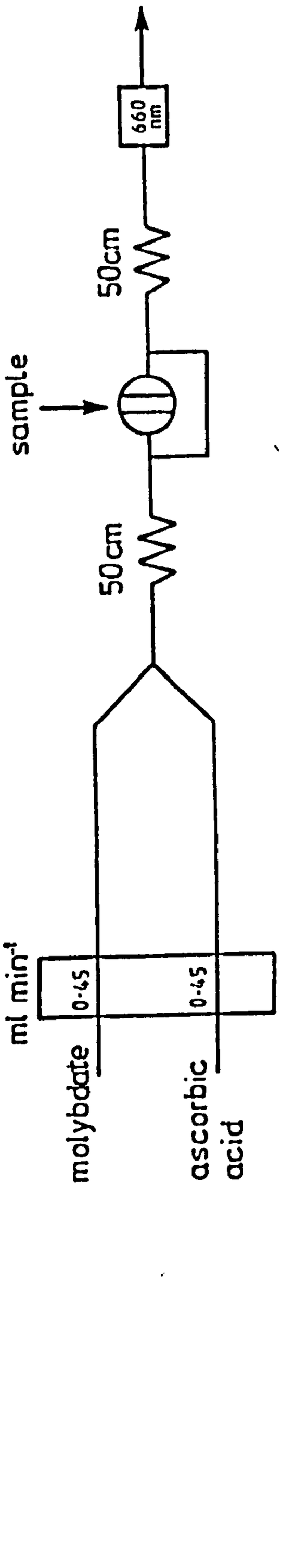


Figure 3.2. Two Stream Manifold

molybdenum blue produced was determined spectrophotometrically at 660 nm.

3.2.3.1. Experimental Conditions

Reagent mixing coil = 50 cm on a 1 cm diameter rod.

Reaction mixing coil = 50 cm on a 1 cm diameter rod.

Sample volume = 120 µl.

Ammonium molybdate concentration = 6 g l^{-1} in 0.4 M nitric acid.

Ascorbic acid concentration = 80 g l^{-1} in a 100 g l^{-1} glycerol solution.

Flow rate = 0.9 ml min^{-1} overall.

3.2.4 Three Stream Manifold

This manifold (figure 3.3.) introduced the sample into a stream of distilled deionised water which was then merged with the reagent stream. This manifold compensated for any refractive index differences between the sample and the reagent stream.

3.2.4.1. Experimental Conditions

Reagent mixing coil = 50 cm on a 1 cm diameter rod.

Reaction mixing coil = 90 cm on a 1 cm diameter rod.

Sample volume = 300 µl.

Ammonium molybdate concentration = 6 g l^{-1} in 0.4 M nitric acid.

Ascorbic acid concentration = 10 g l^{-1} in a 100 g l^{-1} glycerol solution.

Ammonium molybdate flow rate = 0.6 ml min^{-1}.

Ascorbic acid flow rate = 0.6 ml min^{-1}.

Distilled deionised water flow rate = 1.3 ml min^{-1}.

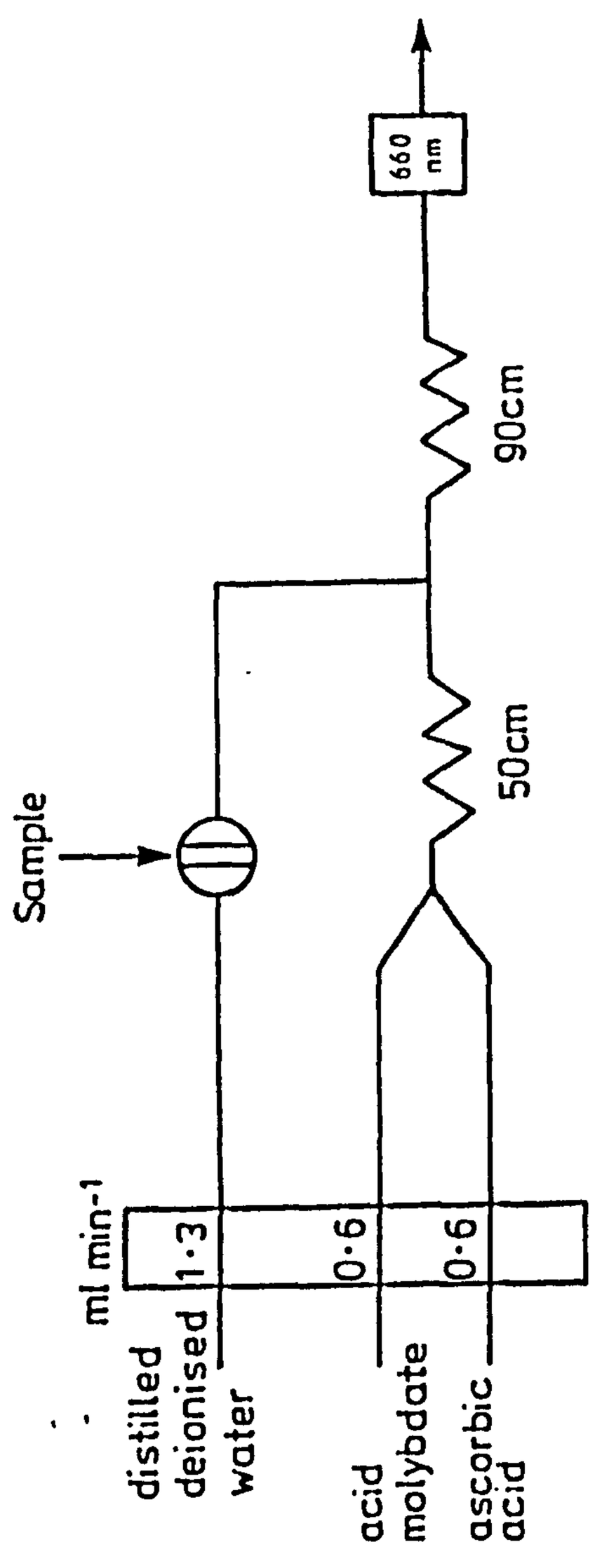


Figure 3.3. Three Stream Manifold

3.2.5. Reagent Injection Manifold

This manifold was based on that described by Johnson and Petty (24) where the reagents were mixed on-line and injected into a flowing sample stream (figure 3.4.). This method used very small volumes of reagent (typically 15 - 60 μl) and was thus most suitable for incorporation into a remote field monitor in conjunction with a solid state photometric detector.

3.2.5.1. Experimental Conditions

Reaction mixing coil = 200 cm on a 1 cm diameter rod.

Reagent mixing coil = 50 cm on a 1 cm diameter rod.

Reagent injection volume = 30 μl.

Ammonium molybdate concentration = 10 g l^{-1} in 0.4 M nitric acid.

Ascorbic acid concentration = 80 g l^{-1} in a 100 g l^{-1} glycerol solution.

Sample flow rate = 1.1 ml min^{-1}.

3.3. Results and Discussion

3.3.1. Two Stream Manifold

3.3.1.1. Optimisation

Optimisation of this manifold was carried out using a univariate approach and a 1 mg l^{-1} PO_4-P standard was used throughout.

Reaction coils with lengths in the range 25 to 200 cm were constructed on 1 cm diameter aluminium rods, and their effect on the signal obtained was studied. The signal for both a 0 and 1 mg l^{-1} PO_4-P standard decreased with increasing coil length due to increased dispersion, but the sensitivity (i.e. the difference between the

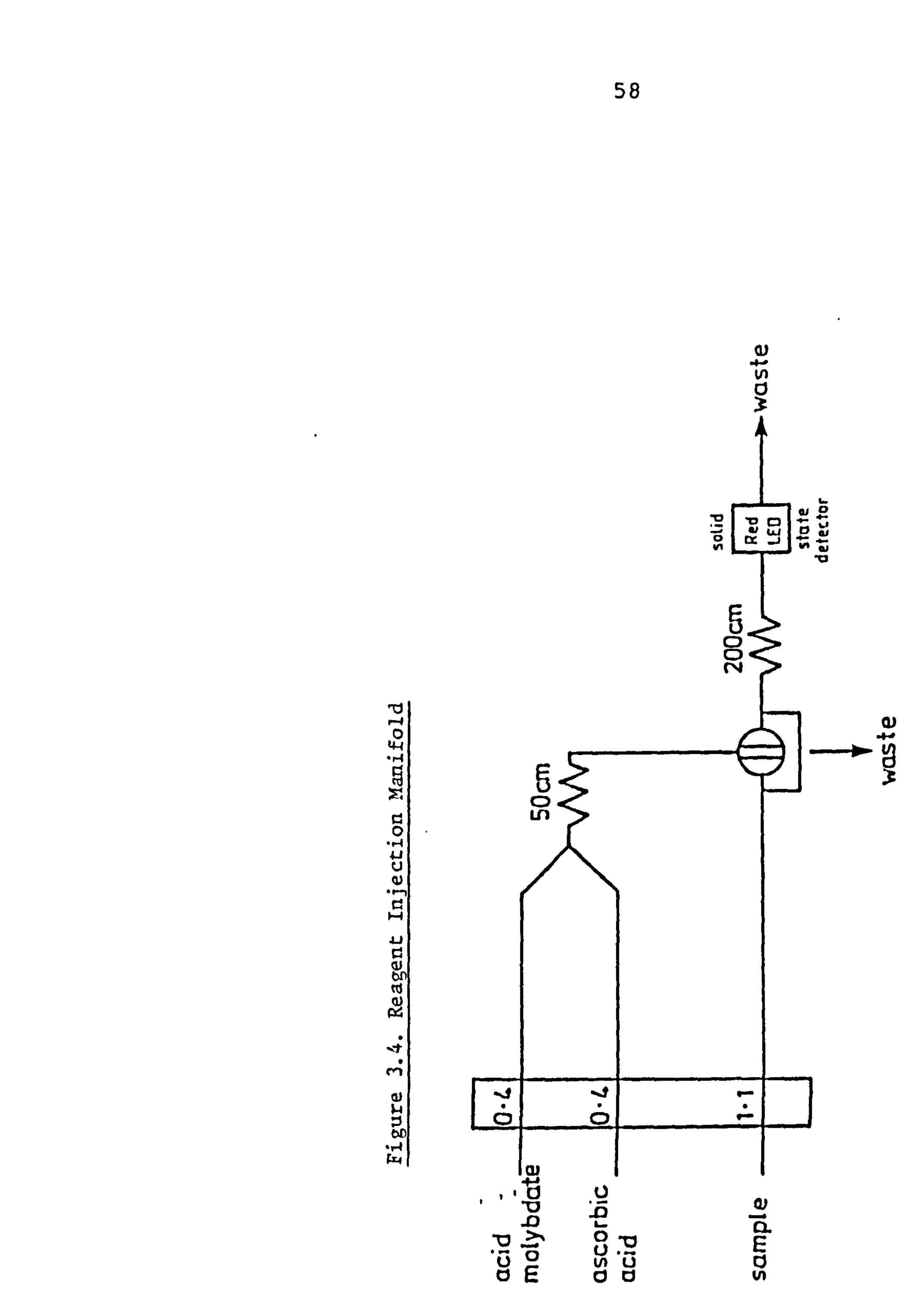


Figure 3.4. Reagent Injection Manifold

blank signal and the 1 mg l^{-1} standard signal) increased. This was due to the reaction proceeding further in the longer coil length because of the longer residence time. A coil length of 50 cm was chosen for subsequent work as being the best compromise between sample throughput and sensitivity.

The effect of flow rate was studied by varying the diameter of the peristaltic pump tubing used. As the flow rate was increased, the signal obtained decreased due to the shorter residence time of the sample zone in the manifold. The optimal flow rate was chosen as 0.9 ml min^{-1} so as to give a good rate of sample throughput.

Sample volumes in the range 30 to 300 µl were studied and, the signal obtained increased with increasing sample volume until 180 µl, above which no further increases in the signal obtained were found. A sample volume of 120 µl was chosen as the optimum.

Ascorbic acid solutions in the range 5 to 100 g l^{-1} were prepared in a 100 g l^{-1} glycerol solution. The signal was found to increase with increasing ascorbic acid concentration, and an 80 g l^{-1} solution was chosen as the optimum, as the 100 g l^{-1} solution was found to be difficult to prepare due to solubility problems.

Ammonium molybdate solutions in the range 1 to 12 g l^{-1} were prepared in 0.4 M nitric acid. The signal obtained increased with increasing ammonium molybdate concentration up to 4 g l^{-1}, above which further increases in molybdate concentration had only a small effect on the signal. This was due to an excess of

molybdate over phosphate in the sample zone, and a concentration of 6 g l^{-1} was chosen as giving the best signal.

3.3.1.2. Calibration

Calibration of the two stream manifold was obtained using phosphate standards in the range 0 to 5 mg l^{-1} PO_4-P. A calibration was obtained which was described by the equation,

signal (mV) = 166.6 x Concentration (mg l^{-1}) + 514.5

and had a correlation coefficient of 0.9998.

The mean of six replicate injections, standard deviation, and relative standard deviation for each standard solution are shown in table 3.1.. The linear calibration range was 0 to 8 mg l^{-1} PO_4-P and the limit of detection (2σ) was calculated from the blank signal as 50 μg l^{-1} PO_4-P.

3.3.2. Three Stream Manifold

3.3.2.1. Optimisation

The manifold was optimised using a univariate approach and a 1 mg l^{-1} PO_4-P standard solution was used throughout.

Reaction coils with lengths in the range 25 to 250 cm were evaluated and the signal obtained was found to increase with increasing coil length up to 90 cm, due to the increase in residence time of the sample, allowing more molybdenum blue to be formed. Above 100 cm, the signal decreased due to the effect of the increased dispersion causing sample zone broadening. A reaction coil length of 90 cm was chosen for subsequent work.

PO_4-P Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation[a] (mV)	Relative Standard Deviation[a] (%)
5.0	1348	8.4	0.6
2.5	927	4.5	0.5
1.0	692	4.1	0.6
0.5	591	4.9	0.8
0.0	514	5.4	1.1

[a] n=6

Table 3.1. Calibration of Two Stream Manifold

The effect of sample volume on the signal obtained was studied in the range 15 to 600 µl and the signal increased with increasing sample volume. The increase in signal obtained became less noticeable at sample volumes above 450 µl. A sample volume of 300 µl was chosen for subsequent work.

The overall flow rate was varied in the range 0.7 to 4.1 ml min^{-1} and the signal obtained was found to decrease with increasing flow rate. This was due to the faster flow rates lowering the amount of molybdenum blue formed due to less reaction time and increased dispersion. An overall flow rate of 2.5 ml min^{-1} was chosen so as to give the best compromise between signal and sample throughput.

Ascorbic acid solutions in the range 1 to 40 g l^{-1} were prepared in a 100 g l^{-1} glycerol solution. The signal obtained increased with increasing ascorbic acid concentration up to 20 g l^{-1} above which further increases produced no increase in the signal obtained, due to there being an excess of ascorbic acid. A concentration of 10 g l^{-1} was used in all subsequent work.

Ammonium molybdate solutions in the range 0.5 to 10 g l^{-1} were prepared in 0.4 M nitric acid and, their effect on the signal obtained was studied. The signal increased with increasing concentration up to 6 g l^{-1}, above which further increases in the ammonium molybdate concentration gave only a small increase in the signal due to their

being an excess of ammonium molybdate over phosphate. A concentration of 6 g l^{-1} was used in subsequent work.

3.3.2.2. Calibration

Calibration of the three stream manifold was obtained using phosphate standards in the range 0 to 5 mg l^{-1} PO_4-P. A typical calibration was described by the equation,

signal (mV) = 38.92 x concentration (mg l^{-1}) + 0.97

with a correlation coefficient of 0.9999. The mean of eight injections, standard deviation and relative standard deviation for each standard are given in table 3.2.. The linear range was 0 to 10 mg l^{-1} PO_4-P and the limit of detection (2σ) was calculated from the detector noise as 20 μg l^{-1} PO_4-P.

Molybdenum blue is normally determined at 660 nm, not at it s peak absorption maximum of 837 nm (figure 3.1). A calibration was obtained for phosphate at 837 nm which was described by the equation,

signal (mV) = 0.062 x concentration (μg l^{-1}) - 0.17

and had a correlation coefficient of 0.9999. The limit of detection (2σ) was determined from the detector noise as being 10 μg l^{-1} PO_4-P.

Antimony potassium tartrate has been widely reported as increasing the sensitivity of the molybdenum blue method (1,15). ~~It is thought that the antimony acts as a catalyst in the formation of the molybdenum blue complex.~~ Antimony potassium tartrate was not soluble in dilute nitric acid, so a 6 g l^{-1} ammonium molybdate solution was made up in 0.6 M sulphuric acid, and antimony potassium tartrate was added at a concentration of 0.195 g l^{-1}. A

PO_4-P Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation[a] (mV)	Relative Standard Deviation[a] (%)
5.00	194.7	0.45	0.2
2.00	79.6	1.03	1.3
1.00	40.9	0.42	1.0
0.75	31.8	0.38	1.2
0.50	19.5	0.50	2.6
0.25	10.2	0.49	4.8
0.0	0.0	--	0.0

[a] n=8

Table 3.2. Calibration of Three Stream Manifold

calibration was obtained which was described by the following equation,

signal (mV) = 0.17 x concentration ($\mu g\ l^{-1}$) -0.77

and had a correlation coefficient of 0.9997. The baseline was unstable but the limit of detection (2σ) was calculated from the detector noise as 10 $\mu g\ l^{-1}$. The ammonium molybdate solution containing antimony potassium tartrate had to be prepared daily as it became cloudy after a day's use.

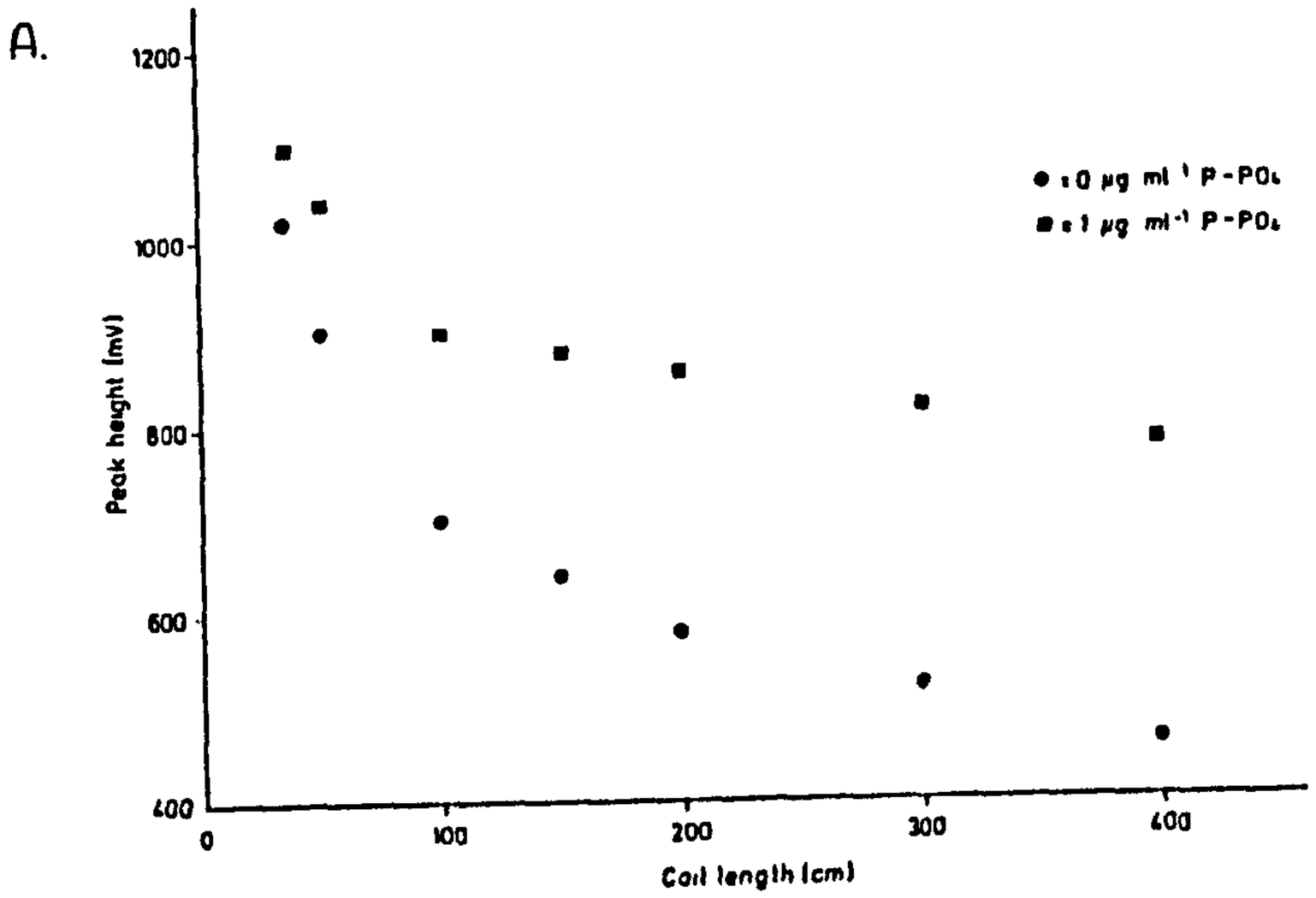
3.3.3. Reagent Injection Manifold

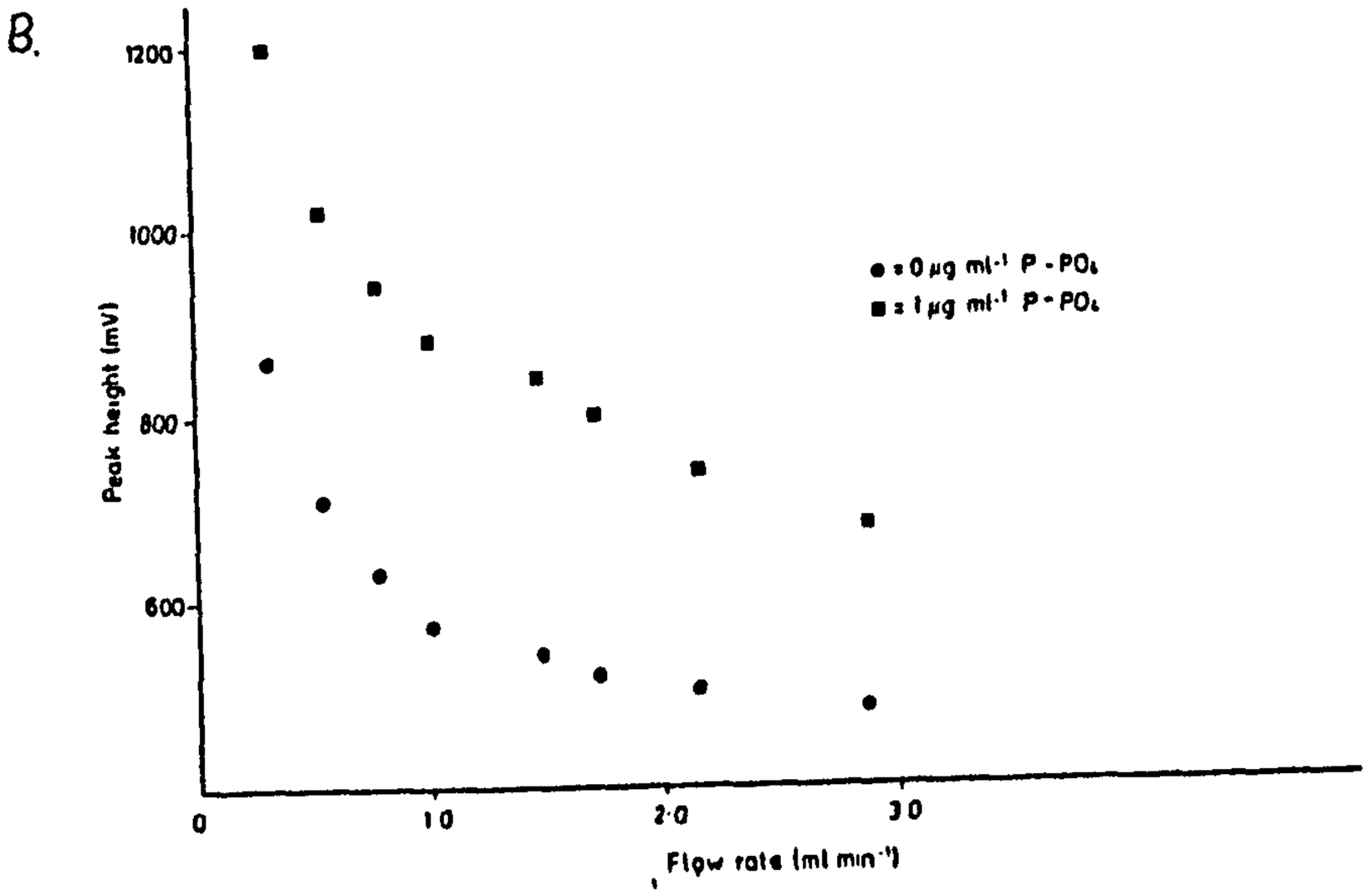
3.3.3.1. Optimisation

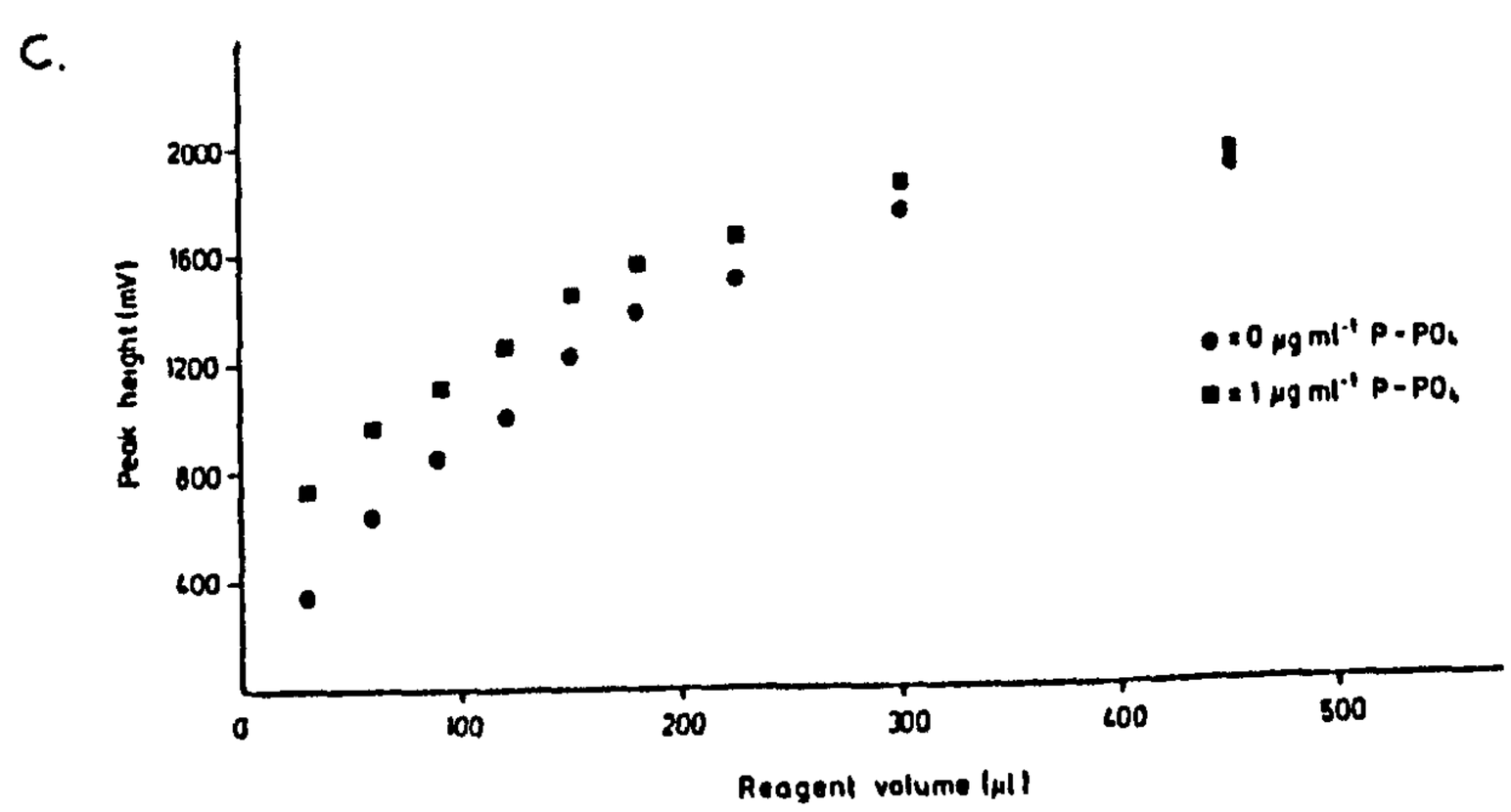
The optimisation of this manifold was carried out using a univariate approach using both 0 and 1 mg l^{-1} PO_4-P standards.

Reaction coils with lengths in the range 25 to 250 cm were constructed on 1 cm diameter aluminium rods. The signal obtained for the blank (0 mg l^{-1} PO_4-P) was due to refractive index differences between the sample stream and the injected reagent zone, which were minimised with larger coil lengths because of the increased dispersion of the reagent slug (figure 3.5.A.). The response for the 1 mg l^{-1} PO_4-P standard showed an increase in the extent of reaction at longer coil lengths because of the increased residence time, in addition to the change in signal caused by the effect of the dispersion on the refractive index of the injected reagent zone. The sensitivity of the manifold, represented here as the difference between the blank and the 1 mg l^{-1} standard, therefore, increased with increasing coil length, and a

Figure 3.5. (A) Effect of Coil Length, (B) Effect of Flow Rate, (C) Effect of Reagent Volume.







200 cm reaction coil was chosen as the optimum between sample throughput and sensitivity.

The effect of sample flow rate on sensitivity was studied in the range 0.3 to 2.9 ml min^{-1} (figure 3.5.B.). The sensitivity was improved at slower flow rates, because the increased extent of reaction more than compensated for the greater effect of refractive index changes. A flow rate of 1.1 ml min^{-1} was used for all subsequent work.

Reagent injection volumes in the range 30 to 450 µl were studied (figure 3.5.C.) and it was found that smaller reagent volumes gave improved sensitivity, with 30 µl being chosen as the optimum as it gave the best sensitivity.

Ascorbic acid solutions were prepared in the range 0.5 to 100 g l^{-1} in a 100 g l^{-1} glycerol solution. The signal obtained increased with increasing ascorbic acid concentration and an 80 g l^{-1} concentration was chosen for all subsequent work.

Ammonium molybdate solutions in the range 0.5 to 20 g l^{-1} were prepared in 0.4 M nitric acid and the signal obtained was found to increase up to 10 g l^{-1}, above which no further increase in signal was obtained. A 10 g l^{-1} concentration was used for all subsequent work.

3.3.3.2. Calibration

A calibration of the reagent injection manifold was obtained using standards in the range 0 to 1 mg l^{-1} PO_4-P which was described by the equation,

signal (mV) = 0.272 x concentration (µg l^{-1}) + 228

with a correlation coefficient of 0.9995. The mean of six replicate injections, standard deviation and relative standard deviation for each standard are given in table 3.3.. The limit of detection (2σ) was calculated from the blank signal as 12 $\mu g\ l^{-1}$ PO_4-P and the linear range was found to be 0 to 2 mg l^{-1} PO_4-P.

3.3.3.3. Reagent Stability

Reagent stability is an important prerequisite for a remote water quality monitor. Ammonium molybdate and ascorbic acid solutions were stable over a period of at least 30 days, the linear calibration obtained on day 1 was described by the equation,

$$\text{signal (mV)} = 0.272 \times \text{concentration } (\mu g\ l^{-1}) + 228$$

$$(r=0.9995)$$

and on day 31 was,

$$\text{signal (mV)} = 0.213 \times \text{concentration } (\mu g\ l^{-1}) + 234$$

$$(r=0.9967)$$

The high level of glycerol (100 g l^{-1}) added to the ascorbic acid solution stabilised the reducing agent by complexing with metal ions in the solution, thus preventing oxidation. It was also helpful in preventing molybdenum blue from precipitating on the walls of the teflon tubing. This is very important because if this is not added, the tubing quickly becomes coated with a blue deposit. When ascorbic acid solutions were made up in glycerol solutions of a lower strength they soon lost their ability to reduce phosphomolybdic acid to molybdenum blue (within 3 days).

PO_4-P Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
1.0	502.5	--	0.0
0.5	358.5	2.55	0.7
0.3	311.5	2.60	0.8
0.2	283.0	2.45	0.9
0.1	254.0	2.05	0.8
0.0	230.5	1.95	0.9

[a] $n=6$

Table 3.3. Calibration of Reagent Injection Manifold

3.3.3.4. Interferences

Many species interfere with this assay (1), e.g. arsenic, silicon and germanium, by forming heteropoly acids with the molybdate in direct competition with phosphate. Other interfering species include oxidising agents which either react with ascorbic acid or, reoxidise the molybdenum blue, such as chromium or vanadium. Sulphide also interferes by reacting with the molybdate. Of all the interfering species only silicate is present in natural waters at high concentrations (typically 2-10 mg l^{-1} SiO_2-Si). The effect of silicate concentration on the signal obtained from a 1 mg l^{-1} PO_4-P solution was studied in the range 0 to 1000 mg l^{-1} SiO_2-Si (silicon as silicate). Although large silicate concentrations gave rise to serious interferences, 50 mg l^{-1} SiO_2-Si resulted in only a 1% increase in the signal obtained and, 10 mg l^{-1} SiO_2-Si had no effect.

3.4. Comparison of Methods

Of the three methods studied, the reagent injection manifold was the most suitable for incorporation into a remote photometric water quality monitor due to it s low reagent consumption and high sensitivity (12 μg l^{-1} PO_4-P). Another factor in it s favour was that the sample or one of the standards was always flowing through the flow cell and so it was always being flushed out to prevent a build up of molybdenum blue. The two stream manifold suffered from a large blank signal due to the refractive index differences between the sample and the reagent streams, and also had the lowest sensitivity of the

manifolds studied (L.O.D.=50 $\mu g\ l^{-1}$). The three stream manifold overcame this blank signal and, was also more sensitive (L.O.D.=10 $\mu g\ l^{-1}$ PO_4-P) and would be suited to a laboratory based phosphate analyser.

3.5. References

1. "Phosphorus in Waters, Effluents and Sewages, 1980", Methods for the Examination of Waters and Associated Materials, H.M.S.O., London, 1980.

2. "Standard Methods for the Examination of Waters and Wastewater", American Public Health Association, 14th Edition, Washington, 1975, pages 466-483.

3. I.L. Marr and M.S.Cresser, "Environmental Chemical Analysis", International Textbook Company, New York, 1983, Pages 126-127.

4. F.W.Roberts, Effluent and Water Treatment, 17(1977)599.

5. J.K.R.Gasser, Chem. Brit., 24(1988)129.

6. "Eutrophication : Causes, Consequences, Correctives", Proceedings of a Symposium, National Academy of Sciences, Washington, 1969.

7. D.Barnes and F.Wilson, "Chemistry and Unit Operations in Sewage Treatment", Applied Science Publishers, London, 1978.

8. R.L.Culp and G.L.Culp, "Advanced Wastewater Treatment", Van Nostrand Rheingold, New York, 1971

9. M.Speigel and T.Forrest, J. San. Eng. Div., 95(1969)803

10. J.Gardinier and G.Mance, "United Kingdom Water Quality Standards arising from European Community Directives", Technical Report TR 204, WRC Environment, Stevenage, 1984.

11. J.Murphy and J.P.Riley, J. Marine Biol. Assoc. U.K., 37(1958)9.

12. J.Murphy and J.P.Riley, Anal. Chim. Acta, 27(1962)31.

13. J.Ruzicka and E.H.Hansen, "Flow Injection Analysis", Wiley Interscience Series, Volume 62, New York, 1977.

14. M.Valcarcel and M.D.Luque de Castro, "Flow Injection Analysis - Principles and Applications", Ellis Horwood, Chichester, 1987.

15. T.A.H.M.Janse, P.F.A.Van der Wiel and G.Kateman, Anal. Chim. Acta, 155(1983)89.

16. S.Motomizu, T.Wakimoto and K.Toei, Talanta, 30(1983)333.

17. A.G.Fogg and N.K.Bsebsu, Analyst, 106(1981)1288.

18. A.G.Fogg and N.K.Bsebsu, Analyst, 107(1982)566.

19. T.Fujinaga,N.K.Bsebsu and B.J.Birch, Bunseki Kagaku, 29(1980)367.

20. M.Braungart and H.Russel, Chromatographia, 19(1984)185.

21. "Ion Chromatography", Ed. J.G.Tarter, Marcel Dekker Inc., New York, 1987.

22. J.F.Kennedy and P.A.Westman, Anal. Chim. Acta, 55(1971)448.

23. Ibid. 13, pages 133-134.

24. K.S.Johnson and R.L.Petty, Anal. Chem., 54(1982)1185.

CHAPTER FOUR

DETERMINATION OF NITRATE

4.1. Introduction

4.1.1. Nitrate in Natural Waters

In natural waters the forms of nitrogen that are of greatest interest are nitrate, nitrite, ammonia and organic nitrogen (1,2). All of these forms of nitrogen, as well as atmospheric nitrogen, are biochemically interconvertible and thus are components of the nitrogen cycle (3).

Nitrate contains nitrogen in its highest oxidation state and is regarded as an end product of the nitrogen cycle. In natural waters nitrate usually comes from one or more of four sources, fertilisers, sewage effluent, acid rain and the nitrogen cycle.

Nitrate has become essential to modern agriculture and large quantities of fertiliser (containing up to 25% by weight of nitrogen) are added to agricultural land each year. Due to the high water solubility of nitrate compounds most of this finds its way into surface and ground waters by means of atmospheric precipitation (5,6). Nitrogenous fertiliser usage in Britain has trebled between 1960 and 1963 (7), and the amount now applied is at the optimum for most arable crops. Nitrogenous fertiliser is now being applied to grassland in increasing quantities to increase the amount of hay and silage produced and, it has been suggested that the consumption of nitrogenous fertiliser will increase by a

further 50% by the year 2000, with most of this increase being applied to grassland (8).

Nitrate is present in domestic wastewater in small quantities, but the effluent of a nitrifying biological treatment works may contain nitrate at levels of up to 30 mg l^{-1} NO_3-N (nitrate as nitrogen), due to the oxidation of ammonia and organic nitrogen compounds present in raw sewage. Nitrates can also be found in the effluents from industrial processes which are either discharged to the sewage system or directly into surface waters.

Another source of nitrate of growing concern is that of acid rain, large industrial complexes and fossil fuel burning power stations emit large quantities of nitrogen oxides which combine with rain to form nitrous and nitric acid. Nitrates can also be formed as part of the nitrogen cycle, giving an indication of the state of the cycle at that point.

Nitrate is an essential nutrient for many photosynthetic autotrophs and in some cases has been identified as the growth limiting nutrient for some bodies of water. In such cases, the discharge of large amounts of nitrate to the body of water can stimulate the growth of photosynthetic autotrophs in nuisance quantities, which may cause a lowering of the dissolved oxygen level to such an extent that other aquatic life may suffer.

4.1.2. Nitrate and Health

High levels of nitrate in drinking water can cause an illness in babies up to six months old known as infant

methaemoglobinemia (9). Nitrate is reduced to nitrite by bacteria present in the gut which then stimulates red blood cells to produce methaemoglobin and, in babies under six months old who have low levels of methaemoglobin reductase, the level of methaemoglobin builds up (10). Methaemoglobin prevents red blood cells from carrying oxygen and thus the babies turn blue, hence the alternative name for this disease, 'blue baby syndrome'.

Nitrates have also been linked to gastric cancer (7) although this has yet to be proven conclusively. The theoretical route is that nitrates can be reduced to nitrite in the mouth and stomach, particularly when the gastric acidity is low, as occurs in infancy, old age, and certain disease states. Nitrite can then combine with other nitrogenous substances (e.g. amines, amides, etc.) normally present to form N-nitroso compounds, some of which are known to be acutely carcinogenic in animals.

It is for these reasons that the European Economic Community (E.E.C.) has set a guideline level of 5.65 mg l^{-1} NO_3-N and a maximum admissible concentration (which must not be exceeded) of 11.3 mg l^{-1} NO_3-N for surface waters intended for abstraction into the drinking water system (11).

Nitrate levels in ground and surface waters have increased steadily over the last twenty years (3) due to increasing use of nitrogenous fertilisers in farming. Groundwater sources are becoming increasingly contaminated with nitrate and the trend is such that most

sources will increase to levels above the E.E.C. maximum in the next twenty years. The majority of groundwater sources in the United Kingdom are from chalk aquifers, and studies that have been undertaken on the time that it takes for water to travel through the rock to reach the saturated water zone of the aquifer, show that it can take between ten and thirty years (12-14). This means that the current levels of nitrate in groundwaters relate to land usage up to thirty years ago and thus, in the future, we must expect a further increase in levels due to the increase in fertiliser usage over the last thirty years. This problem is particularly relevant in eastern and southern parts of the United Kingdom where chalk aquifers and intensive farming are common. Other developed countries are also suffering from nitrate contamination of their aquifers, such as the Netherlands where 70% of the water supply is obtained from groundwater sources (15) and New Zealand where the public health hazard is becoming serious (16).

Nitrate removal from water destined for public supply has been considered but found to be either too expensive at present or unsafe due to health reasons. Ion-exchange columns remove nitrate but at present are very expensive and need frequent replenishment due to the presence of other anions which will also be removed by the column. Another method involves the reduction of nitrate to gaseous nitrogen using bacteria, but because this method uses methanol as an additive, health concerns over the effects of prolonged exposure to residual levels of

methanol prevented this method from gaining acceptance. The method currently being used by some water authorities involves blending high nitrate level water with water from a low nitrate source. This is only practicable in certain areas where such supplies are available, as some areas have no water sources which are low enough in nitrate.

In the future greater controls will need to be placed over water supply catchment areas in order to minimize the leaching of nitrate from farming activities into the groundwater. In Avon, Wessex Water have taken control of the land in part of a water supply catchment area near Bath and have thus been able to control the agricultural practices used and study their effects on the quality of the groundwater (17).

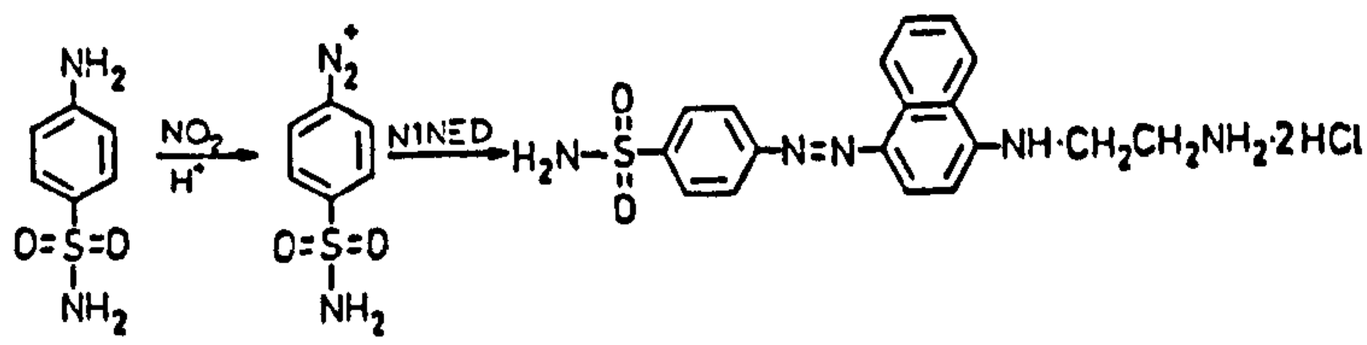
4.1.3. Methods for the Determination of Nitrate

Many methods for the determination of nitrate in natural waters have been reported, but most involve the reduction of nitrate to nitrite followed by a diazo coupling reaction to produce a deeply coloured azo dye which can then be determined photometrically.

Many methods of reducing nitrate to nitrite have been suggested, including the use of solid reductors incorporating either zinc (18,19) or various forms of cadmium (20-22), and the use of soluble reducing agents such as hydrazinium sulphate (23,24) and, titanium (III) tetrachloride (25). The most widely used method is that involving cadmium which has been coated chemically with a layer of copper and is then used either as a packed

column or coil in either continuous flow or a batch (manual) method. The reduction step is interfered with by most oxidising or reducing agents which either oxidise the resultant nitrite back to nitrate or are preferentially reduced, thus lowering the reducing capacity of the cadmium. The copper coating is used because it forms a galvanic cell with the cadmium, with the copper acting as the cathode. The e.m.f. of the cell formed is 0.740 volts and this provides a much greater reduction potential than cadmium on its own (20).

The diazo coupling step is specific for nitrite and the most favoured coupling reagents are N-1-naphthylethylenediamine dihydrochloride (N1NED) and sulphanilamide. These are coupled together to form a deep pink coloured azo dye which exhibits a maximum absorbance at 542 nm (figure 4.1.). The procedure involves the nitrite reacting with the sulphanilamide under acid conditions to form the highly unstable diazonium ion. The diazonium ion then couples with the N1NED to form the deeply pink coloured azo dye.



Although the diazo coupling step makes this method specific for nitrate, if nitrite is present in the sample then it will affect the result obtained. However, nitrite levels found in natural waters are low, typically in the 10-100 $\mu g\ l^{-1}$ NO_2-N range.

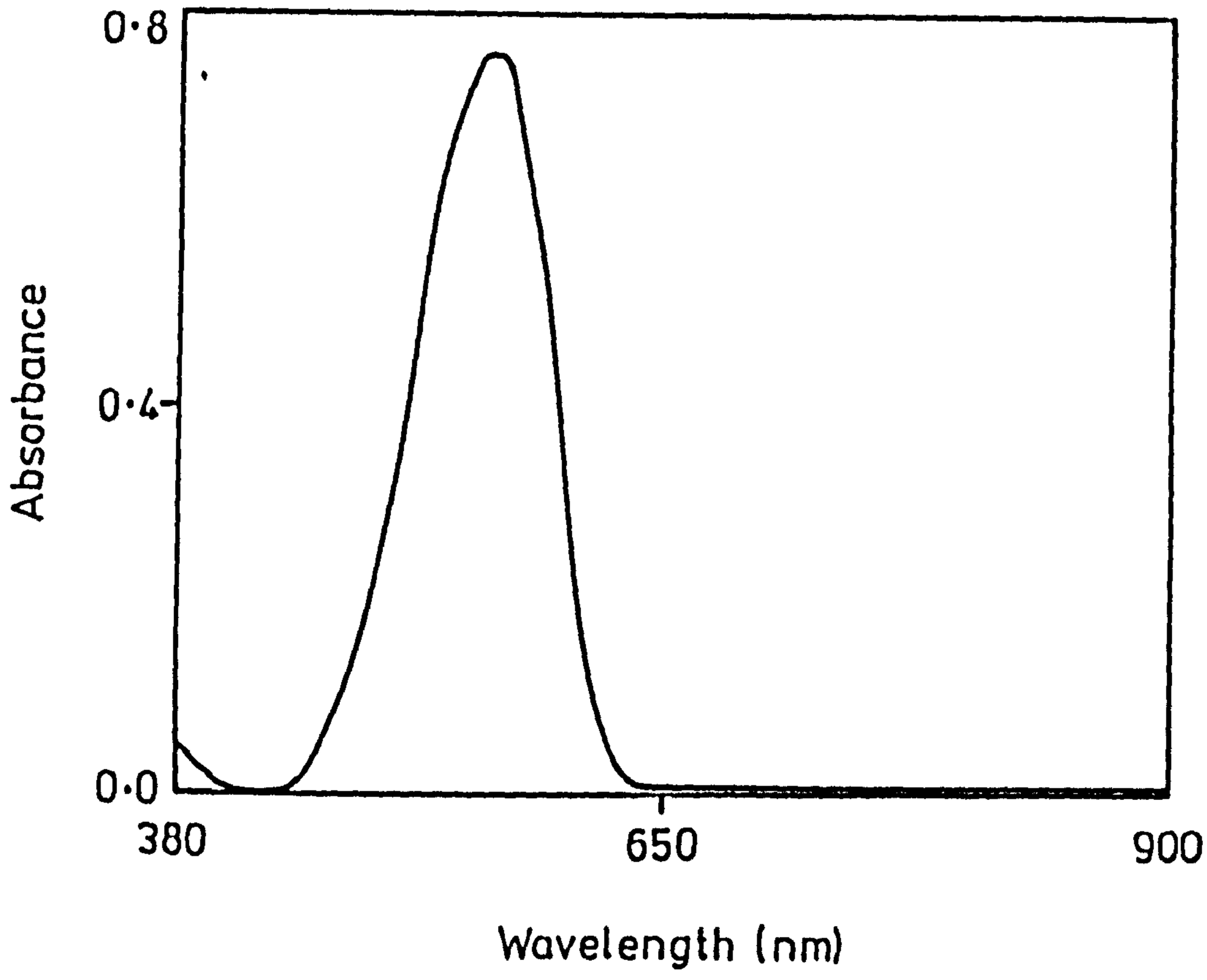


Fig. 4.1. Absorption spectrum of $^{am}_{n}$Azo dye formed between sulphanilamide and N1NED.

Many other methods have been proposed for the determination of nitrate in natural waters and these include :

(i) Direct U.V. spectroscopic method - this is based on the direct determination of nitrate at 210 nm after removing organic interferences with an activated carbon filter (2,26).

(ii) Ion chromatography - the nitrate ion is separated from other anions using an anion exchange column and is then determined either using a U.V. detector (27) or by electrical conductivity (28).

(iii) Nitrate ion-selective electrode - a liquid membrane based nitrate selective electrode is used, either in a batch sampling procedure (29,30) or combined with flow injection analysis as a sample handling technique (31).

(iv) Voltammetric determination - nitrate is reduced to nitrite at a glassy carbon electrode in a flow injection system (32).

(v) Spectrophotometric coupling reagents - many chromogenic reagents have been reported for nitrate including orange I (33), crystal violet (34) and brucine (35).

Most of the above methods have been adapted to flow injection analysis and, for incorporation into an automated water quality monitor the method involving the

reduction of nitrate to nitrite followed by the diazo coupling reaction was studied.

4.2. Experimental

4.2.1.Reagents

All solutions were made up in distilled deionised water.

Nitrate stock solutions (100 mg l^{-1}) 0.7220 g of potassium nitrate 'AnalaR' (BDH - dried at 105 ^{o}C for 2 hours) was dissolved in 1 litre of water.

Sodium nitrite 'AR' (Fisons).

N-1-naphthylethylenediamine dihydrochloride (N1NED) 'AnalaR' (BDH).

Copper (II) sulphate 'AnalaR' (BDH).

Zinc sulphate 'AnalaR' (BDH).

Sodium hydroxide 'AR' (Koch-light).

Ammonium chloride 'AnalaR' (BDH).

Cadmium wire 1.0 mm diameter 'Gold Label' (Aldrich).

Cadmium powder 100 mesh (Johnson Matthey Metals).

Sodium hydroxide 'AR' (Koch-Light).

Ammonium chloride 'AnalaR' (BDH).

4.2.2. Equipment

A solid state detector (as described in chapter 2) incorporating green light emitting diodes as the light source was used throughout. The pump was an Ismatec mini-S-820 (Labdata services) and the injection valve used was a Rheodyne type 5020 six port rotary injection valve. The manifold was constructed using teflon tubing (Anachem) which had an internal diameter of 0.5 mm.

4.2.3. Determination of Nitrate using Hydrazinium Sulphate as a Reducing Agent

This manifold (figure 4.2) used hydrazinium sulphate (2) to reduce the nitrate to nitrite which was then used to couple N1NED and sulphanilamide. The sample was introduced into a stream of sodium hydroxide which was then merged with the reductant solution (containing hydrazinium sulphate with copper sulphate added as a catalyst; zinc sulphate was added to prevent chelation of the copper catalyst by organic species present in the sample). After mixing, the diazo coupling reagents were then merged and the resultant azo dye determined photometrically.

4.2.3.1. Experimental Conditions

Diazotisation reagent coil = 100 cm on a 1 cm diameter rod.

Reduction coil = 400 cm on a 1 cm diameter rod.

Diazotisation reaction coil = 200 cm on a 1 cm diameter rod.

Sample volume = 15 μl.

Sodium hydroxide concentration = 15 g l^{-1}.

Reductor solution = 0.75 g hydrazinium sulphate, 0.1 g copper (II) sulphate and 0.4 g zinc sulphate in 1 litre of water.

N1NED concentration = 0.5 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid solution.

Sulphanilamide concentration = 25 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid solution.

Overall flow rate = 1.6 ml min^{-1}.

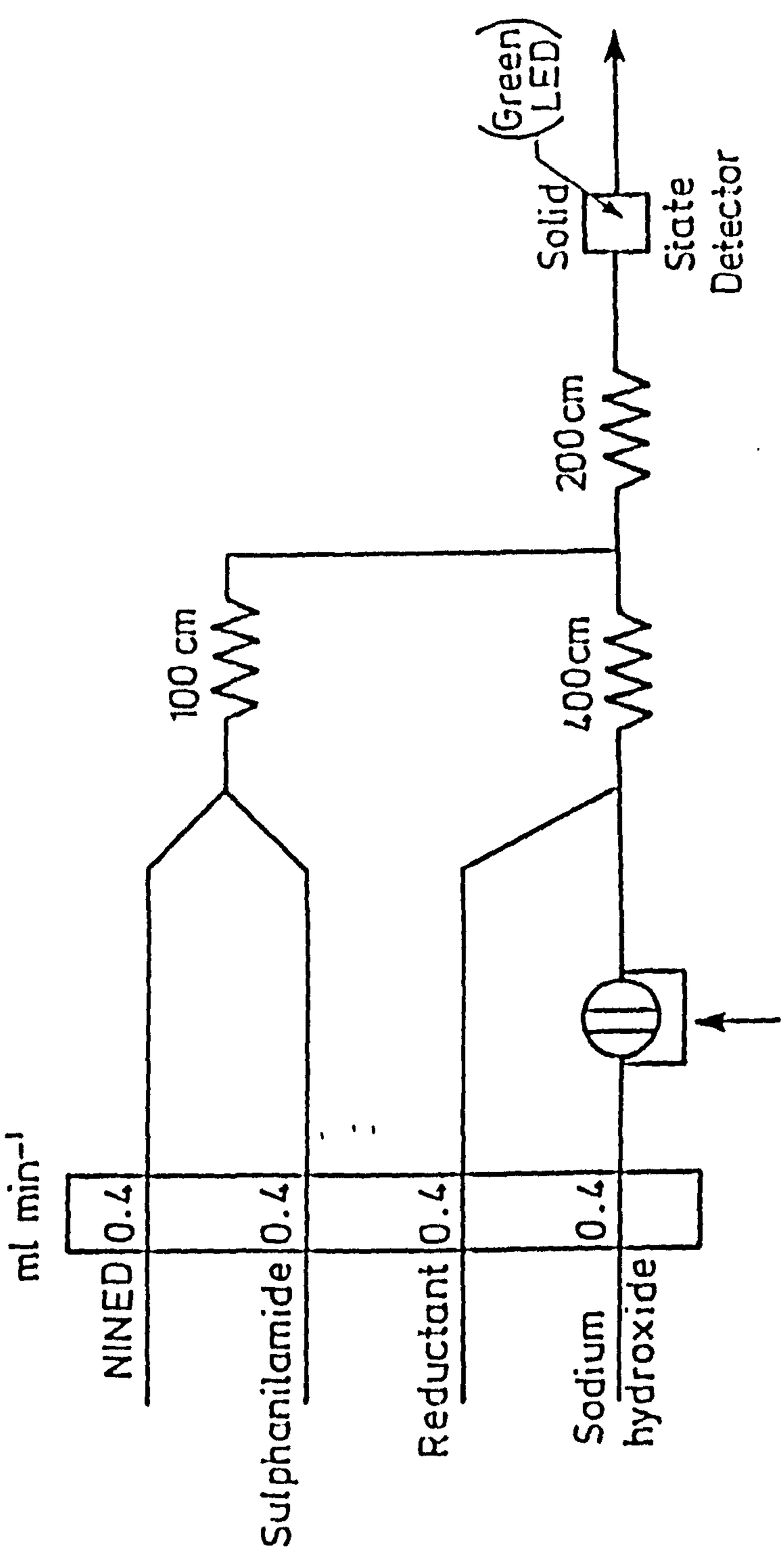


Fig. 4.2. Hydrazinium sulphate manifold.

4.2.4. Determination of Nitrate using Copperised Cadmium Wire

This manifold (figure 4.3.) was based on that described by Nakashima et al. (36). The sample was introduced into a stream of ammonium chloride buffer and then passed through a coil containing copperised cadmium wire. The resultant nitrite was determined photometrically using the diazo coupling reaction.

4.2.4.1. Experimental Conditions

The cadmium reductor coil was prepared by cleaning a 25 cm length of 1.0 mm diameter cadmium wire with emery cloth and then washing with 2 M hydrochloric acid. The wire was inserted into a 1.5 mm internal diameter silicone tube, which was then wound onto a 1 cm diameter rod. The cadmium was copperised by passing 15 ml of copper (II) sulphate solution (20 g l^{-1}) slowly over it. The coil was then washed to remove any particulate matter with ammonium chloride solution (10 g l^{-1}).

Diazotisation reagent coil = 100 cm on a 1 cm diameter rod.

Diazotisation reaction coil = 200 cm on a 1 cm diameter rod.

Sample volume = 15 μl.

Ammonium chloride concentration = 10 g l^{-1}.

NlNED concentration = 0.5 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid solution.

Sulphanilamide concentration = 25 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid solution.

Ammonium chloride flow rate = 0.7 ml min^{-1}.

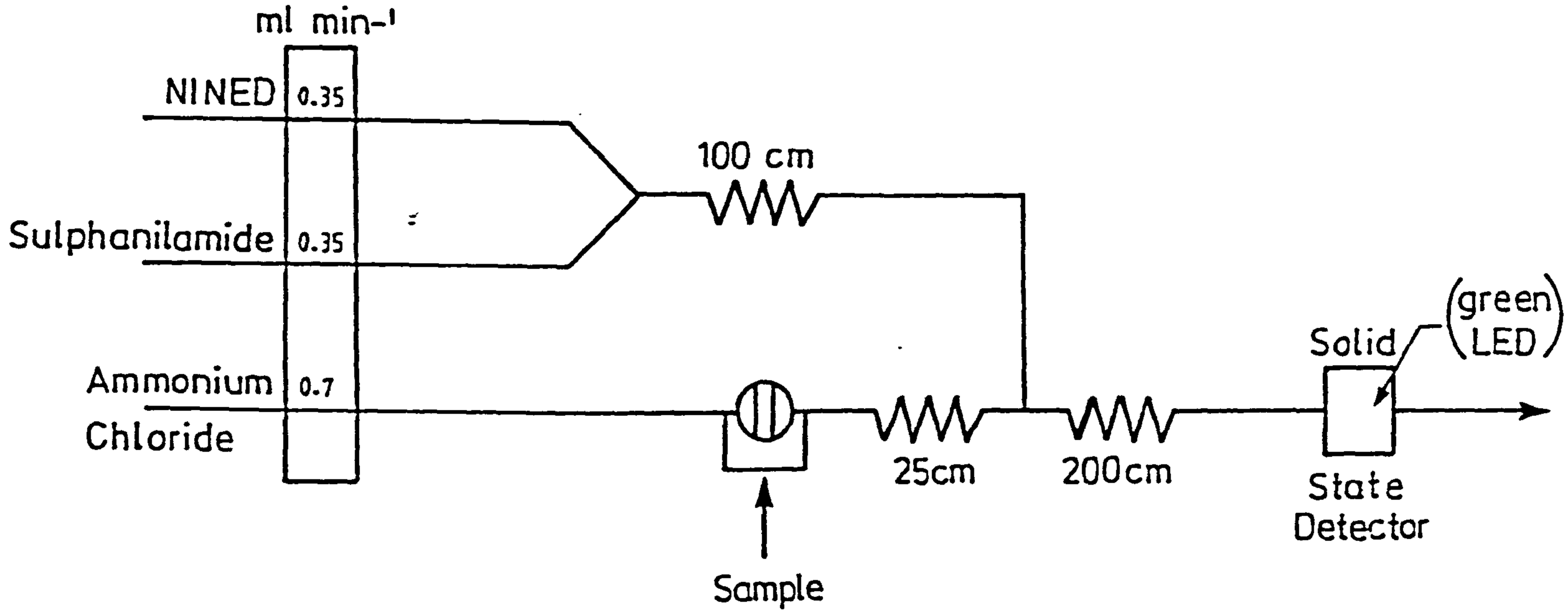


Fig. 4.3. Copperised Cadmium wire manifold.

N1NED flow rate = 0.35 ml min^{-1}.

Sulphanilamide flow rate = 0.35 ml min^{-1}.

4.2.5. Reverse Flow Injection Manifold

The reverse flow injection manifold (figure 4.4.) was adapted from one described by Johnson and Petty (37). A flowing sample stream was merged with an ammonium chloride buffer stream and passed through a six port rotary injection valve. The sample loop of the injection valve was replaced with a copperised cadmium reductor coil. Whilst the valve was in the 'fill' position, ammonium chloride buffer was passed through the reduction coil and the sample stream passed straight through to waste. When the valve was switched to 'inject', the sample stream was passed through the copperised cadmium reductor coil and the resultant stream was then merged with the diazotisation reagents. The resultant azo dye was then determined photometrically. This manifold had the advantage that the reductor coil lifetime could be extended by reducing the time for which the sample was flowing through the reduction coil. When the valve was returned to the 'fill' position the remaining sample in the coil was washed out with ammonium chloride buffer.

4.2.5.1. Experimental Conditions

A cadmium reductor coil, 50 cm long was prepared as described in section 4.2.4.1..

A cadmium reductor column was prepared by adding 5 g of cadmium powder (100 mesh : 0.15 mm diameter) to 50 ml of copper (II) sulphate solution (10 g l^{-1}) and stirring for 2 minutes. The resultant copperised cadmium was

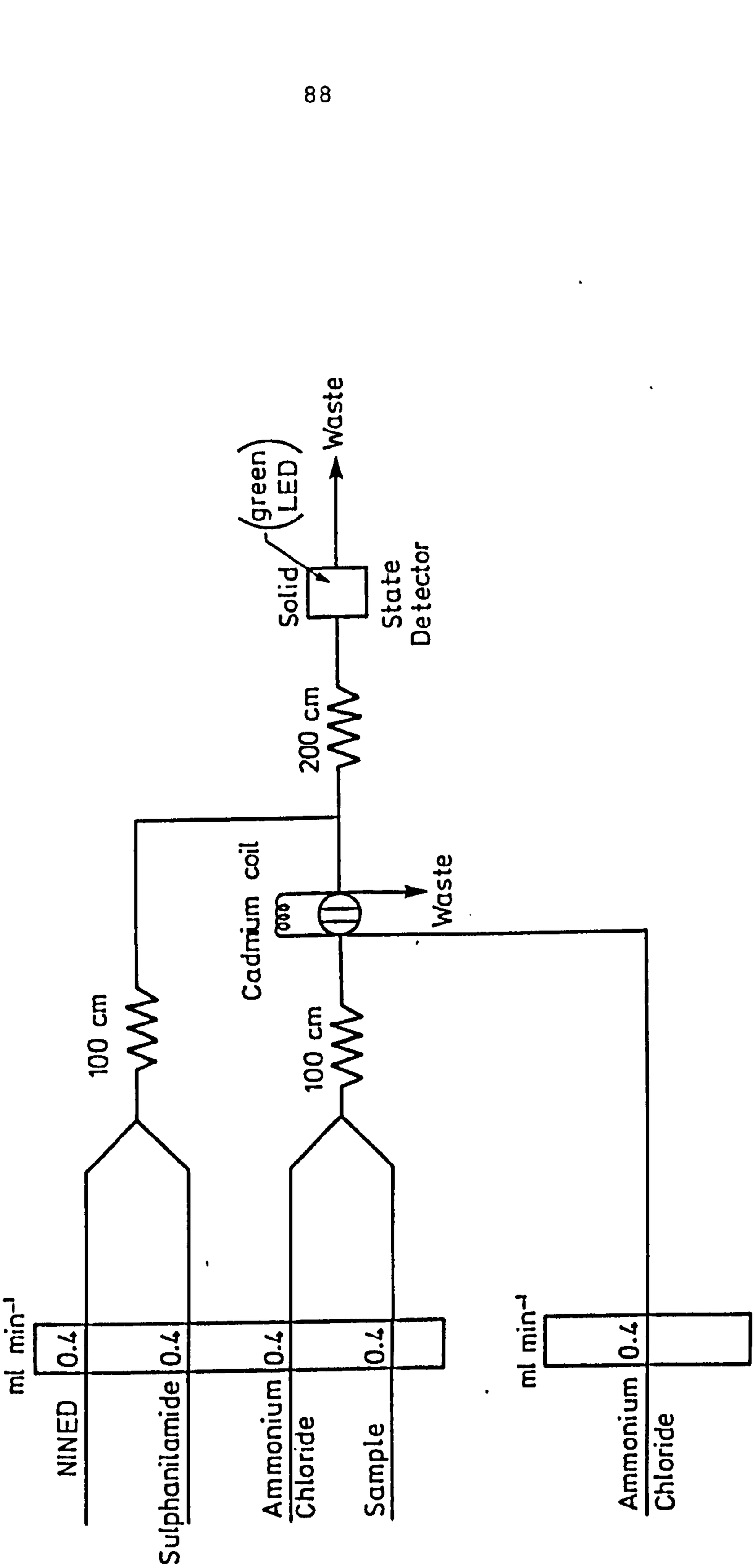


Fig. 4.4. Reverse flow injection manifold.

washed with 2 M hydrochloric acid and ammonium chloride solution (10 g l^{-1}), packed into a glass column (40 mm long, 2 mm i.d.) plugged with glass wool. The columns were stored in ammonium chloride solution (10 g l^{-1}) until required and connected to the teflon tubing of the manifold by short lengths (10 mm) of red/red pump tubing.

Sample/ammonium chloride coil = 100 cm on a 1 cm diameter rod.

Diazotisation reagent coil = 100 cm on a 1 cm diameter rod.

Diazotisation reaction coil = 200 cm on a 1 cm diameter rod.

Ammonium chloride concentration = 10 g l^{-1}.

N1NED concentration = 0.5 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid.

Sulphanilamide concentration = 25 g l^{-1} in a 10 % $^{v}/_{v}$ orthophosphoric acid solution.

Sample flow rate = 0.4 ml min^{-1}.

Ammonium chloride flow rate = 0.4 ml min^{-1}.

N1NED flow rate = 0.4 ml min^{-1}.

Sulphanilamide flow rate = 0.4 ml min^{-1}.

4.2.6. Determination of Nitrate using a Copperised Cadmium Column

This manifold (figure 4.5.) was similar to that described in section 4.2.4. except that a column packed with copperised cadmium powder was used instead of a copperised cadmium wire to reduce the nitrate to nitrite. The resultant nitrite was then determined photo metrically as the azo dye.

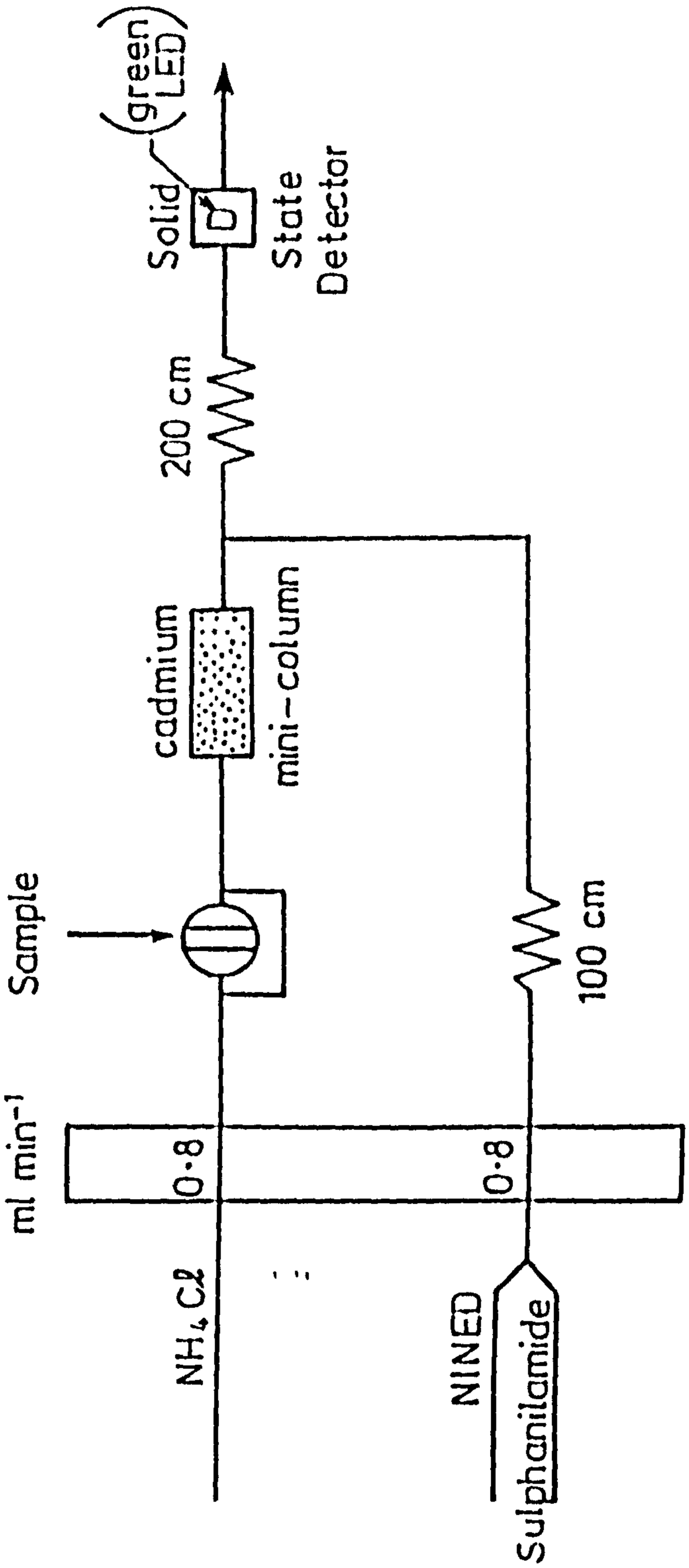


Fig. 4.5. Copperised cadmium column manifold.

4.2.6.1. Experimental Conditions

The cadmium reductor column was prepared as described in section 4.2.5.1..

Diazotisation reagent coil = 100 cm on a 1 cm diameter rod.

Diazotisation reaction coil = 200 cm on a 1 cm diameter rod.

Sample volume = 30 µl.

N1NED concentration = 0.5 g l^{-1} in a 10% $^{v}/_{v}$ orthophosphoric acid solution.

Sulphanilamide concentration = 25 g l^{-1} in a 10% $^{v}/_{v}$ orthophoshoric acid solution.

Ammonium chloride concentration = 10 g l^{-1}.

Ammonium chloride flow rate = 0.8 ml min^{-1}.

N1NED flow rate = 0.4 ml min^{-1}.

Sulphanilamide flow rate = 0.4 ml min^{-1}.

4.3. Results and Discussion

4.3.1. Optimisation of the Diazotisation Step

This was carried out using a univariate approach on the manifold shown in figure 4.5. without the cadmium reductor column and using a 2 mg l^{-1} NO_2-N (nitrite as nitrogen) solution.

Diazotisation reaction coils with lengths in the range 35 to 400 cm were constructed on 1 cm diameter aluminium rods and their effect on the signal obtained was studied. The signal was found to increase with increasing coil length up to 200 cm due to increasing residence time allowing more azo dye to be formed. Above 200 cm, increasing the coil length produced smaller signals due

to the increased dispersion of the sample zone in the carrier stream. A coil length of 200 cm was chosen as providing the maximum signal.

The effect of N1NED concentration was studied in the range 0 to 1.5 g l^{-1} in 10% $^{v}/_{v}$ orthophosphoric acid. As the N1NED concentration was increased, the signal increased up to 0.5 g l^{-1}, above which further increases in N1NED concentration produced no further increase in the signal obtained. An N1NED concentration of 0.5 g l^{-1} was chosen so as to ensure an excess of N1NED was present.

Sulphanilamide solutions in the range 0 to 30 g l^{-1} were prepared in a 10% $^{v}/_{v}$ orthophosphoric acid solution and their effect on the signal was studied. The signal obtained was found to increase with increasing sulphanilamide concentration up to 20 g l^{-1}, above which no further increases in signal were obtained. A concentration of 25 g l^{-1} was used for all subsequent work so as to ensure an excess of sulphanilamide.

The orthophosphoric acid concentration of the reagent solutions was studied in the range 0 to 40% $^{v}/_{v}$ orthophosphoric acid. Concentrations above 4% $^{v}/_{v}$ showed no further increase in the signal obtained and thus a 10% $^{v}/_{v}$ orthophosphoric acid solution was chosen so as to buffer the diazotisation step against samples of high pH.

4.3.2. Determination of Nitrate using Hydrazinium Sulphate

4.3.2.1. Optimisation

This manifold was optimised using the univariate approach, and a 5 mg l^{-1} NO_3-N solution was used throughout.

Reduction coils were constructed with lengths in the range 50 to 400 cm, and their effect on the signal obtained was studied. The signal was found to increase with increasing coil length up to 200 cm, above 200 cm the increase in signal became smaller due to the increasing dispersion having a greater effect on the signal than the increase in reduction time. A coil length of 400 cm was chosen so as to give 100% nitrate reduction and to give a greater capacity for reduction of higher nitrate concentrations.

The overall flow rate was varied over the range 0.8 to 4.2 ml min^{-1} and, as the flow rate increased, the signal obtained decreased due to the shorter residence time and the increased dispersion. An overall flow rate of 1.6 ml min^{-1} was chosen as a compromise between analysis time and sensitivity obtained.

The effect of varying the sample volume in the range 15 to 150 µl was studied and the signal obtained increased up to 60 µl, but further increases in the sample volume produced no further increases in the signal. This was due to the sample being in excess with respect to the reducing agent at the larger volumes, and

so a 15 μl sample volume was used in subsequent work to give the greatest linear range.

Sodium hydroxide solutions in the range 0 to 40 g l^{-1} were studied and, the signal obtained was found to increase up to 15 g l^{-1}, above which further increases in the sodium hydroxide concentration produced a decrease in the signal obtained. The reason for the decrease in the signal was due to the large excess of sodium hydroxide neutralizing the orthophosphoric acid for the diazotisation step, and so a 15 g l^{-1} sodium hydroxide concentration was used to provide the greatest signal without interfering with the diazotisation step.

Copper (II) ions act as a catalyst in the reduction of nitrate by hydrazinium sulphate and it s effect on the signal was studied in the range 0 to 0.5 g l^{-1} copper (II) sulphate pentahydrate in the reductant solution. The signal obtained was found to increase up to 0.05 g l^{-1}, above which further increases in copper sulphate concentration produced no further increase in the signal obtained. A concentration of 0.1 g l^{-1} was chosen so as to provide an excess of copper ions.

The hydrazinium sulphate reductor concentration was studied in the range 0 to 3 g l^{-1} and the signal was found to increase up to 0.75 g l^{-1}, above which further increases in hydrazinium sulphate concentration produced a decrease in the signal. This was due to the hydrazinium sulphate interfering in the diazotisation step and so a concentration of 0.75 g l^{-1} was used for all subsequent work.

4.3.2.2. Calibration

Calibration of this manifold was obtained using nitrate standards in the range 0 to 7 mg l^{-1} NO_3-N, which was described by the equation,

signal (mV) = 14.8 x concentration (mg l^{-1}) + 0.37

and had a correlation coefficient of 0.9999. The mean of six replicate injections, standard deviation and relative standard deviation for each standard are given in table 4.1.. The limit of detection (2σ) was calculated from the blank signal as 90 μg l^{-1} NO_3-N and the linear range was found to be 0 to 7.0 mg l^{-1} NO_3-N.

The stability of the hydrazinium sulphate was studied with calibrations being made every three days. The slope of the calibration graph decreased slowly until after 18 days calibrations were found to be imprecise. The linear calibration on day 1 was described by the equation,

signal (mV) = 12.42 x concentration (mg l^{-1}) - 0.18

with a correlation coefficient of 0.9999.

On day 19 the calibration was described by the equation,

signal (mV) = 11.19 x concentration (mg l^{-1}) - 2.16

with a correlation coefficient of 0.9992.

4.3.3. Determination of Nitrate using Copperised Cadmium Wire

4.3.3.1. Optimisation

This manifold was optimised using a univariate approach and a 5 mg l^{-1} NO_3-N standard was used throughout.

The effect of overall flow rate was studied in the range 0.6 to 3.2 ml min^{-1} and, the signal obtained was

Mean Concentration (mg l^{-1})	Standard Signal[a] (mV)	Standard Deviation[a]	Relative Standard Deviation[a] (%)
7.0	102.2	1.17	1.1
5.0	74.0	1.00	1.4
3.0	44.5	1.41	3.2
2.0	29.7	1.04	3.5
1.0	14.3	0.45	3.1
0.0	0.9	0.22	24.4

[a] n=6

Table 4.1. Calibration of Cadmium Reductor Manifold

found to decrease with increasing flow rate. This was due to a combination of the time the sample zone passed over the cadmium reductor column and also an increase in dispersion affecting the sample zone. An overall flow rate of 1.5 ml min^{-1} was used in subsequent experiments as the best compromise between signal obtained and analysis time.

Sample volumes in the range 15 to 450 µl were studied and the signal obtained was found to increase with increasing sample volume. A sample volume of 15 µl was chosen so as to limit the amount of nitrate passing over the reductor and thus prolong it s lifetime. If a calibration for a low nitrate range was required (as in the case of lake nutrient studies and depth profiling) then a larger sample volume could be used.

The effect of ammonium chloride concentration was studied in the range 0 to 200 g l^{-1} and, with distilled deionised water the signal obtained was found to be irreproducible. Increasing the ammonium chloride concentration to 10 g l^{-1} gave reproducible results and, further increases in ammonium chloride concentration produced a decrease in the signal obtained. This was because of the high salt concentration interfering with the diazotisation step and thus a concentration of 10 g l^{-1} was chosen for further work.

Calibrations were obtained for cadmium reductor coil lengths in the range 25 to 100 cm (figure 4.6). The 25 cm coil was chosen for further work because it had the

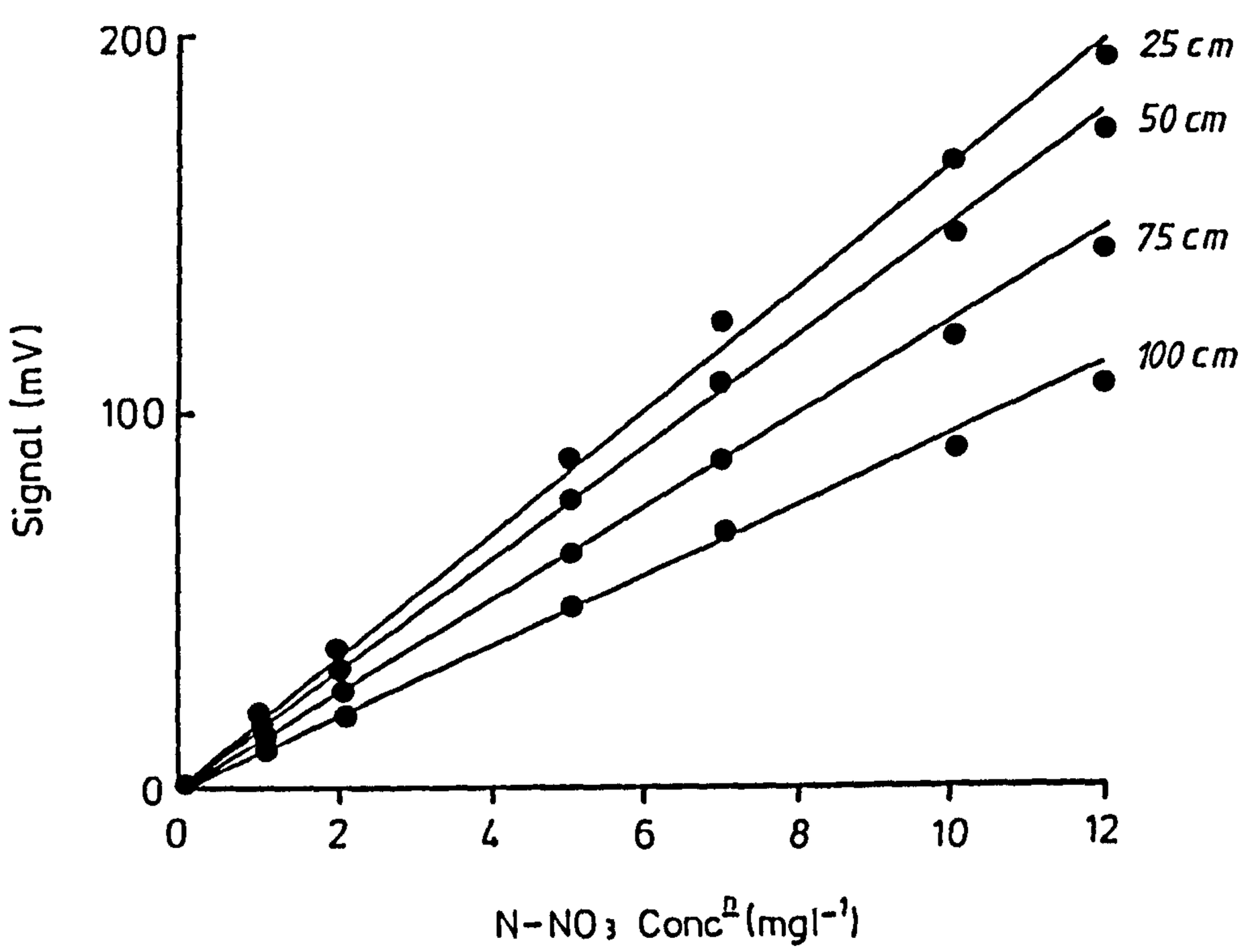


Fig.4.6. Effect of Cadmium reductor coil length.

smallest sample zone dispersion and thus produced the greatest signal.

4.3.3.2. Calibration

Calibration of this manifold was obtained using nitrate standards in the range 0 to 12 mg l^{-1} NO_3-N and was described by the equation,

signal (mV) = 16.4 x concentration (mg l^{-1}) + 0.94

with a correlation coefficient of 0.9994. The mean of eight injections, standard deviation and relative standard deviation are given for each standard in table 4.2.. The linear calibration range was found to be 0 to 20 mg l^{-1} NO_3-N and the limit of detection (2σ) was calculated from the detector noise as 63 μg l^{-1} NO_3-N although this could be decreased by the use of a larger sample volume.

4.3.4. Determination of Nitrate using a Reverse Flow Injection Manifold

4.3.4.1. Optimisation

Cadmium reduction coils with lengths of 50, 75 and 100 cm were evaluated in the injection valve sample loop. Calibrations were obtained for each coil with 'inject' times (i.e. the time for which the sample flows through the coil) of 2 and 3 minutes. This showed that for a linear calibration range greater than 0 to 5 mg l^{-1} NO_3-N, a cadmium reductor coil would be required with a length greater than 100 cm, and thus a longer 'inject' time would also be required, increasing the time of analysis. Another point is that the coils did not last

NO_3-N Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
12.0	182.6	0.53	0.3
10.0	161.9	1.60	1.0
7.0	118.9	0.64	0.5
5.0	84.8	0.38	0.4
2.0	34.1	0.42	1.2
1.0	16.0	0.50	3.1
0.0	0.0	--	0.0

[a] n=8

Table 4.2. Calibration of Cadmium Reductor Manifold

very long (typically 2 calibrations) due to the large amounts of nitrate passing through them.

In an effort to reduce the 'injection' time and hence improve the speed of analysis, a cadmium reductor column of length 10 mm was used. The maximum signal was obtained with an inject time of 60 seconds but the linear calibration range was only 0 to 3 mg l^{-1} NO_3-N. Cadmium minicolumns of lengths 20 and 40 mm were tested and the 40 mm column was found to increase the slope of the linear portion of the calibration graph, but the linear range remained unchanged at 0 to 3 mg l^{-1} NO_3-N.

4.3.4.2. Calibration

Calibration of this manifold was obtained using nitrate standards in the range 0 to 10 mg l^{-1} NO_3-N and the calibration curve obtained was described by the parabolic equation,

$$\text{signal (mV)} = 2.0 + [58.5 \times C] - [2.0 \times C]^2$$

where C was the nitrate concentration in mg l^{-1} NO_3-N. The linear calibration range was 0 to 3 mg l^{-1} NO_3-N and the limit of detection (2σ) was calculated from the detector noise as 20 μg l^{-1} NO_3-N.

4.3.4.3. Comparison of Methods

Samples were obtained from the River Frome (Dorset) catchment area on the 18th June 1986 in acid washed 1 l P.T.F.E. bottles. On return to the laboratory they were immediately filtered through a 0.45 μm GF/C filter paper (Whatman) and then analysed. The samples were analysed for their nitrate content using the proposed method, a

Tecator FIAstar flow injection analyser (38) and a manual spectrophotometric method (39).

The FIAstar method is essentially the same as that described in section 4.2.6. except that instead of having a small minicolumn packed with fine copperised cadmium powder it has a large volume column packed with large pieces of copperised cadmium (3 x 1 mm). The manual method was based on the production of a chromophore by reacting the nitrate with phenyldisulphonic acid and then measuring it spectrophotometrically.

The results (table 4.3.) show good correlation between both automated methods but, the manual method gives values which are consistently lower in nitrate. This was probably due to the phenyldisulphonic acid method being specific for nitrate whereas the other two methods also determine any nitrite that may be present.

4.3.5. Determination of Nitrate using a Copperised Cadmium Column

4.3.5.1. Optimisation

This manifold was optimised using a univariate approach and a 5 mg l^{-1} NO_3-N standard was used throughout.

The effect of overall flow rate was studied over the range 0.5 to 2.3 ml min^{-1} and showed a maximum response at 1.4 ml min^{-1}. The decrease in response at higher flow rates was due the shorter residence time of the sample in the manifold.

The response obtained increased with increasing sample volume (15-180 μl), but a sample volume of 30 μl was used

Location	Grid Reference	NO_3-N content (mg l^{-1})		
		Proposed Method	Automated Method	Manual Method
Sydling Water	SY637947	3.6	3.4	3.0
South Winterbourne	SY712889	4.9	4.9	4.7
River Hooke	SY592976	4.0	4.0	3.4
Woodsford	SY769909	4.6	4.6	4.1
Empool	SY763874	5.7	5.5	5.2
Broomhill Bridge	SY811881	5.1	5.1	4.6

Table 4.3. Comparative Results for Water Samples from the River Frome Catchment Area

in subsequent work in order to prolong the lifetime of the cadmium column by reducing the amount of nitrate passing over it.

The optimum ammonium chloride level was 10 g l^{-1}, above this level the response decreased because chloride ions inhibited the diazotisation step and also the reduction step (20). Below this level the response was erratic because of the decreased buffering capacity of the carrier stream.

Cadmium reductor columns were prepared with lengths of 10, 20 and 40 mm. Calibrations were obtained for each cadmium reductor length with nitrate standards in the range 2 to 10 mg l^{-1} NO_3-N and the results obtained are shown in table 4.4.. Columns of shorter length gave larger signals due to the decreased dispersion of the sample zone but, the longer column lengths had a greater reducing capacity, and so a 40 mm reductor column length was chosen to give the greatest reductor lifetime.

4.3.5.2. Calibration

Calibration of this manifold was obtained using nitrate standards in the range 0 to 10 mg l^{-1} NO_3-N and was described by the equation,

signal (mV) = 56.1 x concentration (mg l^{-1}) + 8.4

with a correlation coefficient of 0.9995. The mean of six replicate injections, standard deviation and relative standard deviation are given for each standard in table 4.5.. The linear calibration range was 0 to 12 mg l^{-1} NO_3-N and the limit of detection (2σ) was calculated from the detector noise as 24 μg l^{-1}.

NO_3-N ($mg\ l^{-1}$)	Cadmium Column Length (signal[a] in mV)		
	10mm	20mm	40mm
2.0	156.7	149.2	127.5
5.0	348.3	336.7	295.0
7.0	451.7	438.3	400.0
10.0	615.0	590.8	565.0
r[b]	0.9970	0.9968	0.9988

[a] n=4 [b] correlation coefficient, n=16

Table 4.4. Effect of Length of the Cadmium Column on Sensitivity

NO_3-N Concentration (mg l^{-1})	Mean Signal (mV)	Standard Deviation	Relative Standard Deviation (%)
10.0	565.0	1.7	0.3
7.0	400.0	0.8	0.2
5.0	295.0	1.5	0.5
2.0	127.5	0.9	0.7
1.0	55.5	0.3	0.5
0.0	0.0	--	0.0

Table 4.5. Calibration of Cadmium Column Reductor Manifold

4.3.5.3. Reagent Lifetime

An important factor in the operation of any water quality monitor is the stability of the reagents which affects the length of time a monitor can be left unattended.

The ammonium chloride solution was found to be stable for at least 30 days in a laboratory at room temperature (20 ^{o}C) after which time some microbial growth could be noticed in the container. It was found that the N1NED and sulphanilamide reagents could be premixed in a 1 : 1 $^{v}/_{v}$ ratio to give a colour reagent which was stable for at least 30 days at room temperature. After this time oxides of nitrogen absorbed from the atmosphere (38) slowly reacted with the reagent giving it a pink colouration. This was not found to be a problem and in one case reagents over three months old gave satisfactory results.

The limiting factor in the unattended operational lifetime of this manifold was the capacity of the cadmium reductor column to reduce nitrate to nitrite. The capacity was investigated by repeated injections of a 10.0 mg l^{-1} NO_3-N standard. No decrease in the signal was found after 5 days (1600 injections equivalent to a nitrate load on the column of 0.48 mg N) and, after a further 400 injections, a decrease in signal of 30% was observed.

4.3.5.4. Comparison of Methods

Six river water samples were collected from various locations in the River Frome (Dorset) catchment area in acid washed P.T.F.E. bottles on the 25th June 1986. On

the same day each sample was filtered through an 0.45 μm GF/C filter (Whatman) and analysed for nitrate content as described previously (section 4.3.4.3.). The results obtained (table 4.6.) show good agreement, although those from the manual method are consistently lower due to the manual method being specific for nitrate whereas the automated methods are for total oxidised nitrogen.

4.3.5.5. Study of the River Aire

Samples of the River Aire (Yorkshire) were obtained at 25 major bridging points along it s length from Malham village near it's source to Chapel Haddelsey near it's confluence with the River Ouse. Samples were obtained in brown acid washed glass bottles and were refrigerated for one day prior to analysis. The two dates were the 29th November 1986, a sunny day with the river at it s normal level, and the 22nd August 1987, a very rainy day with the river in spate. The effects of rainfall on the nitrate levels can be clearly seen from the results obtained (figure 4.7.), as at Malham village (1.5 km from source) the nitrate levels are the same which is what is expected from a groundwater source, and very low as there is little intensive agriculture on the Pennines which serve as the catchment area for the River Aire. Downstream from the source the nitrate level is lower on the rainy day due to the effects of dilution up to Skipton (22.5 km from the source), suggesting that there is little nitrate input from the surrounding land. The increases in the nitrate level found on the dry day are due to discharges from sewage works serving small

Location	Grid Reference	NO_3-N content (mg l^{-1})		
		Proposed Method	Automated Method	Manual Method
Sydling Water	SY637947	3.6	3.5	3.4
South Winterbourne	SY712889	5.0	4.9	4.9
River Hooke	SY592976	3.4	3.4	3.2
Woodsford	SY769909	4.6	4.7	4.5
Empool	SY763874	5.5	5.6	5.2
Broomhill Bridge	SY811881	4.8	4.9	4.8

Table 4.6. Comparative Results for Water Samples from the River Frome Catchment Area 25/06/86

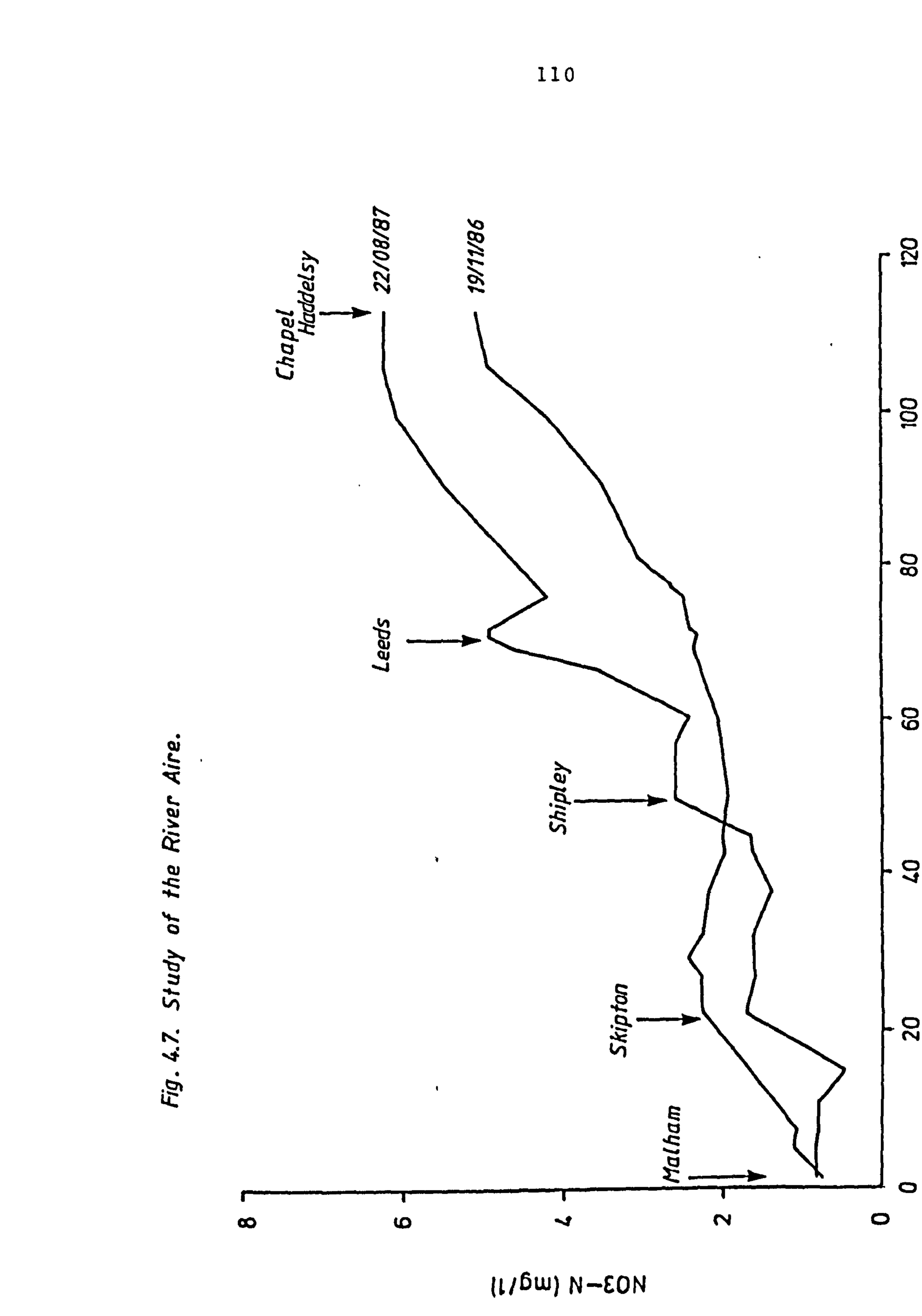


Fig. 4.7. Study of the River Aire.

villages, these being diluted by the increased flow on the rainy day. At Skipton the nitrate level increases on both days due to the discharge from a large sewage works. The nitrate level increases as the river travels further from it s source due to the input from several sewage works. On the rainy day the level increases sharply at Shipley due to the large Victorian sewage works, which when under heavy load, such as in heavy rainfall, discharges effluent directly to the river. Through metropolitan West Yorkshire the level steadily increases on both dates due to further discharges from sewage works and also from industrial effluents. At the lower reaches of the river, the nitrate concentration starts to level off as there are few sewage discharges to the river in this area.

The samples obtained on the 22nd August 1987 were also analysed by a direct ultraviolet spectroscopy method (2) The results (table 4.7.) show good agreement between the proposed method and the direct U.V. method although for the direct U.V. method a variable correction factor, F, had to be applied in order to allow for any organic pollutants which also absorb in the U.V.. This factor varied between 2 (the optimum value - no organic pollution) and 4 (indicating a high level of organic material) according to where you were on the river. In the higher stretches where organic pollution is low it was 2, at the lower reaches where any organic pollutants are greatly diluted it was also 2. In the middle

Site Name	Grid Reference	Distance From Malham Cove (Km)	NO_3-N Concentration (mg l^{-1}) Proposed Method	Direct U.V.
Chapel Haddelsy	SE579263	113.0	6.22	6.28
Beal	SE533255	106.0	6.22	6.30
Ferrybridge	SE482246	99.3	6.05	5.92
Castleford	SE430260	90.5	5.48	5.36
Swillington	SE373294	81.0	4.60	4.21
Stourton	SE331312	76.0	4.19	4.07
Briggate Br.	SE299331	72.0	4.92	4.84
Monks Br.	SE292332	71.0	4.92	4.81
Armley Br.	SE279342	69.5	4.60	4.51
Kirkstall	SE259356	66.8	3.55	3.56
Calvely	SE224369	60.5	2.42	2.51
Apperley Br.	SE195379	57.0	2.58	2.64
Shipley	SE150379	49.8	2.60	2.61
Cottingley	SE113380	45.0	1.65	2.11
Bingley	SE105394	43.0	1.62	1.97
Riddlesden	SE075423	38.0	1.38	1.54
Silsden	SE039452	32.5	1.62	1.71
Cononley	SD995468	27.0	1.60	1.65

Table 4.7. Comparison Between Proposed method and Direct U.V. Method for River Aire Samples, 22/08/87

stretches particularly in metropolitan West Yorkshire it was 3 or 4 depending on the location.

4.3.6. Comparison of Manifolds Studied

A summary of the calibration data for the manifolds studied is given in table 4.8..

The hydrazinium sulphate manifold is not suitable for use in an automated monitor due to a build up of copper which occurs in the flow system during it s operation. If this is not frequently removed with acid it causes the tubing to become blocked. The copperised cadmium wire manifold would at first seem suitable, but the cadmium wire has only a small surface area available for reducing the nitrate and so it has a limited lifespan. The cadmium coils could be regenerated (recopperised) in situ but this would increase the complexity of the manifold used. The reverse flow injection manifold has a very limited linear range and thus would not be suitable for natural waters where nitrate levels normally exceed 3 mg l^{-1} $N\text{-}NO_3$. The copperised cadmium column manifold was chosen as it had a suitable linear range, the reagents were stable for a period in excess of 1 month and, the reduction column had a large surface area because it was made from powdered cadmium, and thus had a large reducing capacity. This manifold was chosen to be automated and incorporated into a spectrophotometric water quality monitor.

4.4. Design and Construction of an Automated Monitor

A block diagram of the automated nitrate field monitor is shown in figure 4.8.. The monitor can be conveniently

Manifold	Linear Calibration Range (mg l^{-1} NO_3-N)	Limit of Detection (μg l^{-1} NO_3-N)	Sensitivity (mV per mg l^{-1} N)
Hydrazinium Sulphate	0 - 7	90	12.42
Copperised Cadmium Wire	0 - 20	63	16.4
r-FIA	0 - 3	20	56.3
Copperised Cadmium Column	0 - 12	24	56.1

Table 4.8. Comparison of Manifolds Studied

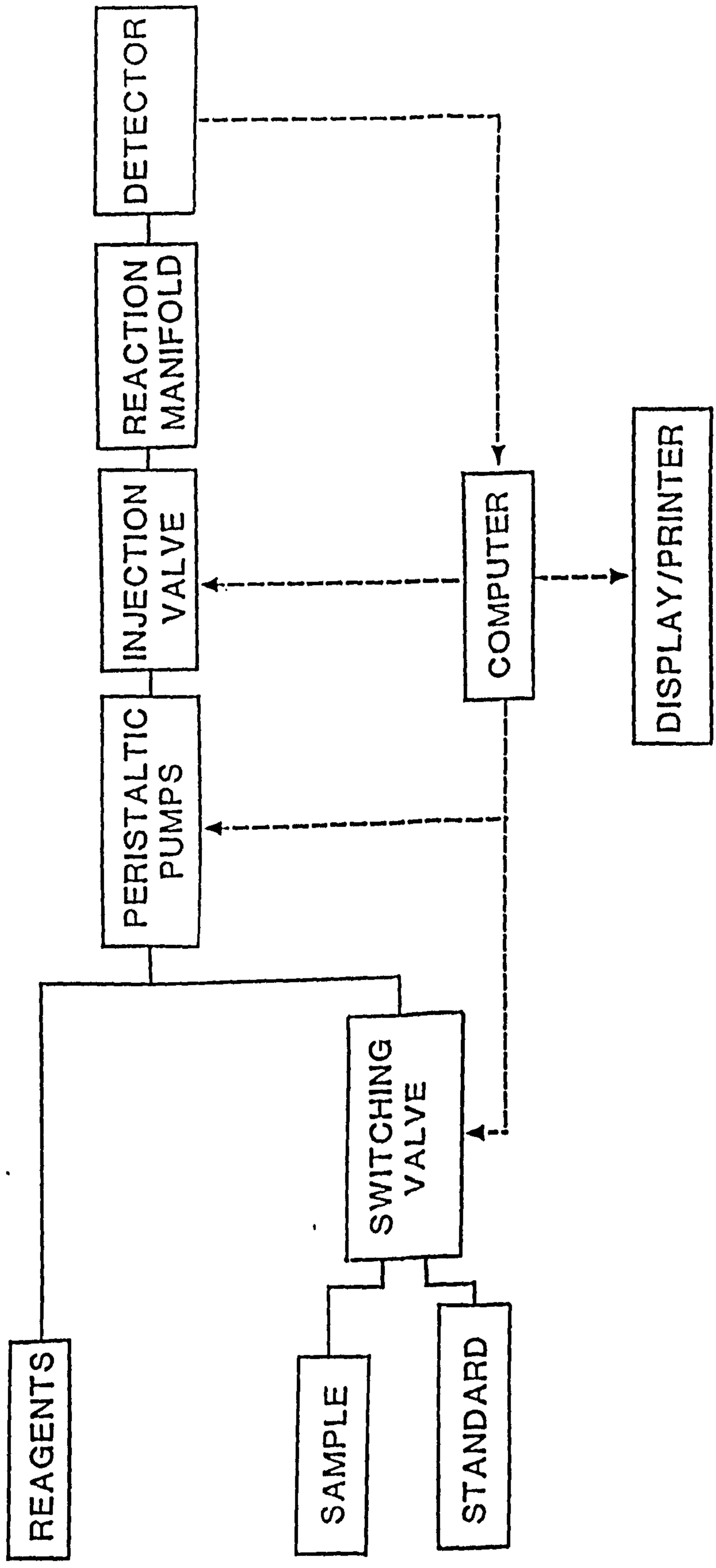


Figure 4.8. Block Diagram of the Automated Nitrate Monitor

split into three parts, the manifold board, the pump box and the computer.

4.4.1. Manifold Board

The manifold board (figure 4.9) contained all the flow injection manifold components and was where the chemistry of the system took place. The manifold was constructed from teflon tubing (0.5 mm i.d.) on a blockboard base (300 x 250 mm). The sample and standard lines were selected by use of a three-way 12 V solenoid valve (LEE, Westbrook, CN) and the resultant line was fed into a 12 V solenoid operated injection valve (Chemlab Instruments). The solenoid injection valve has two 12 V solenoids to move the valve to the inject position and one 12 V solenoid to move it back to the fill position. The valve itself was a standard six port slider valve of the kind used in commercial laboratory flow injection analysers. The solid state photometric detector was also mounted on the manifold board, with the colour reagent being fed into the reference channel and the eluant from the reaction coil fed through the sample channel. The flow injection manifold used was that described in section 4.3.5. with no further modification apart from the sample being interchangeable with a nitrate standard via a three-way solenoid valve (LEE).

4.4.2. Pump Box

The pump box (figure 4.10) contained the power supplies for the detector and valves, the interface between the computer and the valves and the peristaltic pumps which were mounted on the outside of the box. The

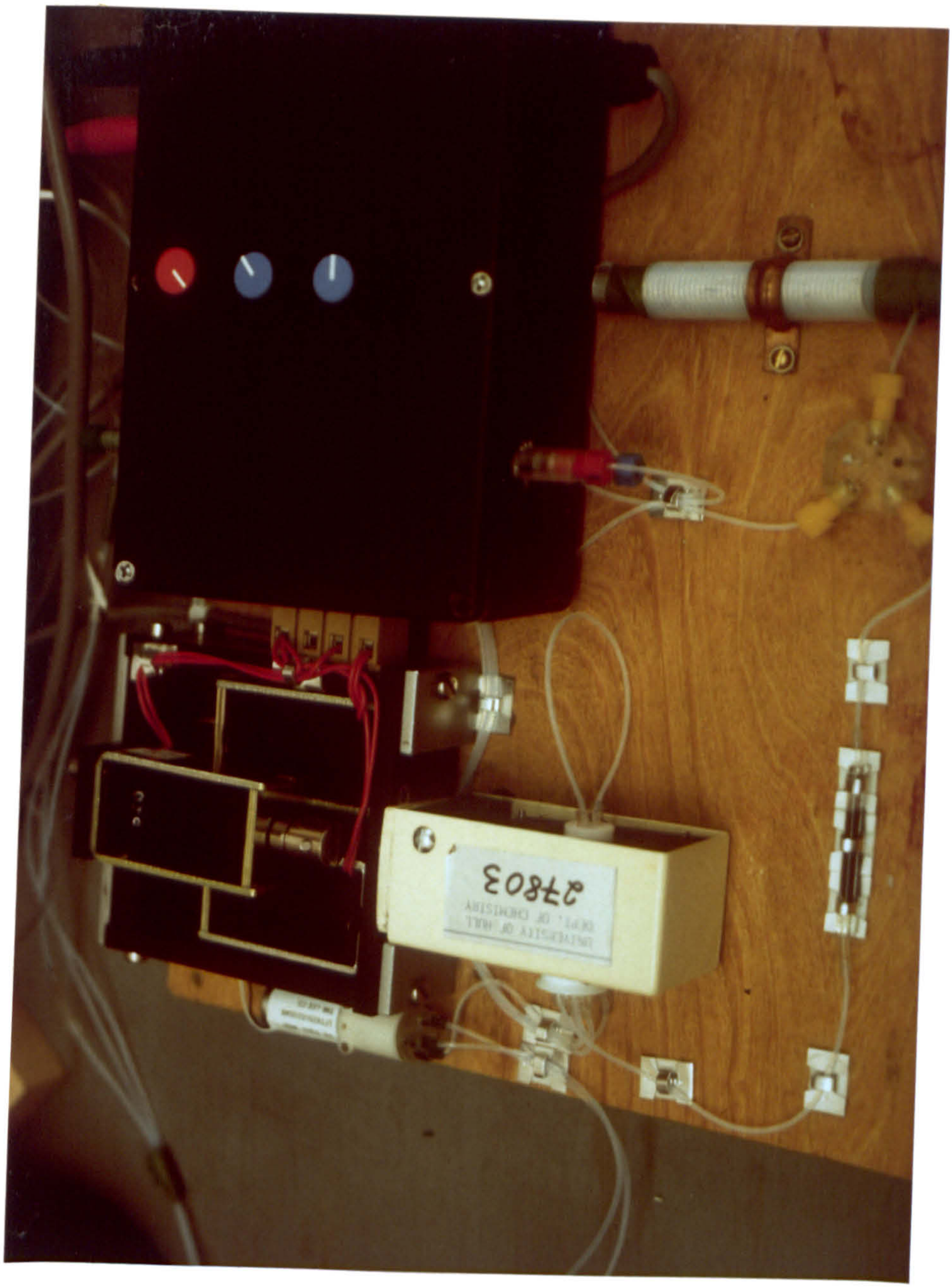


Figure 4.9. Manifold Board

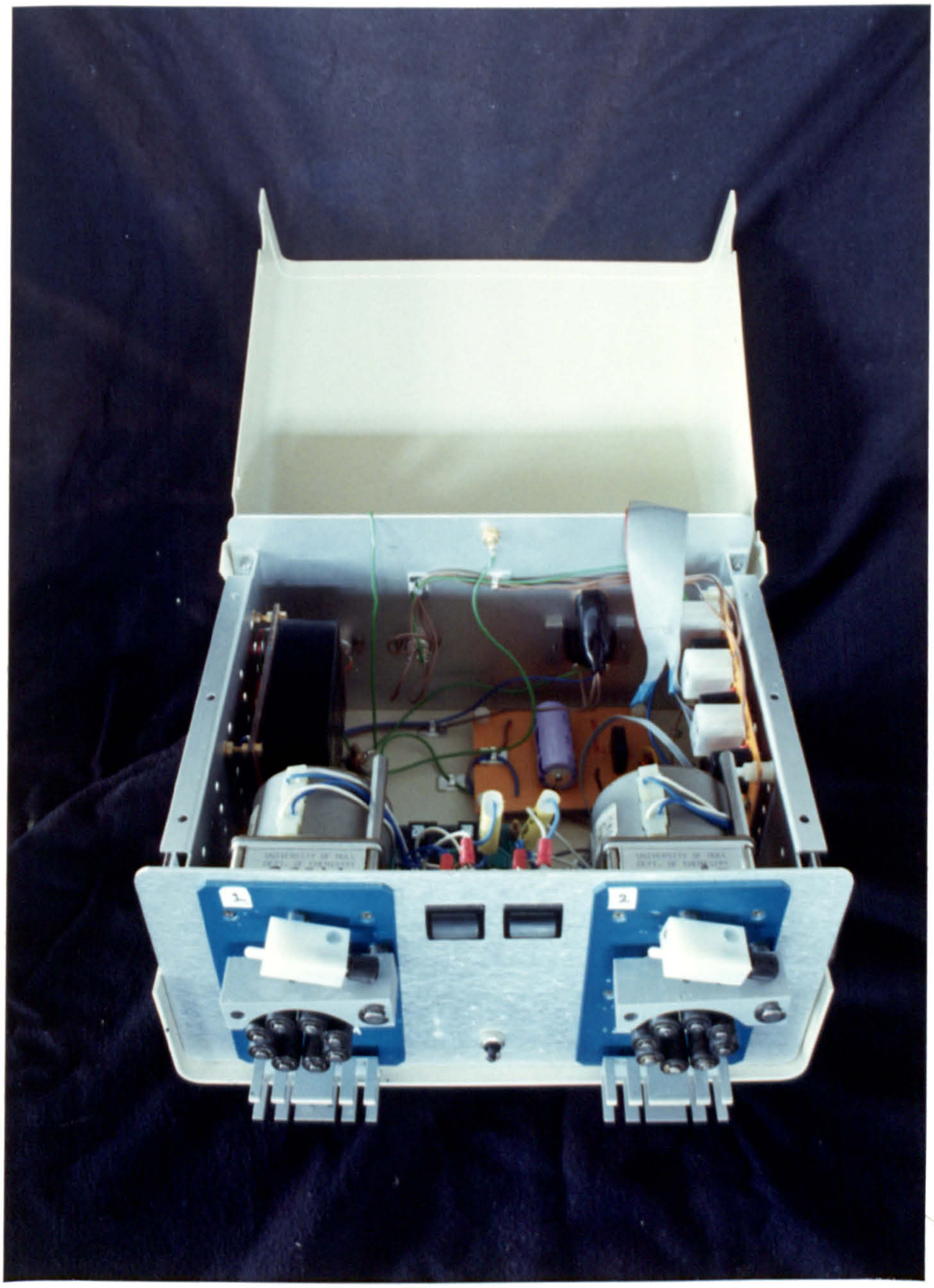


Figure 4.10. Pump Box

circuit diagram is shown in figure 4.11. with a list of components given in table 4.9..

The detector power supply (PSU1) was provided by a ±12 V encapsulated power supply (Radio Spares; n^{o}. 591-102) which provided a highly stabilised ±12 V power source suitable for the detector, which was fed to a 3 way DIN socket on the rear of the case. The valve power supply was constructed from a 12 V/30 VA toroidal transformer (TR1) with a 4 A silicon bridge rectifier to provide a d.c. output and a 2200 μF capacitor to provide some smoothing. This power supply was not regulated and so under no load conditions gave an output of around 18 V and, when under load this dropped to 10 V. The valves were interfaced to the Cube microcomputer via a relay switching system. Three of the input/output lines from the Cube microcomputer were fed through an inverting buffer (IC2) which served two purposes; firstly to protect the input/output (lines PA0 - PA2) port of the Cube and, secondly to invert the logic state of the port. This was necessary because the Cube port was set at ground potential when the software was set to logic 1 and, at +5 V when set to logic 0. The output from the buffer was fed through a 3K3 resistor (R1-3) into the base of a BC183L transistor (T1-3), which served to limit the current drawn from the buffer chip. If +5 V was placed on the base of the transistor then it switched on allowing current to flow between it's collector and emitter. This caused the relay (RL1-3) to be activated allowing a larger current to flow to the coils of either

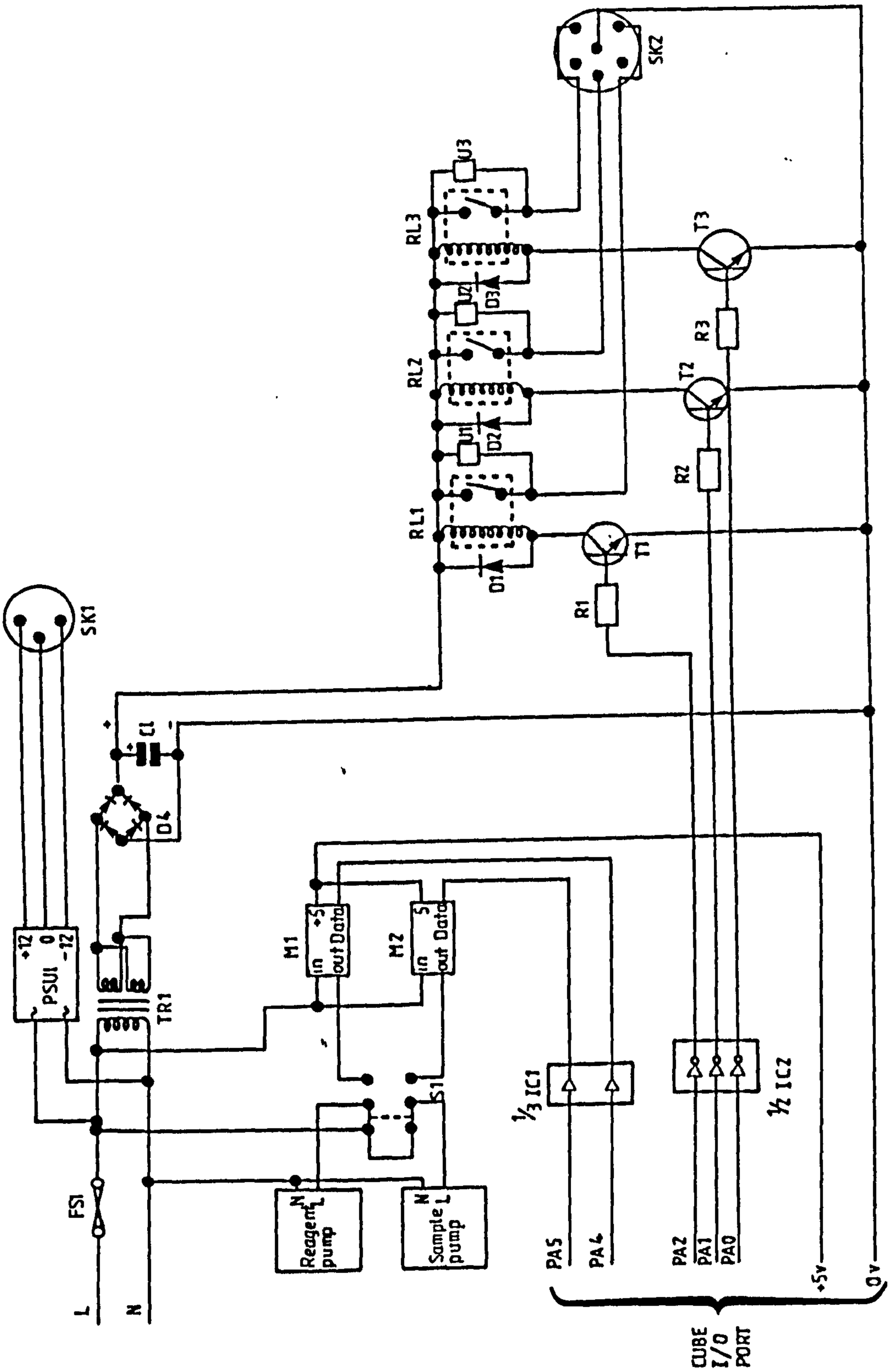


Fig. 4.11. Pump box circuit diagram.

IC1	74LS07 Hex buffer
IC2	4049UB Hex inverting buffer
T1-3	BC183L
D1-3	IN4001
D4	KBU4D 4A/200V Silicon bridge rectifier
R1-3	3K3 0.25W Carbon
C1	2200 μF
U1-3	Contact suppressor (RS n^{o}. 238-463)
M1,2	a.c. output module (RS n^{o}. 348-469)
PSU1	+12V/240V encapsulated power supply (RS n^{o}. 208-462)
TR1	12V 30VA toroidal transformer (RS n^{o}. 208-462)
FS1	500 mA
SK1	3 way 'DIN' connector
SK2	6 way 'DIN' connector
S1	Double pole double throw
RL1-3	12V minature 3A relay (RS n^{o}. 346-621)

Table 4.9. Pump Box Component List

the injection valve or the three-way solenoid valve. The diodes (D1-3) were required to prevent any negative voltages, which may develop as the coil is energised or deenergised, from destroying the transistors. The contact suppressors (V1-3) were used to suppress any RF emissions generated by arcing within the relay contacts. The output from this interface was provided on the rear of the case. The reason that such an interface was required was that the injection valve required 1.7 A for an inject movement and 0.85 A for a fill movement and, the three-way solenoid valve also required 0.2 A for it's operation, all at 12 V, whereas the Cube input/output port only provides TTL (Transistor - Transistor Logic) levels i.e. each line would only provide 5 V at 16 mA.

The two peristaltic pumps were interfaced to the Cube microcomputer using 2 a.c. output modules (M1,2; Radio Spares; n°. 348-469), these provided an isolated output drive for the pumps using an optical isolation technique. When a voltage between 3 and 8 volts d.c. was placed on the input contacts, the output contacts closed allowing 240 V a.c. to flow between them. These a.c. output modules were mounted on a purpose built board (Radio Spares; n°. 632-095) which required that a 5 V line be provided and, then to turn on a module another line was switched to ground. This was achieved by using a hex buffer (IC1) on two lines of the Cube user port (lines PA4 and PA5), which when set at logic 1 in software were at ground potential and thus activated the a.c. output module. When set to logic 0 in software the port line was

at +5 V and the current required to activate the module could not be sunk by the port, hence the module was turned off.

A double pole - double throw switch (S1) was provided so as to enable the pumps to be activated independently of the software when necessary.

4.4.3. Cube Eurobeeb Computer

The computer used in the automated field monitor was a Cube Eurobeeb single board computer. This was a Eurocard based microcomputer using a 6502 central processor unit and running BBC BASIC as it's main language with a few software extensions to allow easier control of the input/output ports. The card as supplied came complete with a battery backed real time clock, 16 Kbytes of RAM, 16 T.T.L. input/output lines and an RS232 serial port. Further cards were available to extend the capabilities of the system and for the nitrate field monitor the complete system consisted of, a Cube Eurobeeb single board computer, a Cuban 12A integrating analogue to digital converter card, a Cumem Selecta 64 Kbyte memory card and a Jobber interface card. All these cards were housed in a 4 - slot minirack complete with a power supply. All these items were supplied by Control Universal Limited, 137 Ditton Walk, Cambridge.

The Cuban 12A card contained a 16 channel multiplexed integrating analogue to digital converter with an accuracy of $\pm$1 bit over a 13 bit range (12 bits with 1 sign bit). This gave the card a dynamic range of $\pm$4.096 V with an accuracy of $\pm$1 mV. The Cumem Selecta card could

be configured to provide up to 64 Kbytes of R.A.M. storage. In our system we used 32 Kbytes of R.A.M. on this card to act as a data buffer for the Cuban 12A. The Jobber interface allowed the connection of a minature printer (24 characters per line) and a 24 x 2 character liquid crystal display. All of the cards were fully supported by the Control BASIC supplied on the Eurobeeb. Figure 4.12. shows the Cube microcomputer system prior to mounting in it's rack.

The Cube input/output port was connected to the pump box via a 26 way I.D.C. plug and ribbon cable and the output from the solid state detector was fed into the Cuban 12A via a 25 way 'D' type socket. The Jobber interface was connected to the minature printer with a 10 way cable and, the L.C.D. display was connected using a 14 way cable. The L.C.D. display and minature printer were mounted on a piece of aluminium (160 x 100 mm) to facilitate transportation.

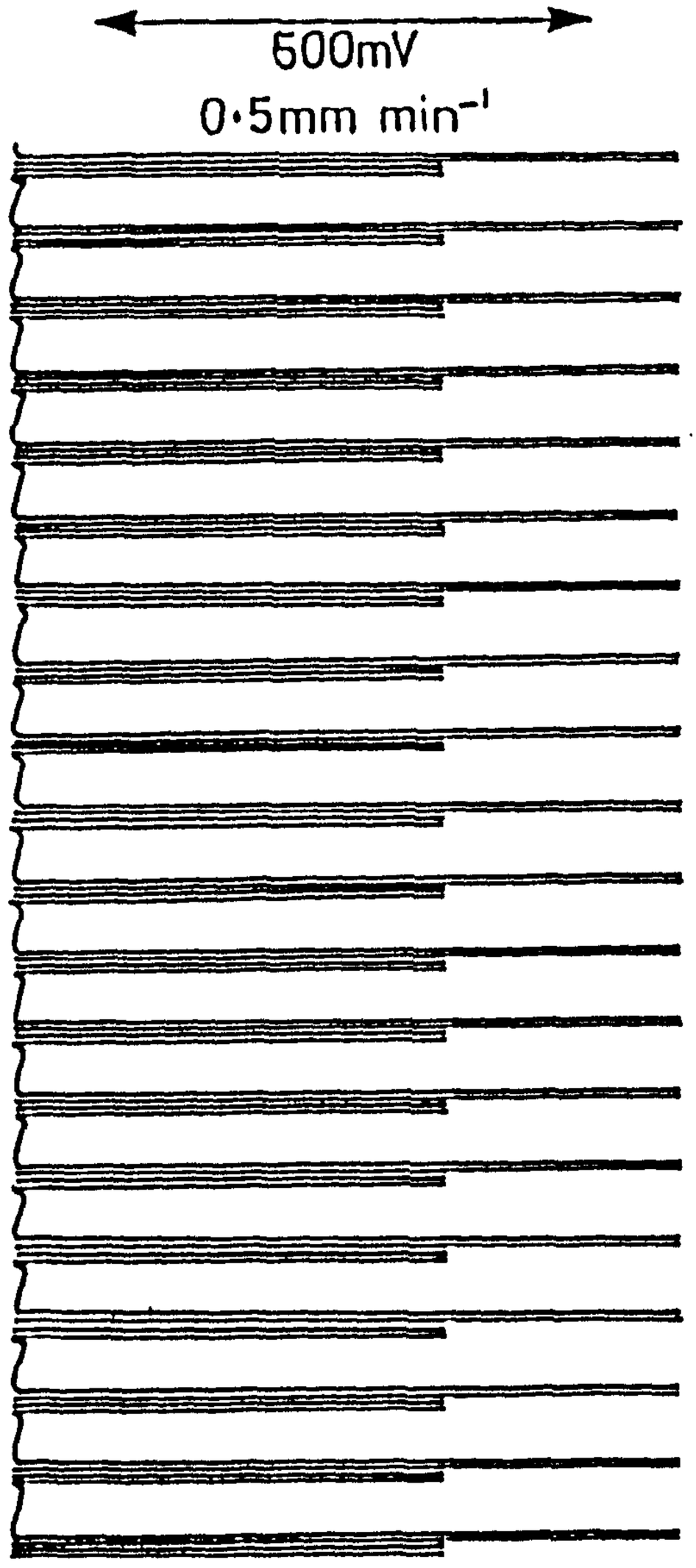
The Cube Eurobeeb computer, being a stand alone computer, has no keyboard and thus a terminal must be connected to it via it's RS232 serial port to enable it to be programmed. The Cube could be interfaced to either a BBC model B or an IBM PC compatible microcomputer with suitable emulation software supplied by Control Universal. The monitor software was developed in the linear R.A.M. of the Cube using a BBC microcomputer as a terminal. When the software was fully developed it was programmed into an 8 Kbyte E.P.R.O.M. (2764 - Eraseable Programmable Read Only Memory) using an E.P.R.O.M.

Figure 4.12. Cube Eurobeeb Computer

programmer (Watford Electronics), this provided a non-volatile copy of the software which was then installed in the Cube in place of one of it´s 8 Kbyte R.A.M. chips. The operating system of the Cube was reprogrammed using software provided so that whenever the Cube was powered up the operating system would automatically run the software in the 8 Kbyte E.P.R.O.M., using the other 8 Kbytes of R.A.M. as storage space for program variables.

The software to control the monitor(appendix 1.) was straightforward and was written in BASIC as a seventy line program. The program was written so that when the minutes portion of the real time clock was either ´00´ or ´30´ then the main program would run. This involved injection of the sample and a 10 mg l^{-1} NO_3-N standard in duplicate whilst the Cuban 12A analogue to digital converter read the signal from the detector every 300 ms and stored it in the Cumem Selecta memory card. When the four injections had been carried out, the software analysed the data from the detector and calculated the signals for each of the four injections. Means were then ratioed and the resultant NO_3-N concentration was displayed on the liquid crystal display and minature printer. A typical chart recorder trace showing the signal obtained from the detector is shown in figure 4.13.. A further feature of the software was that the current NO_3-N reading was compared with the previous reading and, if they differed by more than 10% then the main program was rerun so as to provide a check against any spurious results. Also whenever the Cube was powered

Figure 4.13. Chart Recorder Output from the Nitrate Monitor



up the main program would run regardless of the time, so as to provide an easy way to check whenever the power was down and also to help in maintenance.

4.5. Monitor Trials

4.5.1. Laboratory Trials

The monitor was set up in the laboratory with a 10 mg l^{-1} NO_3-N standard solution and a 7 mg l^{-1} NO_3-N solution as the sample solution to test the reagent consumption, and the precision and stability of the monitor over a 14 day period. It was found that the reagent consumption was 2.25 l of ammonium chloride solution and 2.35 l of the mixed colour reagent, which was equivalent to a reagent consumption of 3.5 ml of mixed colour reagent and 3.4 ml of ammonium chloride solution per determination. The consumption of the 10 mg l^{-1} NO_3-N standard was found to be 0.95 l and, the signal obtained for the standard solution was 420 mV after 14 days compared with 670 mV at the start of the trial. The relative standard deviation of the result obtained for the 7.0 mg l^{-1} NO_3-N sample solution was 1.4% (n=672). Subsequent trials in the laboratory confirmed these figures, and with this data in mind it was recommended that the cadmium reductor column be replaced along with the reagents every 14 days and the pump tubing every 28 days so as to prevent failures occurring which may not be noticed before the next service time.

4.5.2. Field Trials

The nitrate field monitor was tested in the field at two locations, on the River Frome at East Stoke (40) near

Wareham in Dorset and on the River Avon at Limpley Stoke near Bath in Wiltshire. The monitor was also assessed for it's potential use in hydroponic cultivation (41).

4.5.2.1. River Frome at East Stoke

A monitor was installed on the River Frome at East Stoke, Dorset (SY 867868) for a four day period in November 1986. River water was pumped into a 50 l constant head polypropylene container by a submersible pump located on the river bed. The container was flushed with water every 15-20 seconds and sampled every 30 minutes by the monitor through P.T.F.E. tubing (0.8 mm i.d.). The results obtained are shown in figure 4.14., together with flow data and the calculated nitrate load (Kg h^{-1}) for the same period. The nitrate load is more useful than the actual nitrate concentration because it is indicative of the actual amount of nitrate flowing down the river and is useful for work currently being undertaken to study the nitrogen budget for the River Frome catchment. Previous ways of undertaking such studies have involved people taking manual samples every half hour over a period of a week, which is very wasteful of manpower and resources.

Further trials of the nitrate monitor were carried out at East Stoke which led to the permanent installation of two monitors. These are currently located on the same site and are fed from the river by gravity, with coarse filtration of the sample being carried out using nylon mesh honeycomb supports (as used by beekeepers). Fine filtration of the sample was carried out using glass

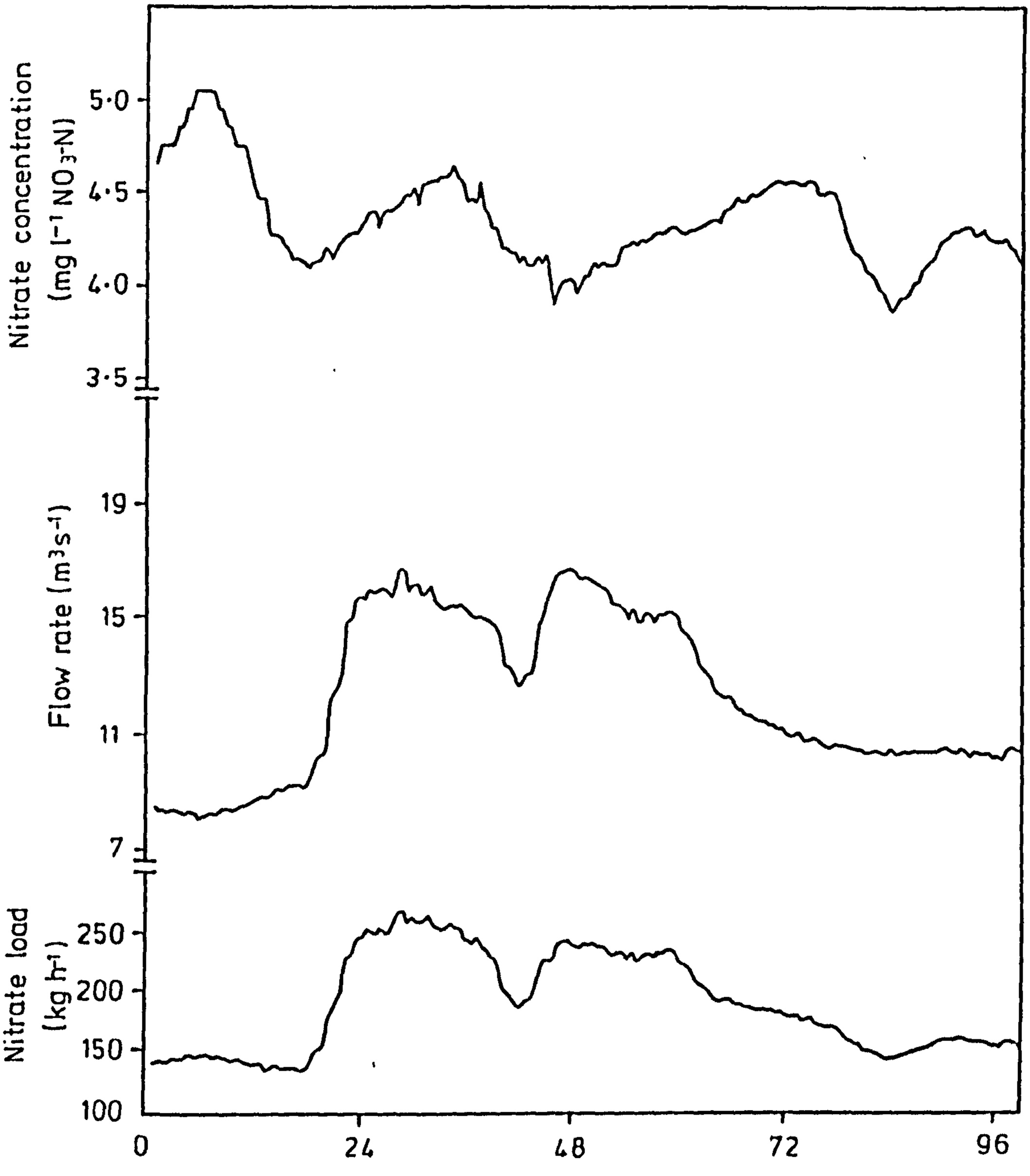


Figure 4.14. Nitrate Levels in the River Frome

tubes (40 x 2 mm i.d.) packed with glass wool which were inserted in the sample line. Random samples were taken over a period of one week and were analysed for nitrate by an automated method (38). The results are shown as a graph of laboratory method (old) versus field monitor (new) in figure 4.15.. The correlation coefficient of a line drawn through the points is 0.913 and the nitrate field monitor showed a mean positive bias of 0.19 mg l^{-1} NO_3-N as compared with the laboratory method. This was found to be due to a fault in the software and was remedied by increasing the time that the reagents were flushed through the system at the end of each run by 15 seconds.

4.5.2.2. Hydroponic Cultivation

In hydroponic cultivation, plants are grown in water to which all the nutrients they require for growth are added as a liquid fertiliser to the water supply prior to it s entry to the growing beds. One example of hydroponic cultivation is that of water cress, in which spring water is fed in at the top of a long bed in which the water cress is growing. A liquid fertiliser containing nitrogen as nitrate, phosphorus as phosphate, potassium and other trace elements is added to the spring water as it enters the bed. No form of continuous monitoring is used to ensure that either sufficient nutrients are available for the plants or, that the fertiliser solution is not being added in excess and thus is being wasted. Currently the fertiliser solution is added periodically to achieve a nitrate concentration at the inlet of 20 mg l^{-1} NO_3-N.

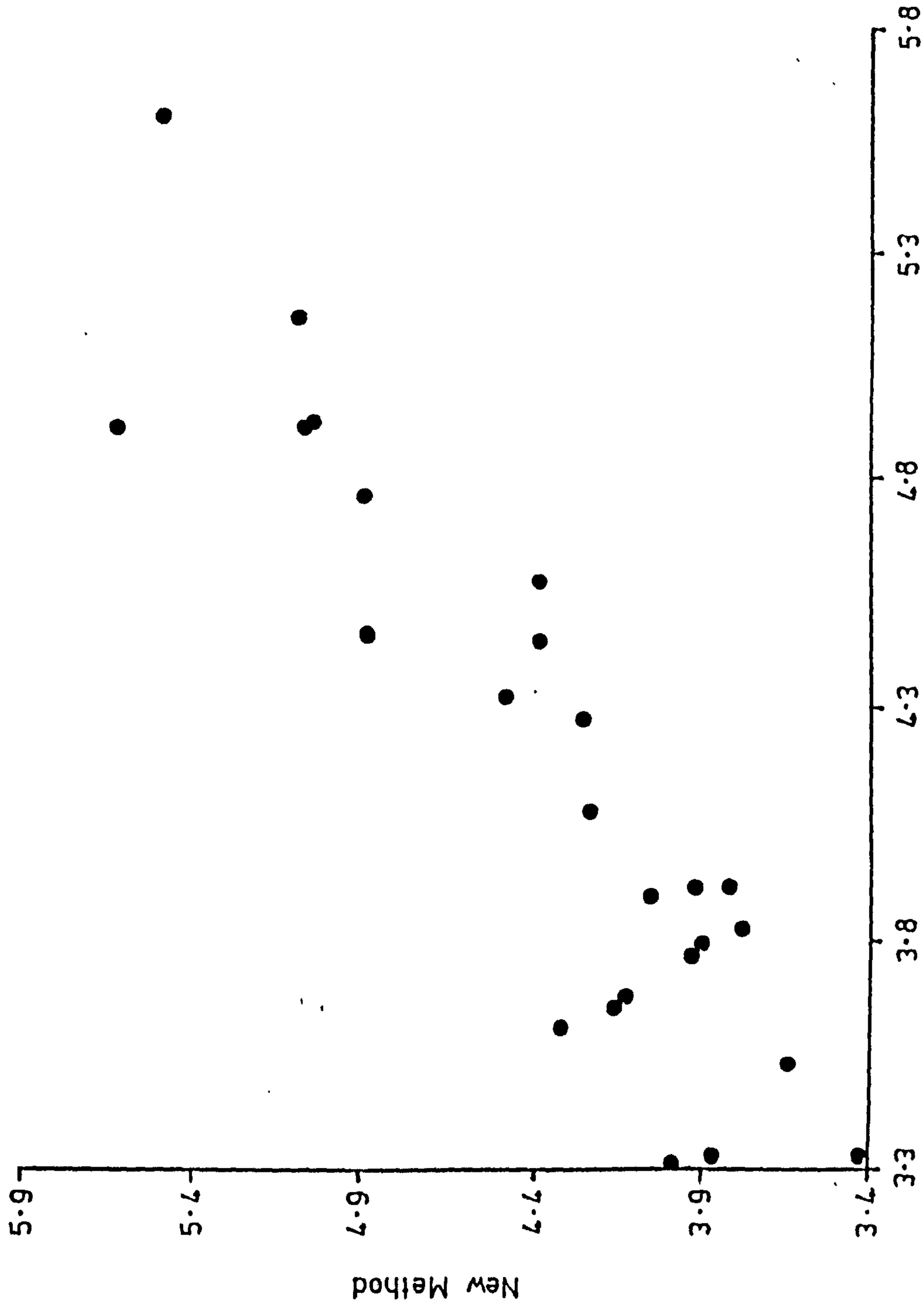


Fig. 4.15. Comparison of nitrate methods.

The nitrate monitor was placed at the outflow of the bed and the nitrate concentration of the effluent was monitored every 30 minutes over a 40 hour period (figure 4.16.) commencing at 4:30 pm on a summer day. The time required for water to pass through the bed was 1-2 hours, which explains the time lag which can be seen when dosing is started or stopped. During dosing the nitrate concentration at the outflow was 16-17 mg l^{-1} NO_3-N, indicating an uptake by the watercress of 3-4 mg l^{-1} NO_3-N, and when dosing was stopped the nitrate concentration of the outflow fell to 2.5 mg l^{-1} NO_3-N, indicating an uptake of 2.0 mg l^{-1} NO_3-N from the spring water (the spring water used had a background nitrate concentration of 4.5 mg l^{-1} NO_3-N). The unexpected rise in signal after 26 hours was probably due to a fluctuation in the flow of springwater through the cress bed.

This short term field trial has clearly shown that the nutrient dosing procedure should be linked to a feedback mechanism from the monitor to the dosing pump, which would result in more efficient and cost-effective dosing, hence saving money by reducing the amount of fertiliser used.

4.5.2.3. River Avon at Limpley Stoke

Wessex Water have constructed a water supply intake protection station on the River Avon at Limpley Stoke near Bath (ST 783619) where water can be abstracted from the River Avon and pumped to a small service reservoir at Tucking Mill 2.5 Km away. Also included in the intake station is water quality monitoring equipment to provide

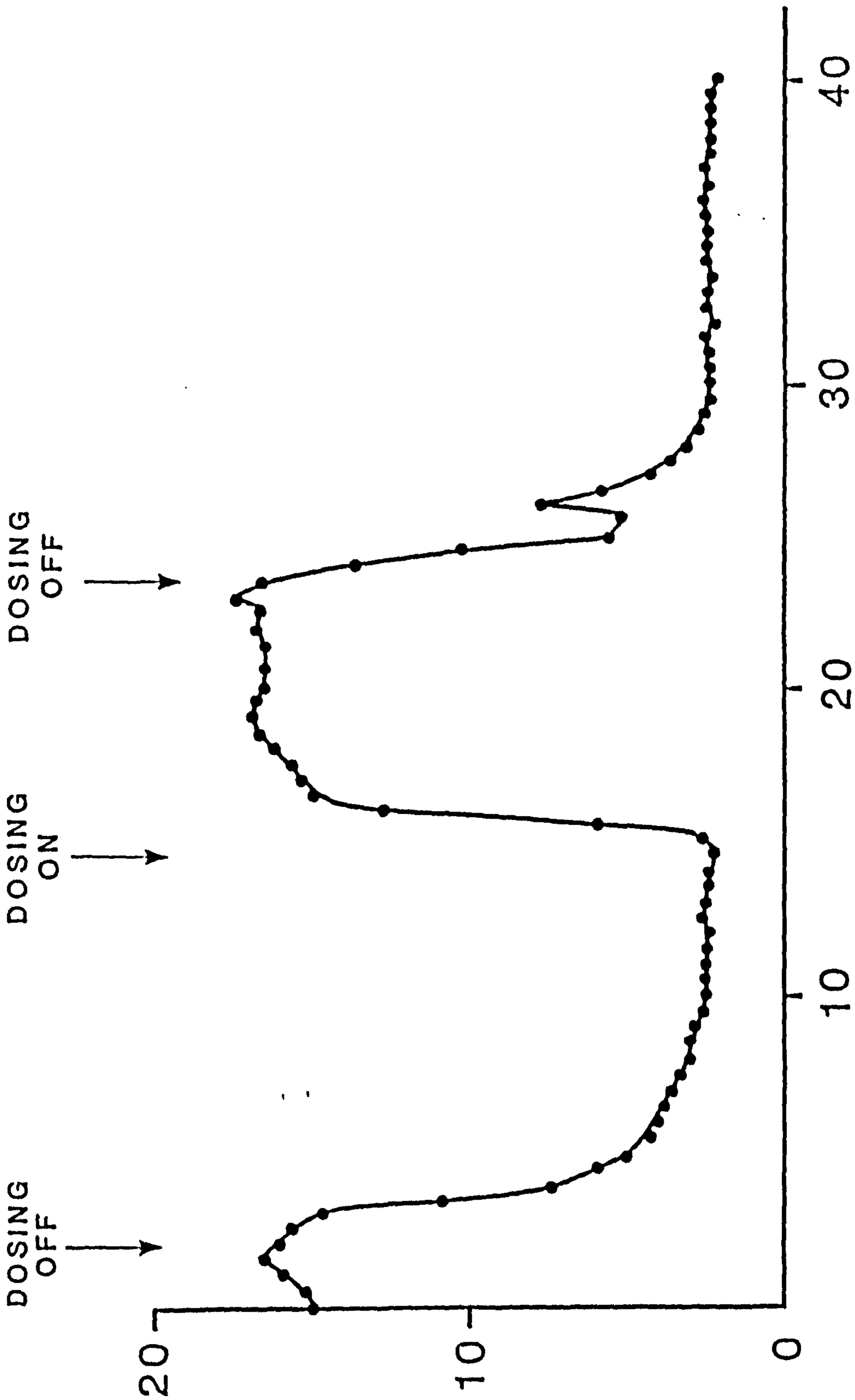


Figure 4.16. Hydroponic Cultivation

data on the quality of the river at that point and, when the water quality drops below preset levels (corresponding to E.E.C. M.A.C.'s) the supply intake can be shut down. Currently the intake is not being used but the site provided an ideal location for field testing the nitrate monitor where it could be compared with ion selective electrode based monitors already on site.

The station is housed in a brick building 90 metres from the river with a railway line between it and the river. A large diameter pipe leads from the river to a sump under the intake station. In this sump are two large pumps which are used to propel the water up to the reservoir. A smaller pump placed in a sump by the river was used to extract a river water supply via a floating strainer placed in the river, which was then fed to a header tank in the intake station via a 4 inch diameter pipe. From this tank a supply was passed to temperature, pH, dissolved oxygen and conductivity probes and also to a turbidimeter. A sample line was taken from this supply and pumped through first a 25 micron and then a 10 micron disposable fabric filter to a constant header tank. Clean river water was fed from this tank to the nitrate and ion selective electrode based monitors. All the monitors in the intake station were duplicated and had the facility to be switched to a tapwater supply if an alarm condition was reached, so that the operation of the monitor could be checked and verified. The output signals of all the monitors were fed, in a 4-20 mA format (4 mA = lowest signal, 20 mA = highest signal) to an interface

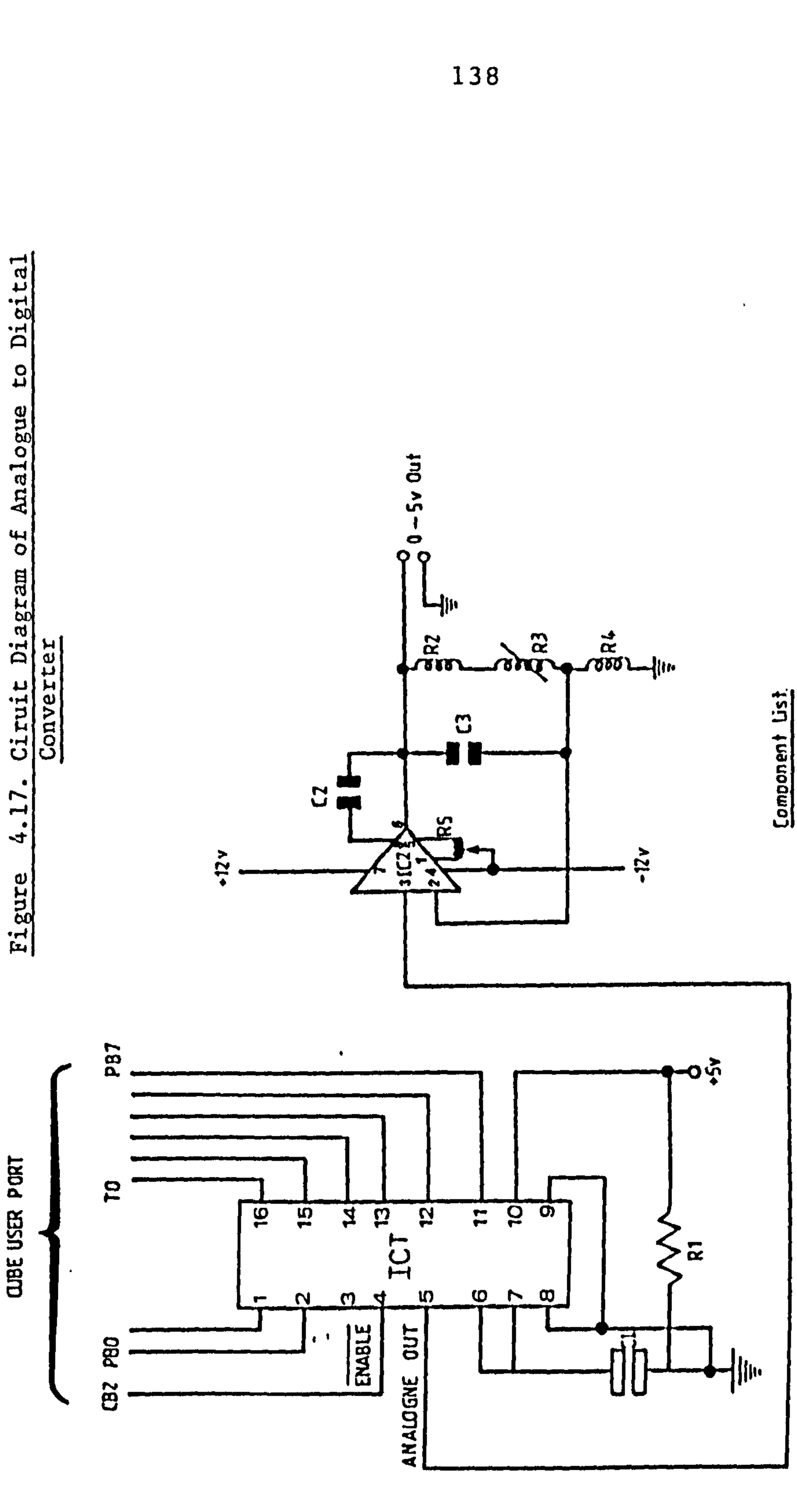
unit (Computronic Services, Letchworth) where they were converted into a digital format. The interface unit also controlled the operation of all the pumps and valves used in the system and could be activated to backflush the sample lines with a mixture of tapwater and compressed air in order to clear any blockage of the inlet strainer. The interface was interrogated by a personal computer (Compaq) which polled the results from each of the instruments every 3 minutes displaying them locally on it s display. Every 15 minutes the current results were also stored on a local hard disk drive. This computer was interrogated once a day (at midnight) by another personal computer (I.B.M.) located at Wessex Water´s regional scientific centre at Saltford near Bristol via an RS232 serial link over a telephone line. The data passed daily to Saltford consisted of 15 minute readings for each of the monitoring devices, any alarm conditions and, any local faults that were detected. The system had the capability for alarms and fault conditions to be transmitted instantaneously to Saltford. The software for this system was known as S.I.P.S. (Supply Intake Protection Scheme) and was commercially written for Wessex Water in FORTRAN, menu driven for ease of use.

S.I.P.S. was reconfigured to allow an extra nitrate monitor to be installed, and the nitrate monitor proposed was modified so as to enable it to be connected to S.I.P.S.. The interface unit required a 4-20 mA current loop signal to be connected to it. The nitrate monitor as constructed had no such output facility although the Cube

computer had an RS232 serial port and a spare 8 bit input/output port with full handshaking, both of which were fully accessible from the software. A signal converter which transforms a 0-5 V signal into a 4-20 mA signal was obtained (Stroud Instruments Ltd., Stroud) and an 8 bit digital to analogue converter was constructed (figure 4.17.) to connect the spare 8 bit input/output port to the signal converter.

The circuit consists of an 8 bit digital to analogue converter (IC1) which produces an output voltage dependant on the status of the 8 lines connected to it from the Cube computer. The converter will only change it s output when the enable pin (pin 4) is driven low and, so when the required binary information was placed on the 8 output lines of the Cube, the CB2 line was driven low and then high. This forces the digital to analogue converter to perform a conversion and when this has been carried out, the output voltage is held at it s new level until CB2 is driven low again. The output from the digital to analogue converter must be linear and vary from 0 to 5 V for values of binary 0 to 255 on the 8 output lines and so a buffering circuit was employed which allowed the output voltage to be offset to zero and, also amplified the maximum output from the converter up to 5 V. This circuit was based on the NE531V operational amplifier (IC2) which, together with discrete components, allowed the offset (R5) and the gain (R3) of the digital to analogue converter to be varied. The output from this circuit was fed into the input of the 4-

Figure 4.17. Ciruit Diagram of Analogue to Digital Converter



Component List

IC1 — ZN428E	C1 — 1µF
IC2 — NE531V	C2 — 33pF
R1 — 390R	C3 — 33pF
R2 — 5K6	
R3 — 5K 10 turn Cermet trimmer	
R4 — 8K2	
R5 — 10K	

20 mA signal converter. This meant that for a value of 0-255 placed on the 8 bit output port of the Cube, a current of between 4-20 mA flowed to the interface unit. Software was written for the Cube to take the current nitrate result and to scale it to a value on the scale 0-255, equivalent to 0 to 12 mg l^{-1} NO_3-N, and then place this value on the 8 bit output port.

The nitrate monitor was installed at the Tucking Mill intake station (at Limpley Stoke) in April 1987 but, was not linked to S.I.P.S. until September 1987 due to problems with the interface between the monitor and the Computronics interface. During the field trial which, which was primarily to assess the long term reliability of the monitor, several minor problems were encountered with the monitor which were all solved (see section 4.5.3.).

A major problem during the trial concerned the S.I.P.S. system, in that on several occasions the software crashed or stopped due to a false alarm event occurring, which then prevented the logging of data from all the monitors in the station. This may have been due to problems with the power supply to the site. The inlet strainers in the river were found to be blocked at regular intervals thus causing the sample pump to burn out and S.I.P.S. shuts itself down if the river water supply fails. On one occasion the strainer had come off and a dead fish was found to be blocking the sample line. These problems with sample introduction have now been resolved with the introduction of two inlet strainers on

the pump inlet line, thus allowing a greater surface area for the inlet.

One advantage of the nitrate monitor was that even when S.I.P.S. had stopped logging data due to a fault condition, a hardcopy of the monitors results was still available when all the data from the ion-selective electrode monitors was lost.

Currently we have monitored the system for 203 days during which time S.I.P.S. did not log data for 92 days due to various problems. These break down as follows,

software faults	2 days
sample supply failure	20 days
computer failure	16 days
alarm conditions	4 days.

The software faults were usually due to the S.I.P.S. software believing that there was a fault in the monitoring station and then shutting itself down, although the river water supply pump continued to provide sample to the monitors. In this case the monitors usually continued to work but the data obtained was lost because S.I.P.S. was not logging. Sample failure was usually due to the intake pump becoming blocked and the motor subsequently burning out. This was a serious fault because it also meant that the monitors ceased to function correctly as they had no sample supply. S.I.P.S. software had the ability to generate alarm signals if a parameter rose above a preset value, but unfortunately S.I.P.S. also shut itself down on that channel when an alarm occurred until the system was reset, thus losing

data. The internal clock on the Compaq computer failed on two occasions, also preventing S.I.P.S. from operating.

The nitrate monitor provided good results for a total of 87 days (43%) of this trial whilst the comparable Kent ion-selective electrode based monitor (model n°. 8006) provided good results for 80 days (39%) out of the total of 203 days. A good result was defined as one which did not vary by more than 10% from it's neighbouring values. It must be born in mind when viewing these figures that the nitrate field monitor used was a prototype which was being tested to discover it's faults whereas the Kent monitor was a commercially available product which has been available for many years. Figure 4.18. shows a histogram comparing the number of days for which good results were obtained for the Hull and Kent monitors with the number of days that S.I.P.S. was operational and, the number of days in that month were added for comparison purposes.

Over the period of the trial the nitrate monitor (Hull) was operational for 87 days out of the 203 days, i.e. it was not producing good data for 116 days. The reasons for this are as follows,

(i) Cube computer faults (23 days) :- the main fault found with the Cube computer was a faulty edge connector on the Eurobeeb card which required the computer to be returned to Control Universal for repair (7 days). Another fault was found with the internal real-time clock

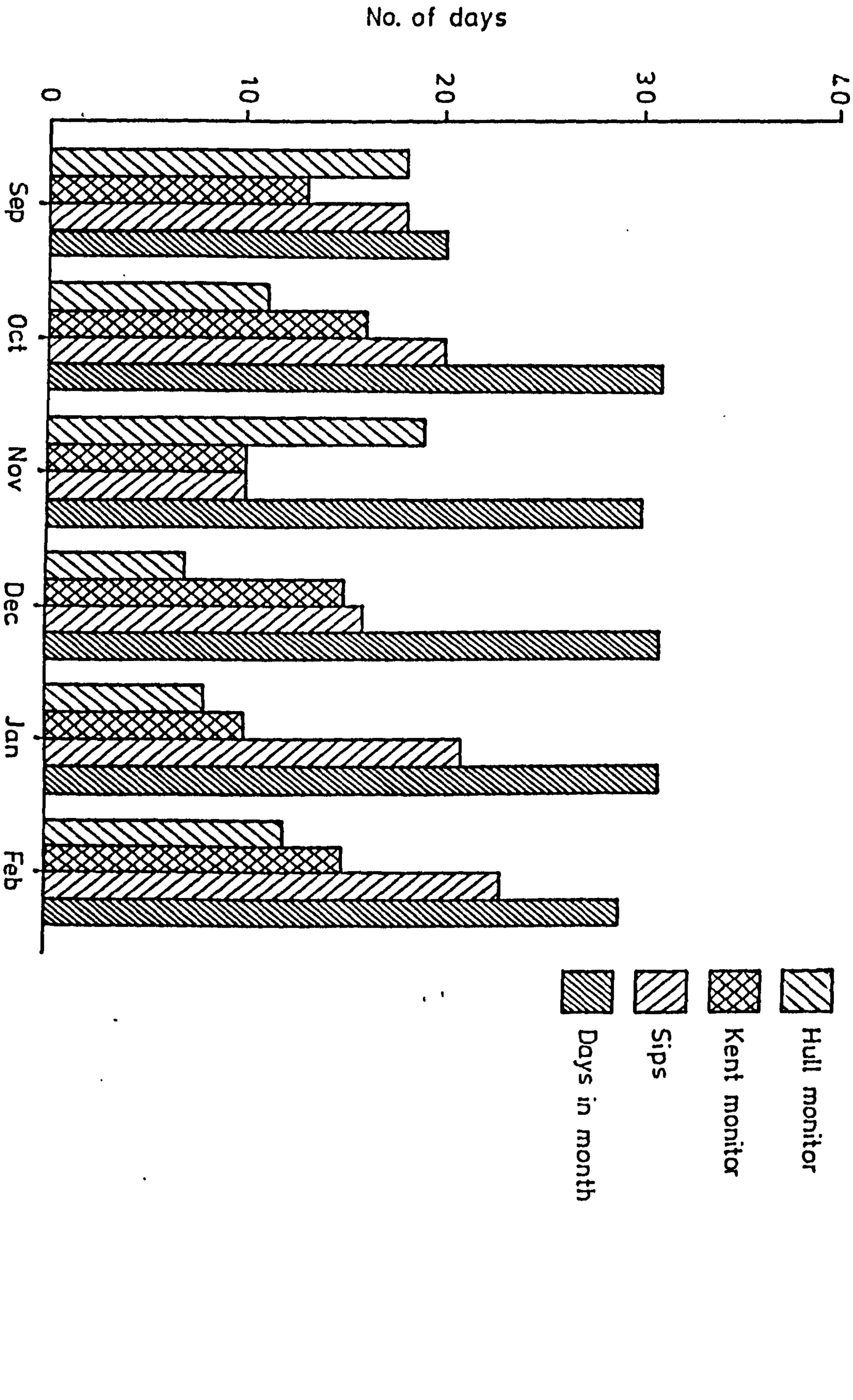


Fig. 4.18. Comparison of uptimes for the two monitors under study.

which worked intermittently and was also rectified by the manufacturers (16 days).

(ii) Sample supply faults (20 days) :- these affected all the monitors at the intake station and were usually due to the sample intake being blocked.

(iii) No reagent/exhausted reductor/system failures (73 days) :- when the reagent/standard supply ran out or the reductor column was exhausted, the system shut itself down until it could be reset by an operator. As we were testing the system we allowed reagents/standards to run out or the cadmium reductor column to become depleted before we actually replaced them. This meant that sometimes up to 7 days could elapse before someone went out to replace them. In a fully commissioned unit, the reagents, standard and reductor column would be replaced weekly and the pump tubes, another source of failure would be replaced monthly, even if still serviceable. Some difficulties were initially experienced by the operator at Wessex Water in the manufacture of the cadmium reductor columns of a suitable quality to last at least 7 days, with the consequence that they failed quite frequently. With experience the columns are now quickly and easily constructed and, last the required period of time.

This trial has proved that the monitor was as reliable as a commercially available ion-selective electrode monitor and, as all the small problems are ironed out it should prove to be even more reliable. This field trial proved the need to routinely replace the cadmium column and replenish the reagents every week and also to replace the pump tubing every 4 weeks.

4.5.3. Problems Experienced During Field Trials

4.5.3.1. Pump Pressure Bar

The bar which holds the pump tubing against the rotating rollers on the peristaltic pump fractured due to the constant stress applied to it over a long period of operation. The pump was a very old one, having previously been used in the research laboratory in a manual flow injection system. The bar was made of a plastic material, and a new one was machined out of nylon, a material with a much greater stress tolerance.

4.5.3.2. Blockages in the Flow System

Problems were experienced with blockages occurring in the reaction coil and also at the flanged joints into the detector housing. Subsequent examination of these blockages found them to be composed of a mixture of glass fibres and cadmium, which had originated from the cadmium reductor columns. The cadmium was due to inadequate plugging of the ends of the columns with glass wool and the glass fibres were from small fragments of glass wool formed when the glass wool was crushed into the column. The solution was to carefully plug the ends of the columns with quartz wool (BDH) which is much finer than

glass wool and not as brittle, so that fibres would not migrate and cause blockages at restriction points.

4.5.3.3. Reductor Column Problems

Several problems were found which were attributed to the cadmium reductor columns. When the columns were packed very tightly, excessive back pressure was generated which resulted in connections to the injection valve 'blowing off' after a short period of operation with the subsequent shutdown of the monitor. There are two solutions to this, one is to be careful with the way in which the columns are packed and the second is to use an injection valve which has flanged connections which will not blow off at the slight back pressures generated by the column.

Also if the columns were packed loosely, the cadmium would slowly pack itself together thus reducing the length of the cadmium and this affected the linear calibration range of the monitor, bringing it down from 0 to 12 mg l^{-1} NO_3-N to 0 to 7 mg l^{-1} NO_3-N or less. The solution was always to pack a firm column of at least 40 mm in length.

4.5.3.4. Lee Solenoid Valves

The Lee solenoid valve which controls the introduction of standard into the monitor is a precision engineered device with a very small internal volume (8 µl). Consequently it is prone to blockages and if these are not remedied the coil may burn out. The solution was to ensure that the sample supplied to the monitor was filtered to remove suspended solids with particle sizes

above 50 μm so as to prevent blockages. Blockages were easily removed by backflushing the valve using a syringe filled with water. In the case of stubborn blockages the valve could be cleared using compressed air.

Another problem found was that the valves on the two monitors in Dorset had colour reagent spilled on their coils and, over a period of time, this dissolved the varnish on the coil winding, helped by the heat generated when the coils were operating. This caused the coils to short out and fuse, rendering them inoperable. The solution in this case was to rewind the coils using 0.2 mm diameter insulated copper wire (Radio Spares; Cat. no. 357-918) until a coil with a resistance of 33 ohms had been built up. A preventative solution was to seal the coils using heat shrink tubing and then to mount them proud of the manifold board to prevent the accumulation of chemicals under them, this also improved the cooling air flow around them.

4.5.3.5. Detector Housing Flanged Joints

On the monitor based at Tucking Mill a fault with the flanged joint taking the colour reagent line into the detector caused a small amount of colour reagent to leak out over the diecast steel case of the detector. Over a period of several months this has slowly become worse to the extent that the detector case is now very thin at this point. This was easily remedied by replacing the flanged joint, a source of potential weakness in most flow injection systems.

All of the problems described above were easily remedied by small alterations to the design of the system. Further trials are being carried out but as yet no other problems have become apparent.

4.6. References

1. "Standard Methods for the Examination of Waters and Wastewaters", American Public Health Association, Washington, 1975, pages 406-410.

2. "Oxidised Nitrogen in Waters 1981", Methods for the Examination of Waters and Associated Materials Series, H.M.S.O., London, 1981.

3. "The Nitrogen Cycle of the United Kingdom - A Study Group Report", The Royal Society, London, 1983.

4. "Standard Methods for the Examination of Waters and Wastewaters", American Public Health Association, Washington, 1985, page 373.

5. F.W.Chichester, J. Environ. Qual., 5(1976)413.

6. D.Winter, Farmers Weekly, March 4, 1983

7. R.J.White, Aqua, 2(1983)51.

8. J.K.R.Gasser, Phil. Trans. Royal Soc. London, B296(1982)303.

9. World Health Organisation, Ambio, 7(1978)134.

10. R.M.Hardisty and D.J.Weatherall, "Blood and its Disorders", Second Edition, Blackwell Scientific Publications, Oxford, 1982, pages 375-379.

11. J.Gardinier and G.Mance, "United Kingdom Water Quality Standards Arising from European Community Directives", Water Research Centre Technical Report TR204, Stevenage, July 1984.

12. S.S.D.Foster and R.I.Crease, J. Inst. Wat. Engrs., 28(1974)178.

13. S.R.Wellings and J.P.Bell, J. Hydrol., 48(1980)119.

14. M.Morgan-Jones, R.J.Flavin and M.J.Hoare, Public Health Eng., 8(1980)49.

15. W.V.Duyvenbooden and J.P.G.Loch, Aqua, 2(1983)59.

16. R.J.Burden, New Zealand J. Science, 25(1982)205.

17. S.B.Tuckwell and M.S.Knight, Paper in Preparation.

18. J.Ruzicka and E.H.Hansen, "Flow Injection Analysis", Wiley Interscience Series, New York, 1977, pages 80-81.

19. S.J.Bajic and B.Jaselskis, Talanta, 32(1985)115.

20. F.Nydahl, Talanta, 23(1976)349.

21. M.N.Jones, Water Res., 12(1978)673.

22. W.Davidson and C.Woof, Analyst, 103(1978)403.

23. M.T.Downes, Water Res., 12(1978)673.

24. J.Hilton and E.Rigg, Analyst, 108(1983)1026.

25. A.Al-Wehaid and A.Townshend, Anal. Chim. Acta, 186(1986)289.

26. J.Slanina, F.Bakker, A.G.M.Bruijn-Hes and J.J.Mols, Fresenius Z. Anal. Chem., 289(1978)38.

27. M.Cooke, J. High Res. Chromatog. and Chromatog. Comm., 6(1983)383.

28. M.Tabatabai and W.A.Dick, J. Environ. Qual., 12(1983)209.

29. R.Sarin and S.P.Pande, J. Indian Inst. Sci., 64(1983)51.

30 D.Capon, Water Treat. Exam., 24(1975)333.

31. E.B.Schalscha, T.Schirado and I.Vergara, Soil Sci. Soc. Am. J., 45(1981)446.

32. A.G.Fogg, A.Y.Chamsi and M.A.Abdalla, Analyst, 108(1983)464.

33. D.L.Heanes, Analyst, 100(1975)316.

34. S.P.Chattopadhyay and A.K.Das, Indian J. Environ. Hlth., 25(1983)282.

35. T.A.Ranney and R.J.Bartlett, Comm. Soil Sci. Plant Anal., 3(1972)183.

36. S.Nakashima, M.Yagi, M.Zenki, A.Takahashi and K.Toei, Fresenius Z. Anal. Chem., 319(1984)506.

37. K.S.Johnson and R.L.Petty, Limnol. Oceanogr., 28(1983)1260.

38. Tecator Application Note n°. ASN 62-02/83.

39. "Standard Methods for the Examination of Waters and Wastewater", American Public Health Association, New York, 1960.

40. J.R.Clinch, P.J.Worsfold and H.Casey, Anal. Chim. Acta, 200(1987)523.

41. J.R.Clinch, P.J.Worsfold, H.Casey and S.Smith, Anal. Proc., 25(1988)71.

CHAPTER FIVE

DETERMINATION OF AMMONIA

5.1. Introduction

5.1.1. Ammonia in Natural Waters

Ammonia is naturally present in surface and groundwaters at low levels, typically 10 to 200 $\mu g\ l^{-1}$ ammoniacal nitrogen (NH_3-N), arising mainly from the deamination of organic nitrogen containing compounds and the hydrolysis of urea, a major waste product of living organisms (1,2). Higher levels of ammonia are usually indicative of pollution e.g. from overloaded sewage treatment works (where the level may exceed 10 $mg\ l^{-1}$ NH_3-N) or from the anaerobic decay of organic matter (3). Surface land runoff does not contribute significantly to the ammonia loading of surface waters as it is readily taken up by plants and adsorbed onto soil particles and clays. Ammonia is also part of the nitrogen cycle although, as it is the reduced form of inorganic nitrogen, it is usually readily oxidised to higher oxidation states.

Ammonia is toxic to most aquatic lifeforms and it is for this reason that the E.E.C. have set a mandatory maximum concentration of ammonia in waters intended to support aquatic life of 780 $\mu g\ l^{-1}$ NH_3-N (4). Water intended for abstraction into the drinking water supply system has a maximum permitted level of 38 - 380 $\mu g\ l^{-1}$ NH_3-N dependant on the type of treatment used. In final

treated water the guideline level is 38 $\mu g\ l^{-1}$ NH_3-N and the maximum admissible concentration is 380 $\mu g\ l^{-1}$ NH_3-N.

Ammonia usually exists in water as the NH_4^+ - ammonium ion, only in waters of high pH will appreciable amounts of dissolved ammonia gas be present. In water analysis the total ammonia concentration is usually quoted, i.e. the sum of ammonia and any ammonium salts present,

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^-$$

This equilibrium (pK_b=4.8) is highly pH dependant and so in order to measure the total ammonia present we usually have to convert all the ammonia into one form.

5.1.2. Methods of Determining Total Ammonia

There are many methods available for the determination of ammonia in natural waters, most of which have been adapted to flow injection analysis,

(i) Distillation/titration (5,6) - the sample is made strongly alkaline and the resultant ammonia gas is distilled off into an acid solution of known strength. The ammonia concentration of the sample is then determined by back titration of the remaining acid.

(ii) Nessler's reagent (7-9) - mercuric (II) iodide in alkaline solution reacts with low concentrations of ammonia to form a brown suspension which can be determined turbidimetrically at 420 nm. This method is very sensitive but requires careful control of the operating conditions to ensure the formation of the brown suspension.

(iii) Indophenol blue (10-14) - this involves the formation of an indophenol blue compound by the Berthelot reaction or a modified form of it. Ammonia is oxidised by hypochlorite to chloramine which then reacts with phenol to form an indophenol blue which can be determined spectrophotometrically at 620 nm. The reagents used vary but the overall reaction is the same. Nitroprusside has also been added as a catalyst to aid in the formation of the indophenol blue.

(iv) Ion-selective electrode (15,16) - the electrode employs a hydrophobic gas permeable membrane to separate the sample solution from the internal electrode solution. Dissolved ammonia gas in the sample diffuses across the membrane dissolving in the internal solution (usually ammonium chloride), changing it's pH and thus creating a potential between the internal solution and the sample solution which is proportional to the ammonia concentration. This means that the sample must be made strongly alkaline in order to determine total ammoniacal nitrogen. Commercial ammonia ion-selective electrodes are available from several manufacturers.

(v) Fluorimetric derivatisation (17,18) - ammonia reacts with o-phthalaldehyde in the presence of 2-mercaptoethanol with the product being determined spectrofluorimetrically at an

excitation wavelength of 410 nm and an emission wavelength of 470 nm.

(vi) Gas diffusion (19-24) - the sample is introduced into an alkaline carrier stream where the ammonia present is converted to the gaseous state. This stream is then passed over one side of a gas permeable membrane, which has an acid base indicator flowing on the other side. Ammonia diffuses across the membrane causing a colour shift in the indicator solution which can then be determined spectrophotometrically. Most reported methods for ammonia using the gas diffusion technique use bromothymol blue as the indicator. Figure 5.1. shows the absorption spectrum of bromothymol blue at pH 6.5 and pH 8.0.

The gas diffusion manifold was investigated further as being suitable for incorporation into an automated ammonia monitor.

5.2. Experimental

5.2.1. Reagents

All solutions were prepared in distilled deionised water.

Ammonia stock solutions (1000 mg l^{-1} NH_3-N) were prepared by dissolving 3.8190 g of ammonium chloride 'AnalaR' (BDH - dried at 105 ^{o}C for 2 hours) in 1 litre of water. Working standards were prepared by serial dilution of this stock standard.

Sodium hydroxide 'AR' (Koch - Light).

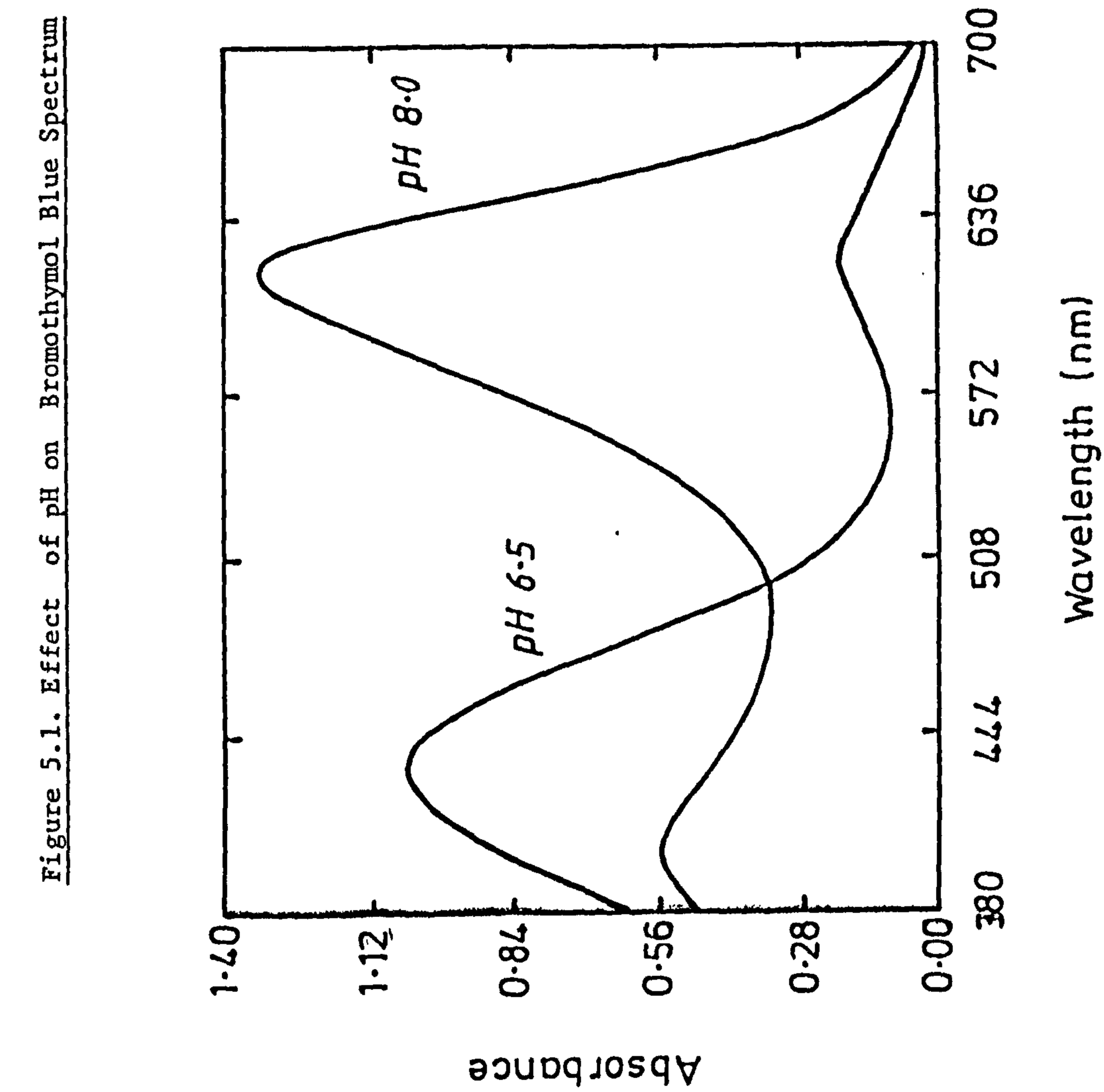


Figure 5.1. Effect of pH on Bromothymol Blue Spectrum

Bromothymol blue 'Indicator grade' (BDH).

5.2.2. Equipment

A solid state photometric detector (as described in chapter 2) incorporating red light emitting diodes as the light source was used throughout. The manifolds were built using 0.5 mm i.d. teflon tubing (Anachem), a Rheodyne type 5020 six port rotary injection valve (Anachem) and an Ismatec mini-S-820 peristaltic pump (Labdata services).

The gas diffusion cell was machined out of perspex (figure 5.2.) with a channel which had a total surface area of 360 mm^2, the channels were 240 mm long by 1.5 mm wide and were machined to a depth of 0.2 mm. The microporous teflon membrane used was thread seal tape (Radio Spares cat. n^o. 512-238) with a width of 12 mm.

5.2.3. Gas Diffusion Manifold

This manifold (figure 5.3.) was similar to that described previously (19); the sample was introduced into a stream of sodium hydroxide where the ammonium ions present were converted to dissolved ammonia gas. This stream was then passed over one side of a gas permeable membrane (P.T.F.E. - 45 μm thick) with a stream of bromothymol blue indicator at pH 6.5 flowing on the other side. The pH gradient across the membrane helps the ammonia to cross into the indicator stream where it causes a change in pH and thus a shift in the colour which can be detected photometrically at 615 nm.

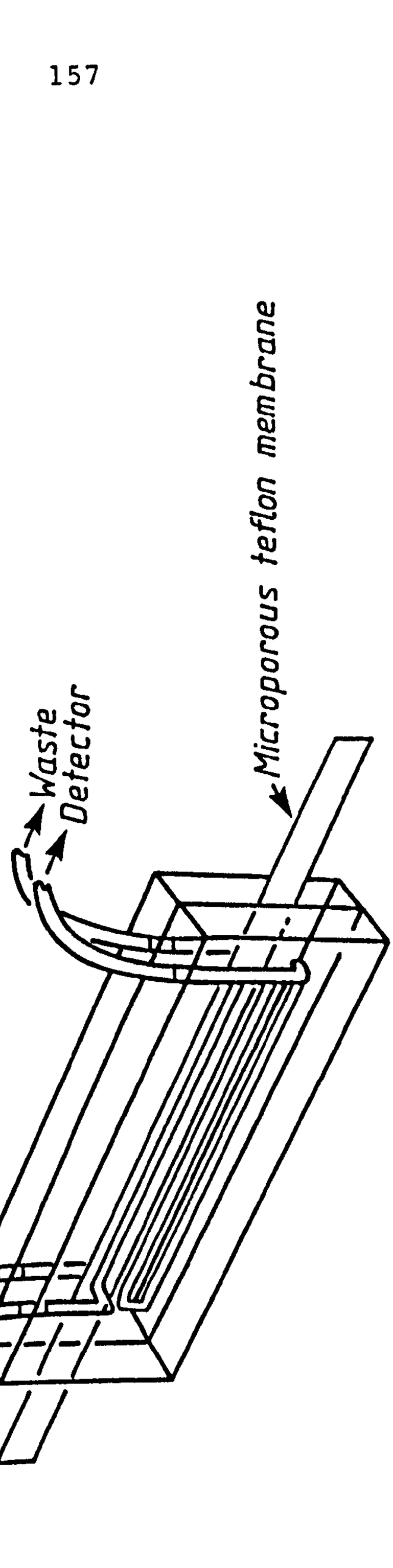


Figure 5.2. Gas Diffusion Cell

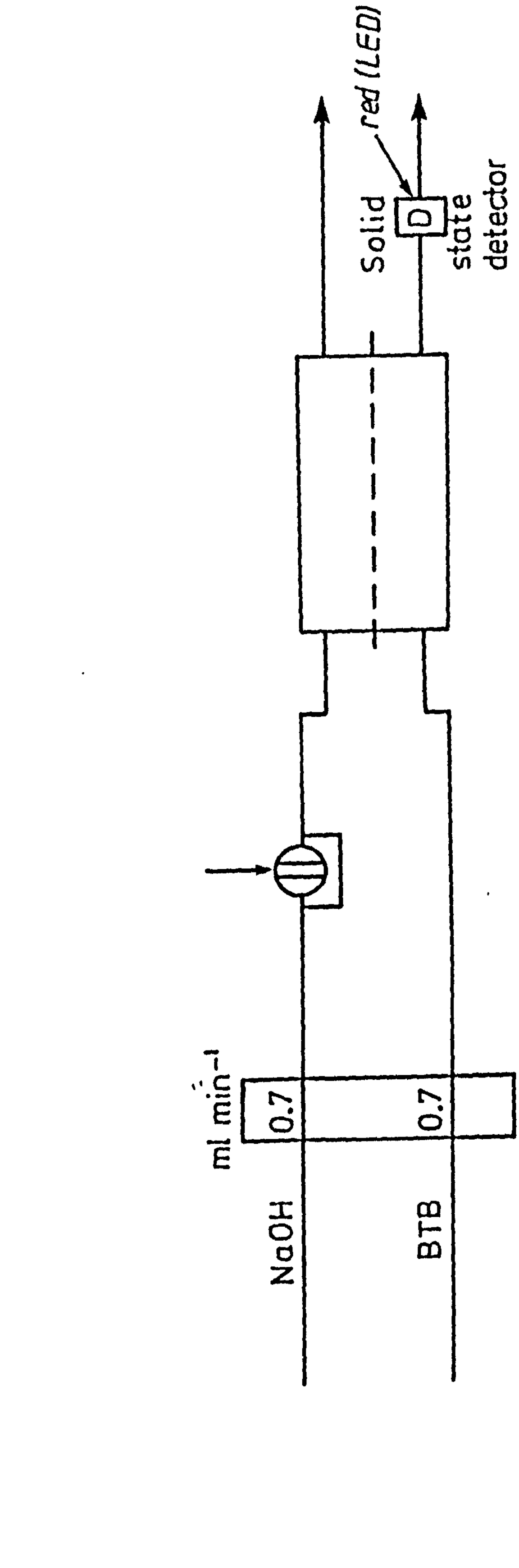


Fig. 5.3. Gas diffusion manifold.

5.2.3.1. Experimental Conditions

Sample/sodium hydroxide mixing coil = 100 cm on a 1 cm diameter rod.

Sample volume = 225 µl.

Sodium hydroxide concentration = 0.4 g l^{-1} (10^{-2} M).

Bromothymol blue concentration = 0.5 g l^{-1} in a 2% $^{v}/_{v}$ ethanol solution.

Sodium hydroxide flow rate = 0.7 ml min^{-1}.

Bromothymol blue flow rate = 0.7 ml min^{-1}.

Gas diffusion cell path length = 240 x 1.5 mm.

5.2.4. Modified Gas Diffusion Manifold

In the modified gas diffusion manifold (figure 5.4.) the sample stream was merged with the sodium hydroxide stream prior to introduction via an injection valve into a distilled deionised water stream flowing on one side of a gas permeable membrane. On the other side an indicator solution containing bromothymol blue at pH 6.5 was flowing prior to passing through the solid state detector cell. This manifold ensured that the microporous teflon membrane was not saturated with hydroxyl ions when the system was not in use.

5.2.4.1. Experimental Conditions

Bromothymol blue flow rate = 0.7 ml min^{-1}.

Distilled deionised water flow rate = 0.7 ml min^{-1}.

Sample flow rate = 0.7 ml min^{-1}.

Sodium hydroxide flow rate = 0.7 ml min^{-1}.

Sample/sodium hydroxide mixing coil = 50 cm on a 1 cm diameter rod.

Sample/sodium hydroxide injection volume = 180 µl.

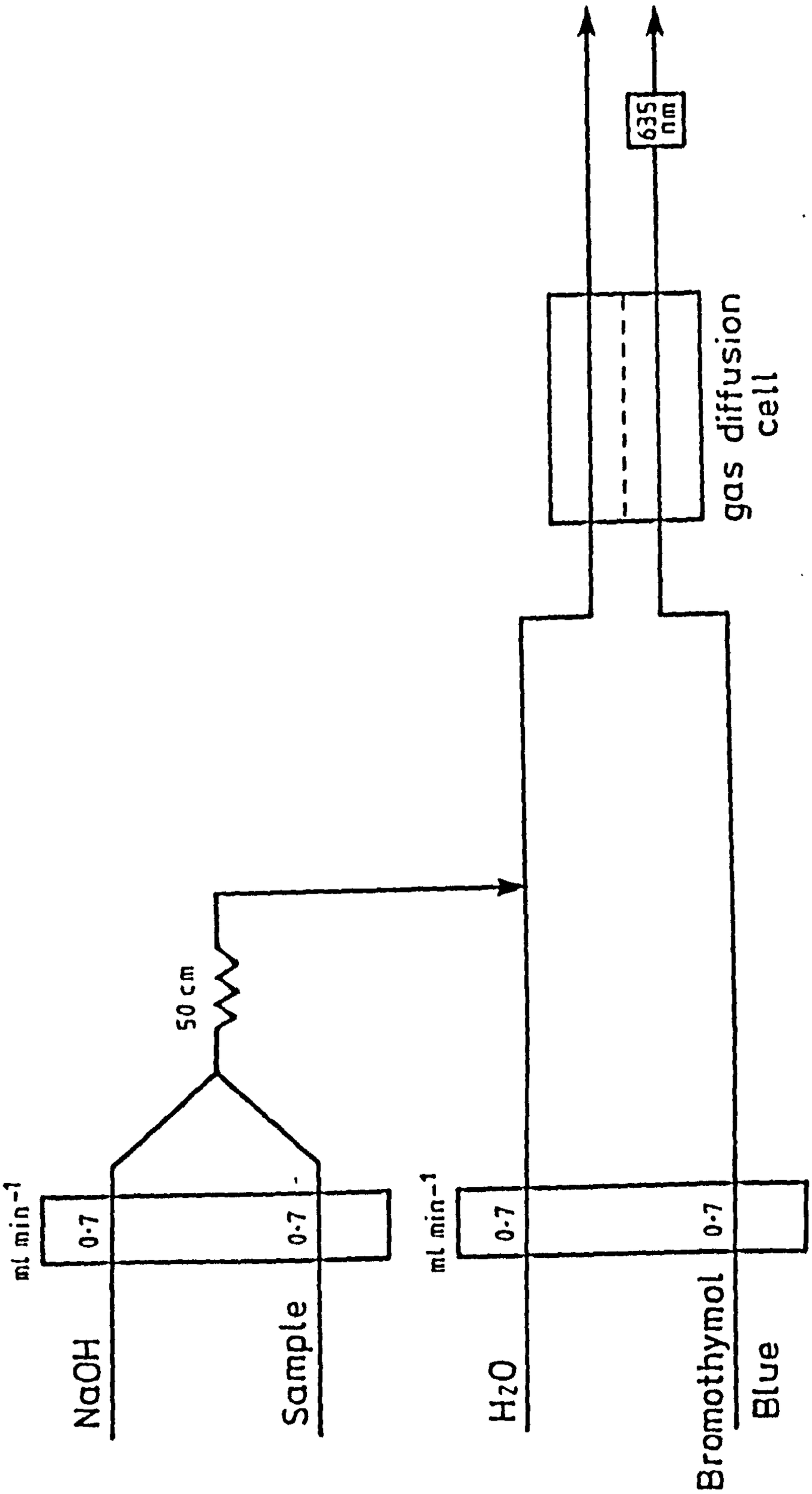


Figure 5.4. Modified Gas Diffusion Manifold

Bromothymol blue concentration = 0.5 g l^{-1} in a 2% $^{v}/_{v}$ ethanol solution.

Sodium hydroxide concentration = 0.4 g l^{-1} (10^{-2} M).

Gas diffusion cell path length = 240 x 1.5 mm.

5.3. Results and Discussion

5.3.1. Gas Diffusion Manifold

5.3.1.1. Optimisation

This manifold was optimised using a univariate approach and a 1 mg l^{-1} NH_3-N standard was used throughout.

The effect of overall flow rate on the signal obtained was studied over the range 0.25 to 1.2 ml min^{-1} for the bromothymol blue and water lines. These flow rates had to be the same to prevent pressure fluctuations affecting the teflon membrane and ultimately weakening it. The signal decreased with increasing flow rate due to the relatively slow rate of transfer of ammonia across the membrane. A flow rate of 0.7 ml min^{-1} per channel was chosen for subsequent work so as to give the best compromise between speed of analysis and signal obtained.

Sample volumes in the range 30 to 450 µl were studied and it was found that the signal increased with increasing sample volume up to 300 µl where a doublet peak was obtained. This was due to the middle part of the sample zone not being mixed with the sodium hydroxide and so producing a zone with only a little free ammonia gas in it. A sample volume of 225 µl was used in subsequent work so as to provide the maximum signal.

A mixing coil was found to be necessary to ensure that the sample was adequately mixed with the sodium hydroxide. It was found that a coil length of 100 cm provided adequate mixing with the maximum signal and minimum analysis time. Coils longer than this reduced the signal obtained due to dispersion of the sample zone and shorter coils produced little mixing of the sample zone in the sodium hydroxide stream.

The effect of sodium hydroxide concentration on the signal obtained was studied in the range 0 to 2 g l^{-1}. A concentration of 0 g l^{-1} gave no signal due to no free ammonia being formed; increasing the sodium hydroxide concentration increased the signal obtained up to 0.2 g l^{-1} above which further increases in sodium hydroxide concentration produced no further increases in the signal obtained. A concentration of 0.4 g l^{-1} was used for subsequent work so as to provide a buffering effect against samples of low pH.

Bromothymol blue solutions with concentrations in the range 0.01 to 0.50 g l^{-1} were prepared in a 2% $^{v}/_{v}$ ethanol solution. The ethanol was used to facilitate easy dissolution of the higher bromothymol blue concentrations. The signal obtained increased with increasing bromothymol blue concentration and 0.5 g l^{-1} was used for subsequent work as higher concentrations were unobtainable.

Bromothymol blue solutions with pH's in the range 4.0 to 8.0 were prepared and their effect on the signal obtained is shown in figure 5.5.. At low pH (<5.5) the

ammonia that does transfer across the membrane does not alter the pH of the bromothymol blue sufficiently to cause a colour shift. At high pH (>7.0) the ammonia that transfers across the membrane causes no appreciable change in the pH of the indicator solution. The optimum pH of the bromothymol blue solution was between 5.5 and 7.0, which corresponds to a colour of dark green. When the bromothymol blue solutions were made up, they were initially orange in colour, sodium hydroxide (0.4 g l^{-1}) was added dropwise until a dark green colour was obtained, if further sodium hydroxide was then added the colour changed to a deep blue.

The effect of the gas diffusion cell path length was studied using path lengths between 80 and 440 mm. The results obtained (table 5.1.) show that above 240 mm the signals obtained decrease due to increased dispersion of the sample zone in the gas diffusion cell. The optimum path length was 240 mm which produced the greatest signal.

5.3.1.2. Calibration

Calibration of this manifold was obtained using ammonia standards in the range 0 to 5000 μg l^{-1} NH_3-N. A typical calibration was described by the equation,

signal (mV) = 0.58 x concentration (μg l^{-1}) - 23.06

with a correlation coefficient of 0.9999. The mean of six replicate injections, standard deviation and relative standard deviation for each standard solution are shown in table 5.2..

NH_3-N ($\mu g\ l^{-1}$)	Signal (mV) Gas Diffusion Cell Path Length (mm) 80	120	200	240	320	440
1000	79.6	211.7	428.3	848.3	709.4	727.5
750	66.7	158.3	313.3	643.3	535.0	555.8
500	45.0	101.7	210.0	419.2	355.0	359.2
250	24.2	51.7	103.3	200.0	170.0	168.3
0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5.1. Effect of Gas Diffusion Cell Dimensions on Calibration

NH_3-N Concentration ($\mu g\ l^{-1}$)	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
5000	2871	18.8	0.7
2000	1172	20.8	1.7
1000	584.2	3.8	0.6
750	406.7	3.4	0.8
500	257.5	1.6	0.6
250	110.8	3.8	3.4
0	-22.5	0.29	6.2

[a] n=6

Table 5.2. Calibration Data for Gas Diffusion Cell Manifold

The linear calibration range of the manifold was 0 to 10 mg l^{-1} NH_3-N and the limit of detection (2σ) was calculated from the blank as 5 μg l^{-1} NH_3-N.

5.3.1.3. Sampling of the River Aire Catchment

Samples of the River Aire were obtained at 25 major bridging points along it s length from Chapel Haddelsy near Goole to Malham village near it's source on the 27th August 1987. Samples were obtained in brown acid washed glass bottles and were refrigerated for one day prior to analysis. On the date that the samples were obtained the river was in spate due to heavy rainfall over the first 50 km of it s length. The results are shown in figure 5.6.. Below Malham the ammonia level increases slowly due to inputs from small village sewage works. The large increase from 60 km and above was due to the inputs from several large Victorian sewage works serving metropolitan West Yorkshire which are old and overloaded, especially in heavy rainfall. The NH_3-N level started to drop off above 80 km as the river left Leeds and dilution effects can be seen. The peak at 98 km was due to the input to the river from the sewage works at Castleford.

This clearly shows that monitoring of ammonia levels in a river system gives a good indication of the state of pollution at that point and can give a good correlation between discharge inputs and the levels found in the river.

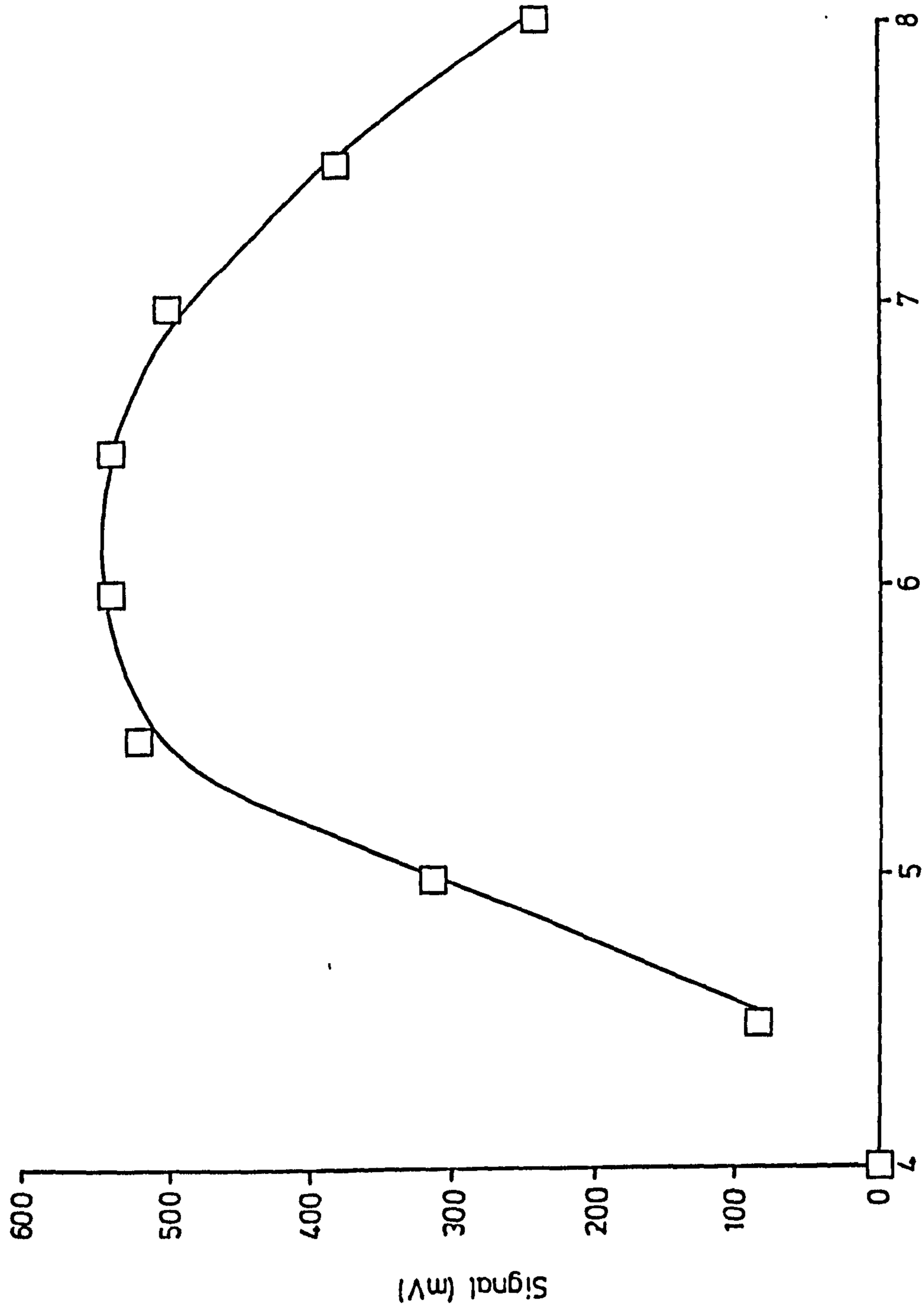


Fig. 5.5. Effect of pH on signal.

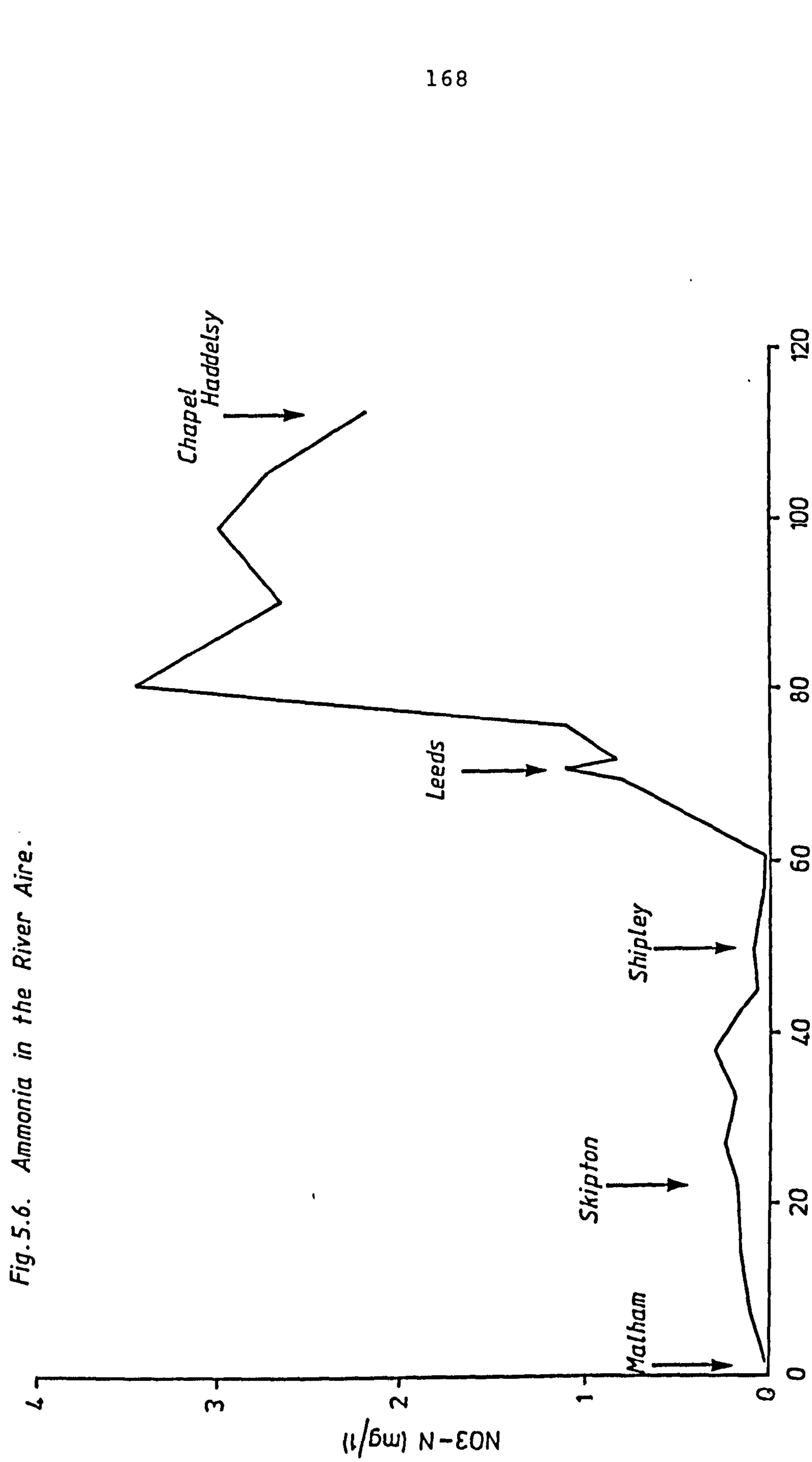


Fig. 5.5.6. Ammonia in the River Aire.

5.3.2. Modified Gas Diffusion Manifold

5.3.2.1. Optimisation

This manifold was optimised using a univariate approach with a blank (0 mg l^{-1} NH_3-N) and a 1 mg l^{-1} NH_3-N standard solution.

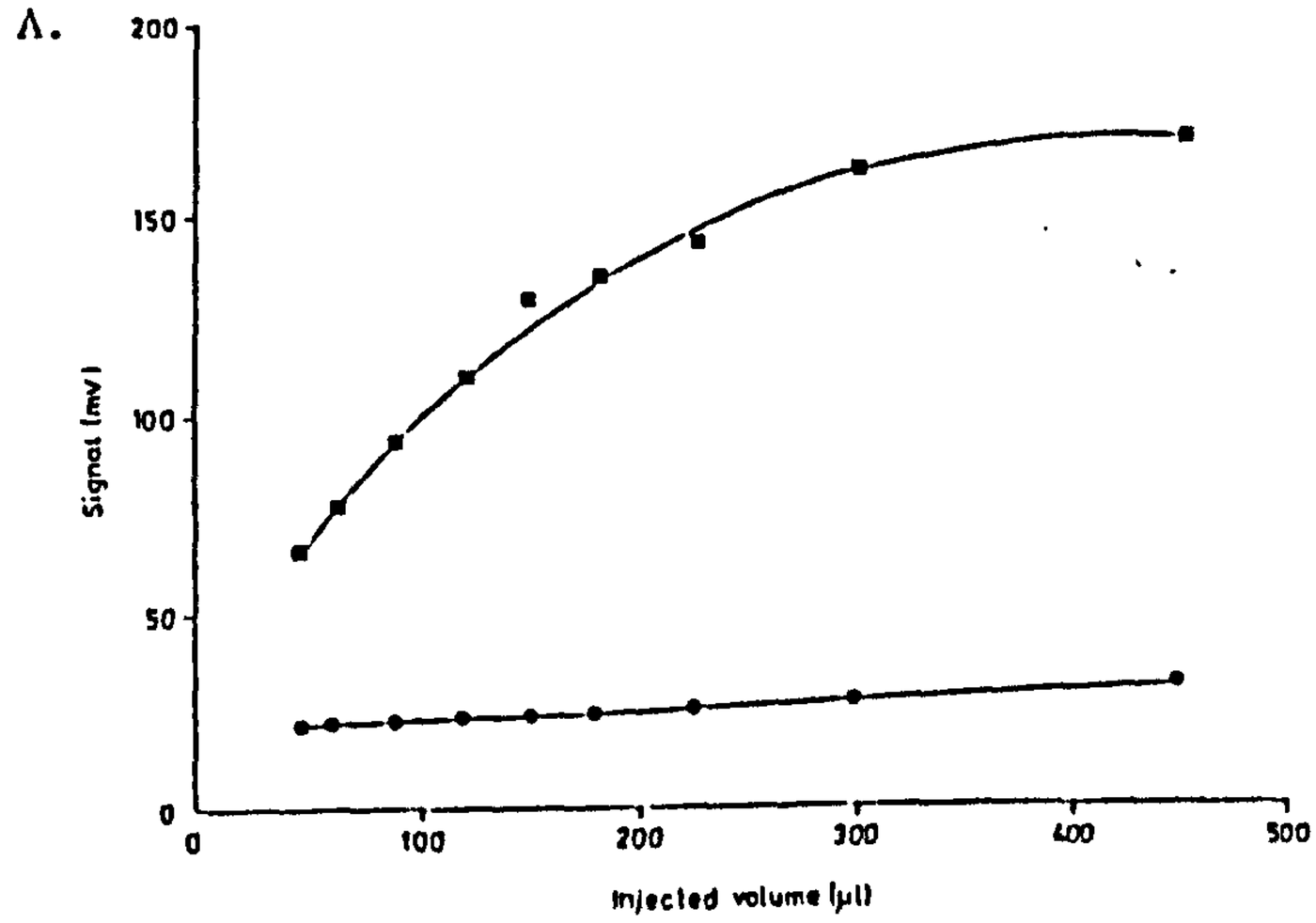
The effect of sample/sodium hydroxide mixing coil length was studied in the range 0 to 300 cm. A coil length of 50 cm or above was found to adequately mix the sample and the sodium hydroxide, and was thus used for all subsequent work.

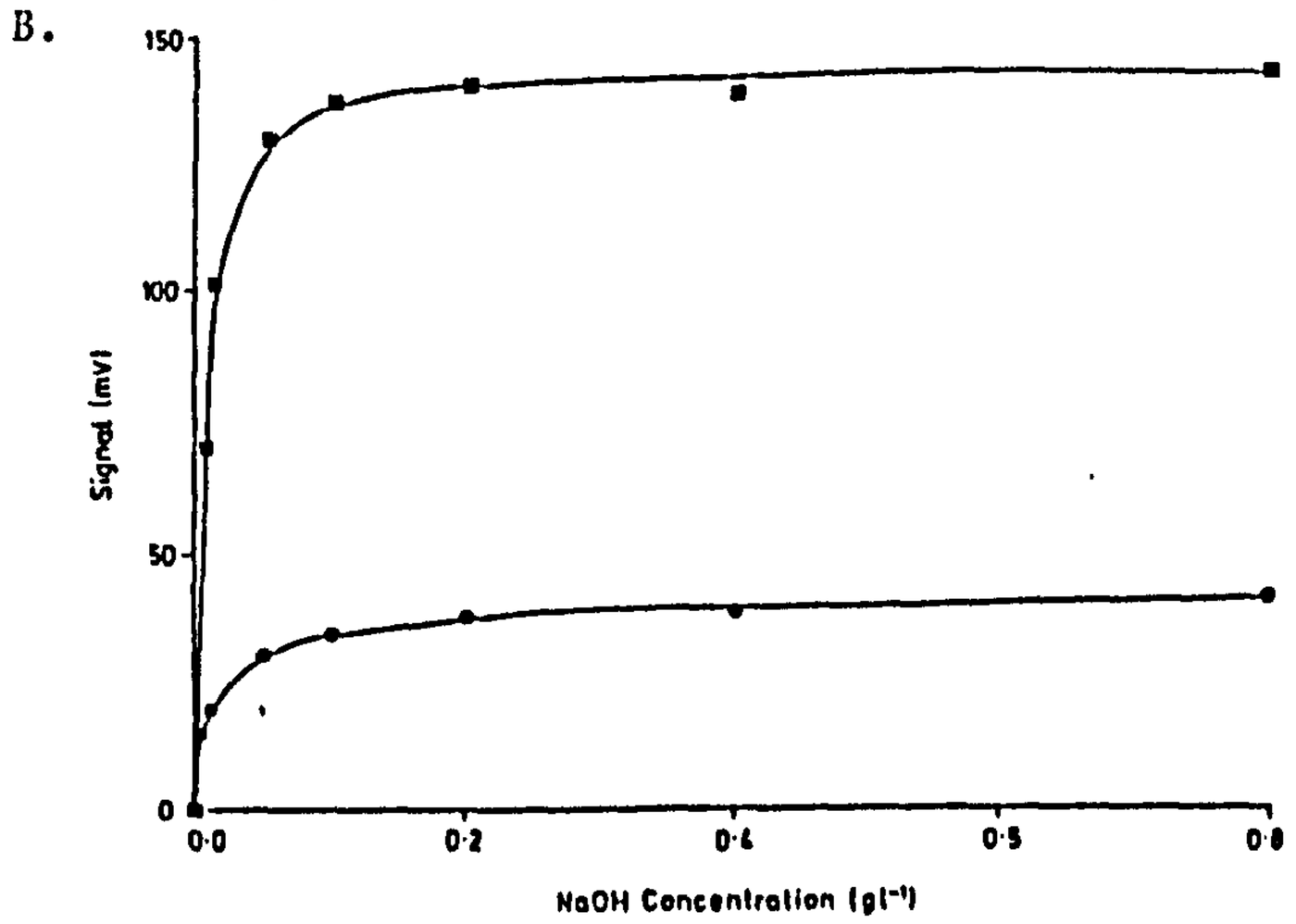
Injection loop volumes were studied in the range 45 to 450 µl and their effect on the sensitivity of the manifold (the difference between a blank and 1 mg l^{-1} NH_3-N solution) is shown in figure 5.7.A.. The blank signal, which was due to the transport of hydroxide ions across the membrane, did not increase significantly with injected volume and the 1 mg l^{-1} NH_3-N signal increased exponentially with sample volume. A volume of 180 µl was used in subsequent work in order to achieve an acceptable analysis time.

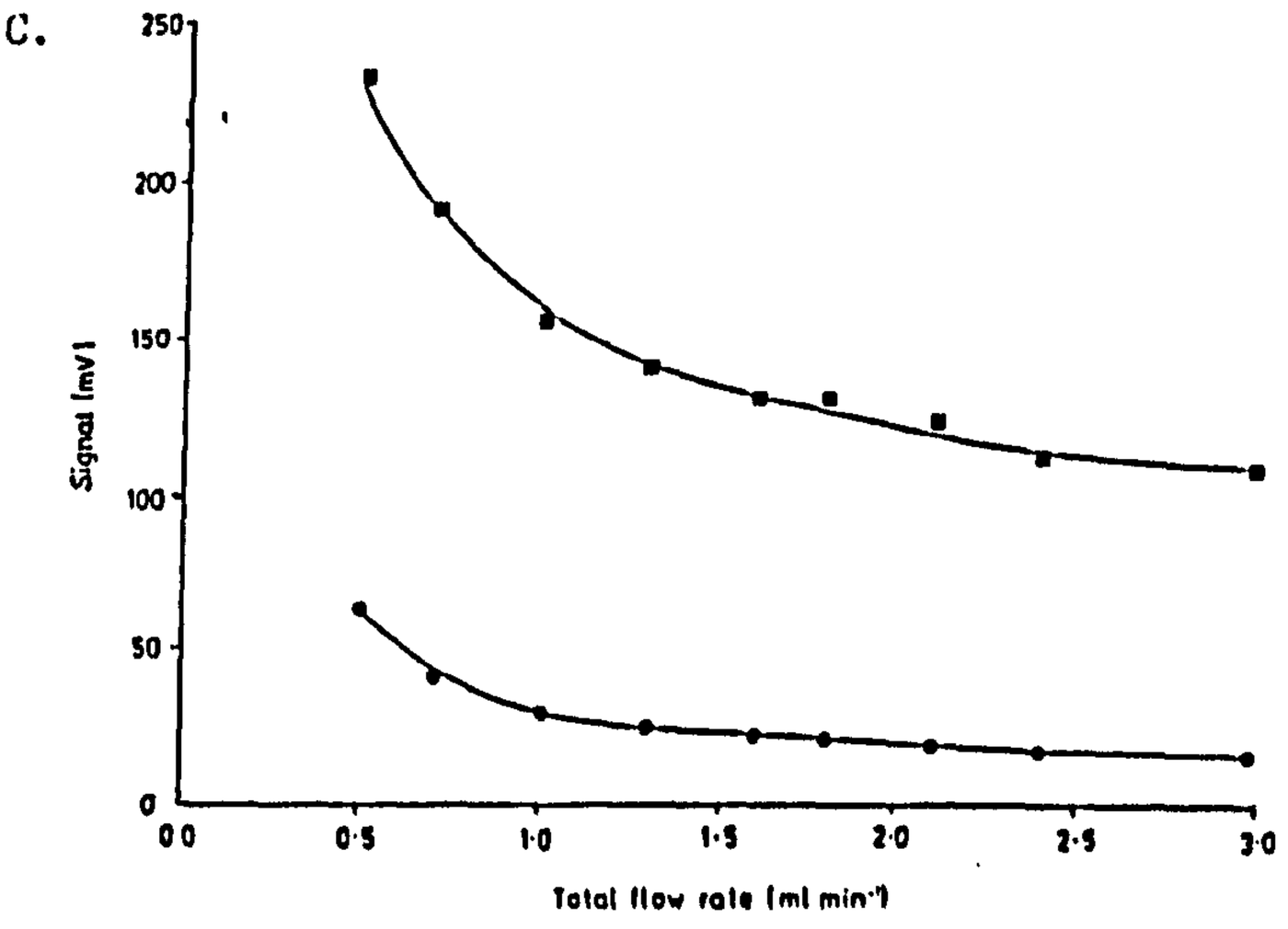
The flow rates of the donor and acceptor streams were matched over the range 0.25 to 1.5 ml min^{-1} per channel and their effect on sensitivity is shown in figure 5.7.B.. Slower flow rates resulted in greater diffusion of ammonia across the teflon membrane but a flow rate of 0.7 ml min^{-1} per channel was used for subsequent work in order to achieve an acceptable analysis time.

The effect of sodium hydroxide concentration on the liberation of ammonia from samples is shown in figure

Figure 5.7. (A) Effect of Injection Volume, (B) Effect of Flow Rate, (C) Effect of Sodium Hydroxide.







5.7.C., for a sample blank and a 1 mg l^{-1} NH_3-N standard. A sodium hydroxide concentration of 0.4 g l^{-1} was chosen so as to ensure maximum sensitivity over the expected pH range of river water samples (5.5 to 8.5) and at the same time maximise the lifetime of the teflon membrane.

Bromothymol blue solutions with concentrations in the range 0.01 to 0.5 g l^{-1} were prepared in a 2% $^{v}/_{v}$ ethanol solution and their effect on the signal obtained was studied. It was found to increase with increasing bromothymol blue concentration and an 0.5 g l^{-1} solution was chosen as giving the optimum signal.

As shown earlier (section 5.3.1.1.) for the original manifold, the optimum pH of the bromothymol blue for the transfer of ammonia was found to be between 5.5 and 7.0 where the maximum sensitivity of the manifold was achieved. This was also found to be the case for the modified manifold.

The effect of gas diffusion cell path length was studied and the results obtained were comparable with those in section 5.3.1.1. and thus a path length of 240 mm was used.

5.3.2.2. Calibration

Calibration of this manifold was achieved using ammonia standards in the range 0 to 5000 μg l^{-1} NH_3-N. A typical calibration was described by the equation,

signal (mV) = 0.244 x concentration (μg l^{-1}) + 25.5

with a correlation coefficient of 0.9999. The mean of six replicate injections, standard deviation and relative standard deviation are shown in table 5.3.. The linear

NH_3-N Concentration ($\mu g\ l^{-1}$)	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
5000	1243	11.5	0.9
2000	517.0	5.8	1.1
1000	275.0	5.0	1.8
500	141.7	1.9	1.3
250	80.0	-	0.0
0	0.0	-	0.0

[a] n=6

Table 5.3. Calibration Data for Modified Gas Diffusion Cell Manifold

calibration range of this manifold was found to be 0 to 7 mg l^{-1} NH_3-N and the limit of detection (2σ) was calculated from the blank signal as 17 μg l^{-1} NH_3-N.

5.4. Design and Construction of an Automated Monitor

A block diagram of the automated ammonia field monitor is shown in figure 5.8.. The computer and pump box were the same as previously described (section 4.4.) with the exception that an extra relay channel was added to the pump box to facilitate the operation of a second solenoid switching valve.

5.4.1. Manifold Board

The manifold board contained the flow injection manifold and the solid state photometric detector. The manifold was constructed from teflon tubing (0.5 mm i.d.) on a blockboard base similar to that described for nitrate. As the manifold used (figure 5.4.) gave rise to a blank signal, two three way solenoid valves (LEE, Westbrook, CN.) were used to switch the sample line between a blank (distiled deionised water), the river water under analysis, and a 1 mg l^{-1} NH_3-N standard solution. The solenoid injection valve was as described previously and, the reference channel of the detector had the bromothymol blue solution flowing through it prior to it s passage through the gas diffusion cell.

5.4.2. Pump Box

This was as described in section 4.4.2. with an additional relay channel so that the extra 12 V solenoid valve (to switch between the sample and the blank) could be operated. This was achieved by taking line PA3 of the

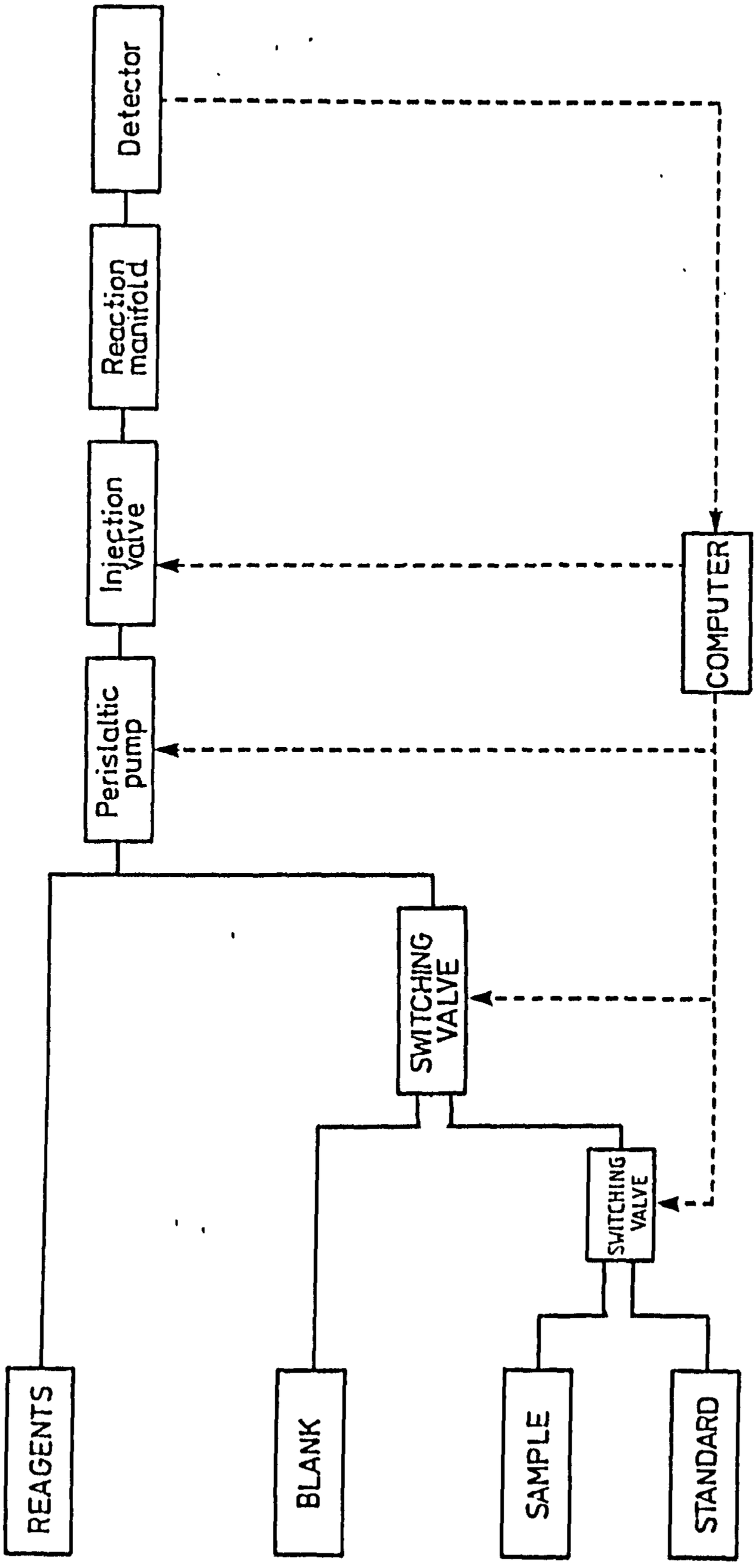


Fig.5.8. Block diagram of the ammonia monitor.

Cube input/output port into a spare buffer of IC2. The resultant line was then fed through a 3K3 resistor into the base of a BC183L transistor, which served to limit the current drawn from the buffer chip. Thus if 5 V was placed on the base of the transistor then it switched on allowing current to flow through the relay coil, thus switching the solenoid valve on. The circuit was the same as used for the other 12 V solenoid valves as in figure 4.11..

5.4.3. Cube Eurobeeb Computer

The Cube Eurobeeb computer used was as described in section 4.4.3.. The software (similar to that shown in appendix 1.) to control the monitor was written in BASIC and programmed onto an E.P.R.O.M. for stand alone operation. the programme was activated on the hour and half hour, and, after a 3 minute delay in order to flush the system, duplicate injections of the blank, river water sample and 1 mg l^{-1} NH_3-N standard were made. The detector output was sampled every 300 ms and the value stored in R.A.M.. After the six injections had been carried out, the mean of each duplicate injection was ratioed as described in section 4.4.3. and the resultant NH_3-N concentration was displayed on the miniature liquid crystal display and printer. A typical chart recorder trace showing the signal obtained from the detector is shown in figure 5.11..

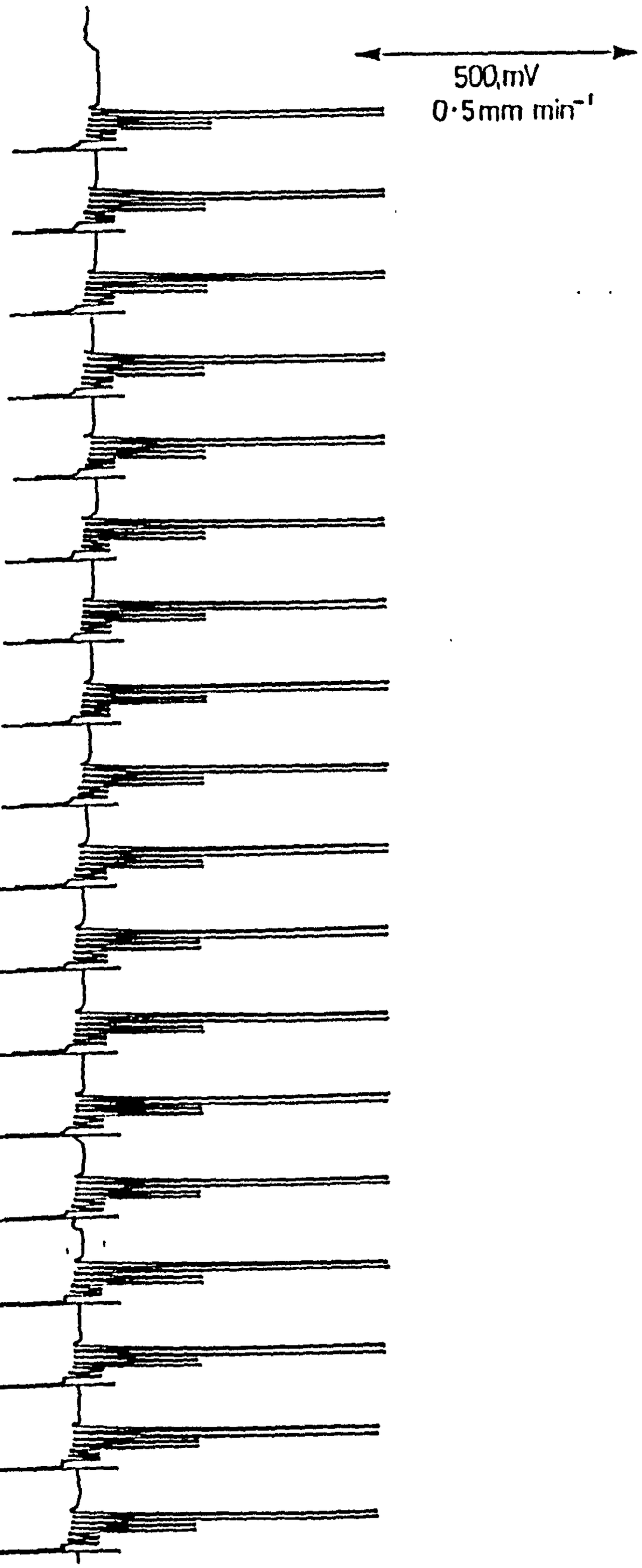


Figure 5.9. Detector Output from the Ammonia Monitor

5.5. Monitor Trials

5.5.1. Laboratory Trial

The ammonia monitor was set up in the laboratory using a 1 mg l^{-1} NH_3-N standard solution and a 0.35 mg l^{-1} NH_3-N solution as the sample so that observations could be made about reagent consumption and the precision and stability of the monitor.

It was found that the reagent consumption over an 8 day period was 3.2 l of each of the bromothymol blue solution and distilled deionised water, and, 2.0 l of sodium hydroxide solution. This was equivalent to a consumption of 8.2 ml of each of the bromothymol blue solution and distilled deionised water and, 5.2 ml of sodium hydroxide solution per determination. The consumption of the 1 mg l^{-1} NH_3-N standard solution was found to be 0.6 l and the blank solution was 0.8 l. The relative standard deviation of the result obtained for the 0.35 mg l^{-1} NH_3-N standard was 2.2% (n=484). Subsequent trials confirmed these figures.

The P.T.F.E. microporous membranes were found to fail after 14-16 days of continuous usage; usually they split, possibly due to fluctuations in the flow rates causing the membrane to oscillate in the gas diffusion cell, eventually weakening sufficiently to fracture at the point of greatest strain. The reagent solutions used were stable for at least 30 days when stored in dark plastic bottles at room temperature. With this data in mind it was recommended that the P.T.F.E. membrane is replaced

weekly, with the pump tubing and reagents being replaced fortnightly.

5.5.2. Field Trials

The ammonia field monitor was installed on the River Avon in Wiltshire at the Bradford on Avon sewage works pumping station (ST 815604) in December 1987. The site consists of a wooden hut used by the Scientific Services section of Wessex Water for river water quality monitoring. River water was pumped up from the river into a 10 l capacity sink and was then allowed to overflow back to the river under gravity. Suspended in the sink were electrochemical probes for temperature, pH, conductivity and dissolved oxygen. The data obtained from these was transmitted via a satellite link to divisional offices at Bath six miles away. The sample inlet to the monitor was taken from the sink using 0.8 mm i.d. teflon tubing with a glass minicolumn (50 mm long; 2mm i.d.) packed with glass wool to remove any suspended solids present in the river water sample.

During the trial the monitor only ran for periods of up to three days due to problems experienced with the Cube computer. The clock on the computer stopped at random, thereby causing the monitor to shut down. The computer was returned to the manufacturer several times without the fault being rectified. The fault was eventually traced to a faulty clock circuit on the issue 7 Eurobeeb card, this was replaced with an issue 10 board which had had the fault remedied. Another problem that "surfaced" in the field trials was that air bubbles in

the reagent and sample/standard lines caused the output signal from the detector to be noisy. This sometimes confused the simple peak finding algorithm causing it to 'hang up' because it could not find any sensible peaks and, the monitor shut itself down. This problem is currently being remedied by rewriting the peak detecting algorithm.

Results for a three day period of operation are shown in figure 5.10. and show that the monitor detected two large flushes of ammonia in the River Avon which would not normally have been detected using conventional manual interval sampling techniques. These two flushes were probably due to either the temporary overloading of one of the sewage works situated upstream of Bradford on Avon or due to an industrial effluent being discharged.

Further field trials of this monitor were curtailed due to the need to use some of the parts in the construction of an aluminium monitor.

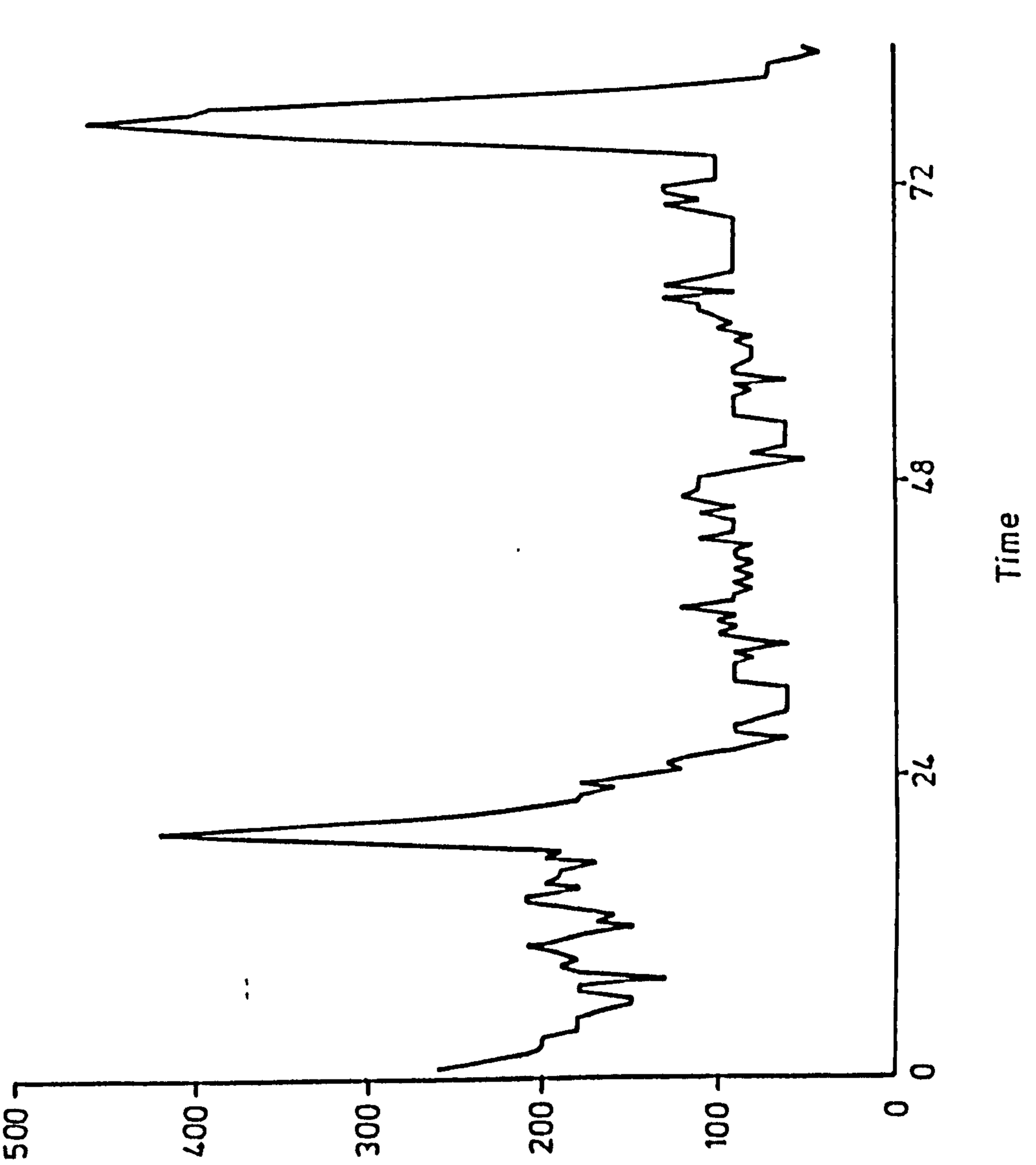


Figure 5.10. Ammonia Levels in the River Avon

5.6. References

1. "Standard Methods for the Examination of Waters and Wastewaters", American Public Health Association, Washington, 1985.

2. "The Nitrogen Cycle of the United Kingdom - A Study Group Report", Royal Society, London, 1983.

3. M.D.Krom, S.Grayer and A.Davidson, Aquaculture, 44(1985)153

4. J.Gardinier and G.Mance, "U. K. Water Quality Standards arising from E.E.C. Directives", W.R.C. Technical Report TR204, Medmeham, 1984.

5. C.L.Ho, J. Test. Eval., 12(1984)100

6. "Ammonia in Waters 1981", Methods for the Examination of Waters and Associated Materials Series", DoE, H.M.S.O., London, 1981.

7. P.B.Sarkar and N.N.Ghosh, Anal. Chim. Acta, 13(1955)195.

8. P.B.Sarkar and N.N.Ghosh, Anal. Chim. Acta, 14(1956)209.

9. F.J.Krug, J.Ruzicka and E.H.Hansen, Analyst, 104(1974)47.

10. D.Scheiner, Water Res., 10(1976)31

11. M.W.Weatherburn, Anal. Chem., 39(1967)971.

12. P.F.Reay, Anal. Chim. Acta, 176(1985)275.

13. J.Slanina, F.Bakker, A.Bruyn-Hes and J.J.Mols, Anal. Chim. Acta, 113(1980)331.

14. Chemlab Instruments Method Sheet No. CFI-008-01, April 1986.

15. R.F.Thomas and R.L.Booth, Environ. Sci. Technol., 7(1973)523.

16. L.R.McKenzie and P.N.W.Young, Analyst, 100(1975)620.

17. A.Rios, M.D.Luque De Castro and M.Valcarcel, Anal. Chim. Acta, 187(1986)139.

18. S.S.Goyal, D.W.Rains and R.C.Huffaker, Anal. Chem., 60(1986)175.

19. W.E.Van Der Linden, Anal. Chim. Acta, 151(1983)359.

20. W.E.Van Der Linden, Anal. Chim. Acta, 155(1983)273.

21. G.Svensson and T.Anfalt, Clin. Chim. Acta, 119(1982)7.

22. M.Van Son,, R.C.Schothorst and G.Den Boef, Anal. Chim. Acta, 153(1983)271.

23. M.Valcarcel and M.D. Luque De Castro, "Flow Injection Analysis - Principles and Applications", Ellis Horwood, Chichester, 1987.

24. Tecator Application Note, AN 50/82, December 1982.

CHAPTER SIX

DETERMINATION OF ALUMINIUM

6.1. Introduction

Aluminium is the third most abundant element within the earth´s crust (1,2). It occurs primarily in aluminosilicate minerals, as feldspars in rocks and as clay minerals in well weathered soils (3). Concentrations of dissolved aluminium in surface waters are naturally low due to the poor solubility of aluminium minerals but, aluminium solubility increases with decreasing pH. Aluminium levels in surface waters can be expected to increase due to problems with acid rain increasing the ability of waters to leach aluminium from rocks and soils.

Aluminium in the form of sulphate is added to surface waters abstracted for use in the drinking water supply as a coagulant to remove dissolved organic compounds (4). These organic compounds (mainly humic acids) cause discolouration and turbidity of the water, and are present mainly in surface waters obtained from upland catchment areas. Aluminium is added, usually as aluminium sulphate, at a concentration of between 5 and 70 mg l^{-1} Al and visible agglomerates or flocs of coagulated material are formed. These can be aided in their formation by the application of gentle agitation and are then removed from the clarified water by sedimentation and filtration. A problem with this process is that with increasing concern over health problems associated with aluminium, it is important to be able to monitor the

level of residual aluminium after the coagulation process. Normally this is done using a simple manual colo rimetric method, but what is really needed is an on-line monitor with feedback control which would also control the dosing of the aluminium sulphate, so that only a minimum amount is added. This would not only prevent high levels of aluminium from being present in the drinking water but would also save money by ensuring that only the absolute minimum amount of aluminium is used in the treatment process.

Alternatives to aluminium as a coagulant include iron, added as chlorinated copperas, a mixture of ferric chloride and ferric sulphate. Some research has also been undertaken into the use of cationic polyelectrolytes (5) but concern has been expressed over the danger of exposure to low levels of these compounds over a long period of time.

6.1.1. Aluminium and Health

Aluminium has been recognised as a selective neurotoxic agent for over 90 years (6) and the list of human neurological conditions for which elevated aluminium levels have been associated with continues to expand. Most of these conditions are related to Alzheimer senile and pre-senile dementia for which the actual cause has not been found, but the evidence that shows that aluminium is involved is very strong. Alzheimers disease is the most common cause of senile brain disease and is progressive, fatal and untreatable. It usually begins with learning-memory deficits and slowly progresses to

involve all aspects of intellectual activity. Movement is also progressively impaired and in some cases seizures are common.

In Alzheimers disease there is a significant loss of brain tissue from all parts of the brain (7) above that due to normal ageing processes. The causes of neuronal loss in Alzheimers disease are still unknown but a number of possibilities have been suggested, including inherited genetic defects (8), although studies of D.N.A. to date have failed to reveal any abnormalities in affected people, and an exogenous toxin, such as aluminium (9).

It must be stressed that although the evidence is strong that aluminium is linked with Alzheimers disease, the link has not yet been proven. The E.E.C. has set limits on the aluminium concentration in drinking water (10), based on this link with neurological disease, which some treatment plants have difficulty in meeting. They have set a guideline level of 50 $\mu g\ l^{-1}$ Al and a maximum admissible concentration of 200 $\mu g\ l^{-1}$ Al for drinking water leaving the plant. They have not set any standards for water intended for abstraction into the water supply as it is assumed that the aluminium is added at the plant.

6.1.2. Methods of Determining Aluminium

There are many methods available for the determination of aluminium in natural waters, some of which have been adapted to flow injection analysis;

(i) Atomic absorption spectroscopy :- aluminium forms refractory oxides and thus either a high

temperature nitrous oxide - acetylene flame or a graphite furnace with a hydrogen atmosphere must be used (11). Alternatively aluminium can be determined by inductively-coupled plasma emission spectroscopy (12).

(ii) Fluorimetric derivatisation (13-15) :- a complex is formed with the aluminium ions using a selective ligand e.g. morin, which can then be determined fluorimetrically.

(iii) Ion chromatography :- a cation exchange column is used to separate the aluminium from the other cations and the eluent is monitored conductimetrically (16).

(iv) cathodic stripping voltammetry (17) :- aluminium is preconcentrated on a hanging mercury drop using a complexing agent and is then determined as the complex.

(v) UV/Visible spectroscopy :- the aluminium is complexed using a chromogenic reagent and the resultant complex determined spectrophotometrically. Many chromogenic reagents have been proposed including,

(a) aluminon (18)

(b) eriochrome cyanine R (19)

(c) xylenol orange (20)

(d) bromopyrogallol red (21)

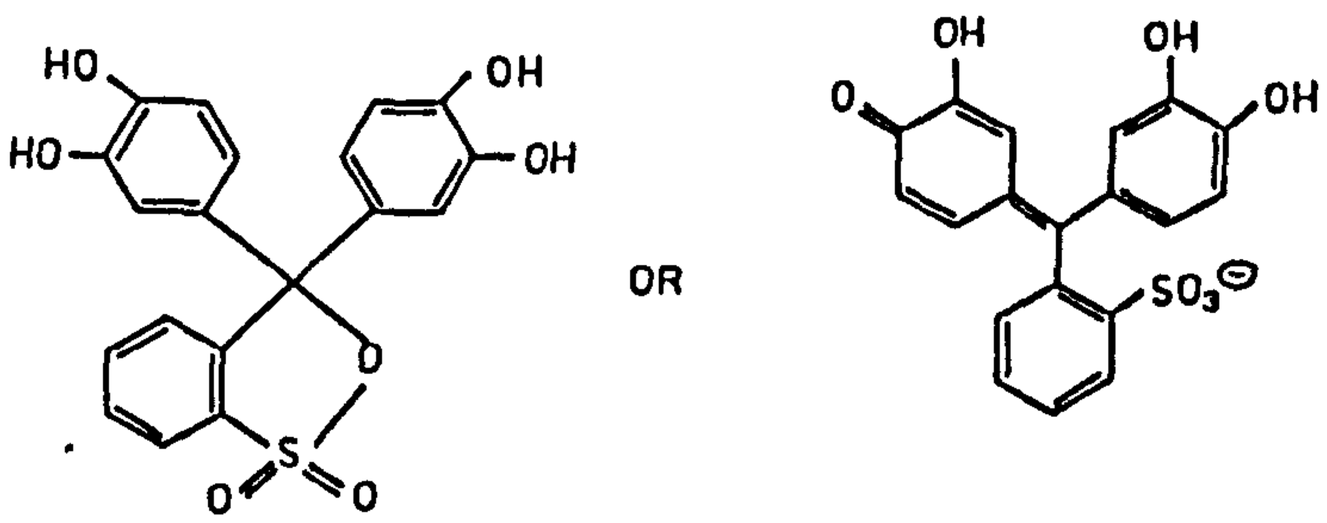
(e) chrome azurol S (22)

(f) pyrocatechol violet (23).

Several comparisons of the various colorimetric reagents available for aluminium have been presented (24-26) and the most widely used method is that involving pyrocatechol violet (27-29).

6.1.3. Pyrocatechol Violet Method

Pyrocatechol violet (sometimes called catechol violet) or 3,3′,4′-trihydroxyfuchson-2′′-sulphonic acid has the following structure,



and when dissolved in water gives a yellow/orange coloured solution with a maximum absorption peak at 444 nm.

Pyrocatechol violet will complex aluminium (III) ions at pH 6.1 to form a blue coloured complex with a maximum absorption at 620 nm (figure 6.1.).

6.2. Experimental

6.2.1. Reagents

All solutions were made up in distilled deionised water and stored in either teflon or polythene containers.

Aluminium working standards were prepared by serial dilution of a 1000 mg l^{-1} aluminium 'Spectrosol' standard solution (BDH).

Ferric nitrate solution, 1000 mg l^{-1} Fe 'Spectrosol' (BDH).

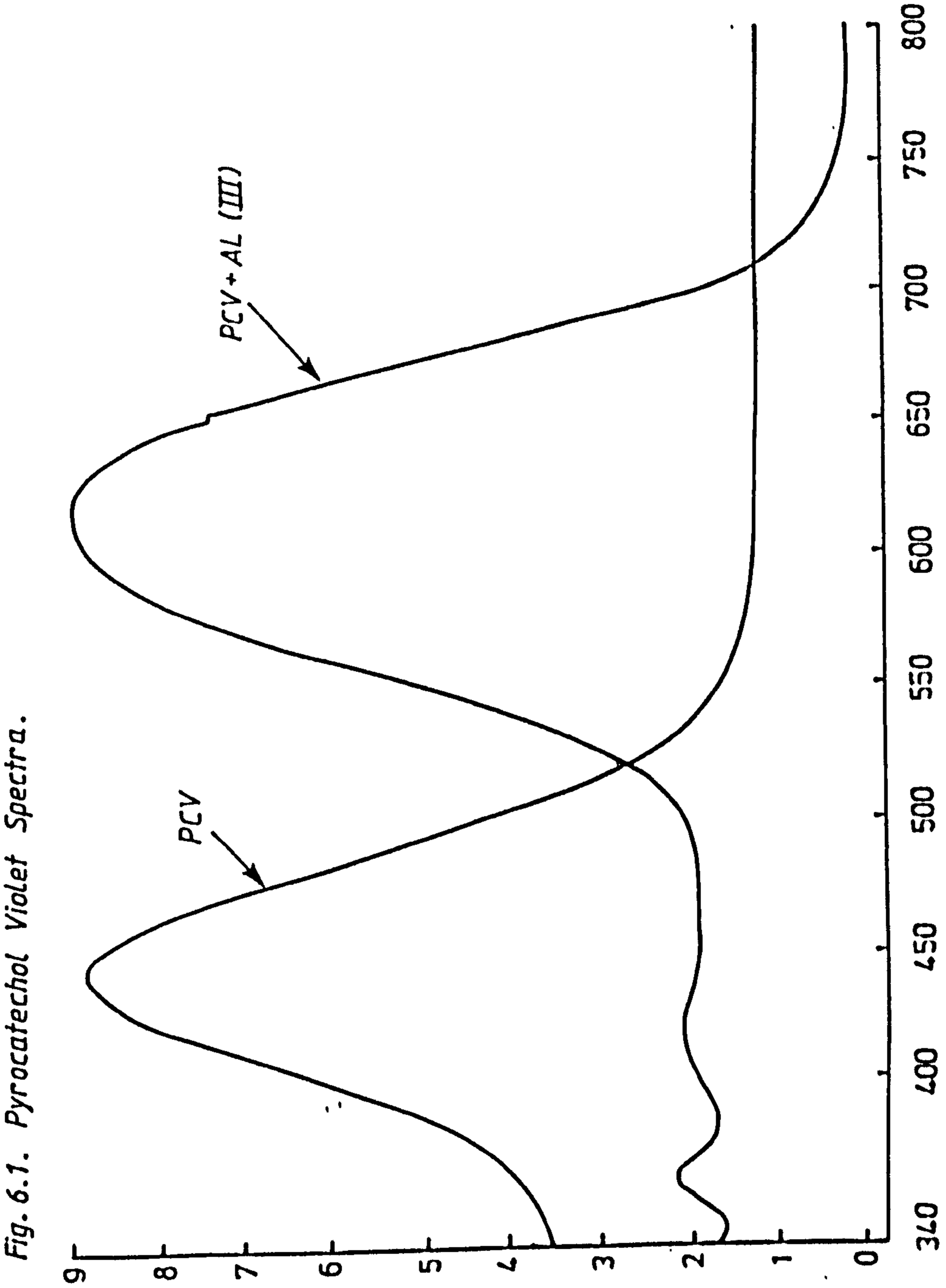


Fig. 6.1. Pyrocatechol Violet Spectra.

Pyrocatechol violet 'gpr' (BDH).

Hexamine 'gpr' (BDH).

1,10-phenanthroline hydrochloride 'gpr' (BDH).

Hydroxylammonium chloride 'gpr' (BDH).

6.2.2. Equipment

A solid state photometric detector (as described in chapter 2) incorporating red light emitting diodes as the light source was used throughout. The manifolds were constructed using 0.5 mm i.d. teflon tubing (Anachem), an Ismatec mini-S-820 peristaltic pump (Labdata services) and a Rheodyne type 5020 six port rotary injection valve (Anachem).

6.2.3. Aluminium Manifold

This manifold (figure 6.2.) involved the introduction of the sample into a distilled deionised water carrier stream which was then merged with the colour reagent stream. The colour reagent contained pyrocatechol violet and hexamine, and the resultant aluminium-pyrocatechol violet complex was determined photometrically using a solid state photometric detector.

6.2.3.1. Experimental Conditions

Reaction coil length = 200 cm on a 1 cm diameter rod.

Sample volume = 225 μl.

Water flow rate = 0.7 ml min^{-1}.

Colour reagent flow rate = 0.7 ml min^{-1}.

Colour reagent composition = 0.75 g of pyrocatechol violet in 1 l of a 50 g l^{-1} hexamine solution.

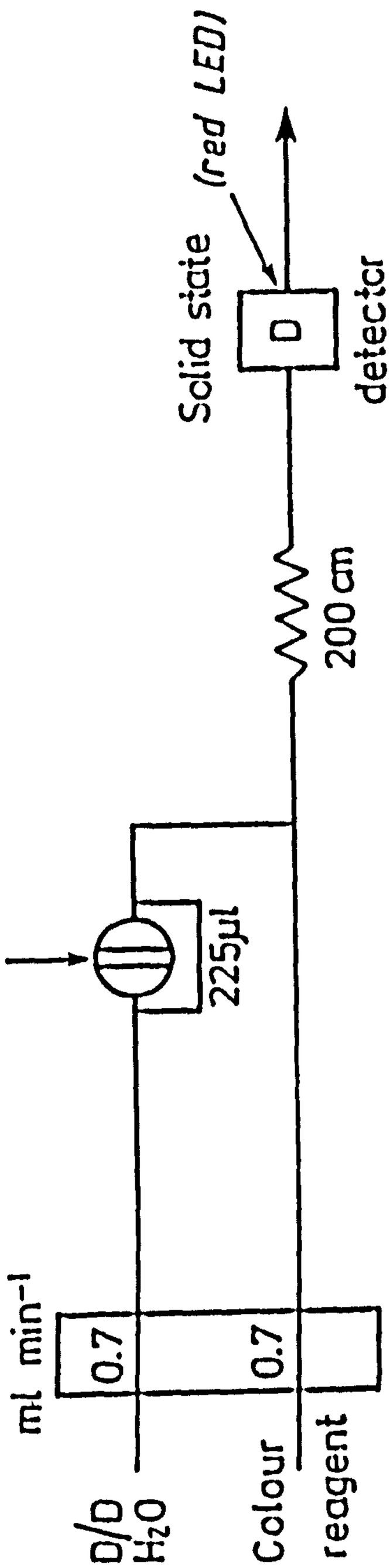


Fig. 6.2. Aluminium manifold.

6.2.4. Aluminium Manifold with Removal of Iron

The previous manifold suffered from interference due to iron present in some samples. The iron can be removed by selectively complexing it with 1,10-phenanthroline after first ensuring that it is all converted to iron (II) by using hydroxylammonium chloride as a reducing agent. This was achieved by merging the water carrier stream (figure 6.3.) with a stream of 1,10-phenanthroline and hydroxylammonium chloride, allowing the iron masking reagents time to remove the iron and, then merging it with the colour reagent.

6.2.4.1. Experimental Conditions

Iron masking coil length = 200 cm on a 1 cm diameter rod.

Reaction coil length = 50 cm on a 1 cm diameter rod.

sample volume = 225 µl.

Water flow rate = 0.35 ml min^{-1}.

Iron masking reagent flow rate = 0.35 ml min^{-1}.

Colour reagent flow rate = 0.7 ml min^{-1}.

Iron masking reagent composition = 1 g of 1,10-phenanthroline in 1 l of a 200 g l^{-1} solution.

Colour reagent composition = 0.75 g of pyrocatechol violet in 1 l of a 50 g l^{-1} hexamine solution.

6.2.5. Reagent Injection Manifold

The reagents were premixed on-line and then injected into the flowing sample stream (figure 6.4.). This manifold saved on reagent consumption and also the flowcell was kept clean because it was constantly being flushed with sample.

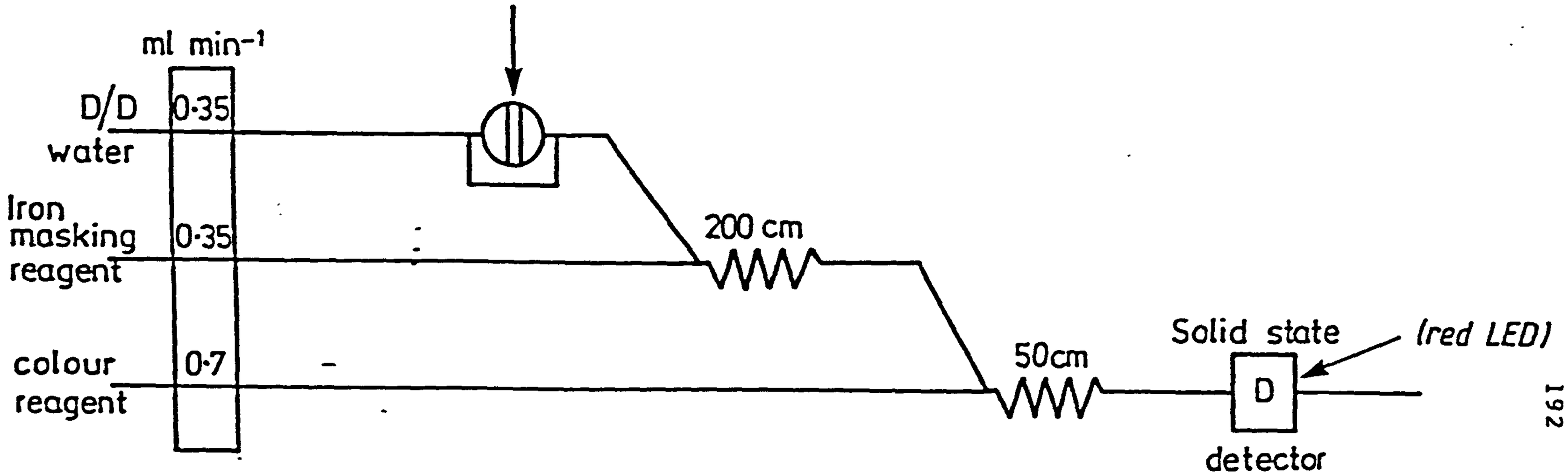


Fig. 6.3. Aluminimum manifold with removal of Fe.

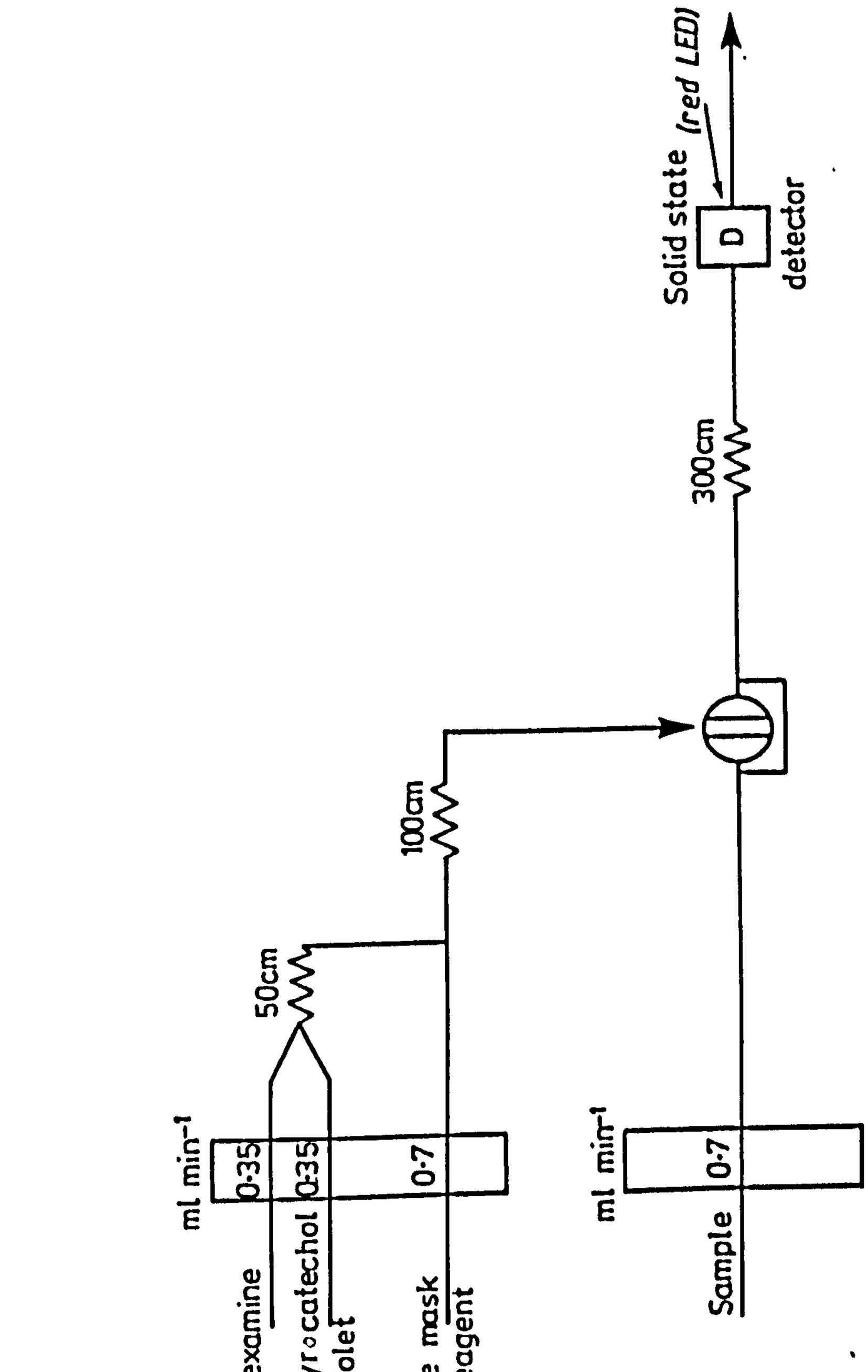


Fig. 6.4. Reagent Injection Manifold

6.2.5.1. Experimental Conditions

Colour reagent mixing coil length = 50 cm on a 1 cm diameter rod.

Reagent mixing coil length = 100 cm on a 1 cm diameter rod.

Reaction coil length = 300 cm on a 1 cm diameter rod.

Reagent volume = 15 µl.

Hexamine flow rate = 0.35 ml min^{-1}.

Pyrocatechol violet flow rate = 0.35 ml min^{-1}.

Iron masking reagent flow rate = 0.7 ml min^{-1}.

Sample flow rate = 0.7 ml min^{-1}.

Hexamine concentration = 200 g l^{-1}.

Pyrocatechol violet concentration = 1.5 g l^{-1}.

Iron masking reagent composition = 2 g of 1,10-phenanthroline in 1 litre of a 100 g l^{-1} hydroxylammonium chloride solution.

6.3. Results and Discussion

6.3.1. Aluminium Manifold

6.3.1.1. Optimisation

This manifold was optimised using a univariate approach and a 1 mg l^{-1} Aluminium standard was used throughout.

The effect of varying the overall flow rate was studied in the range 0.6 to 3.2 ml min^{-1}. The signal obtained increased with increasing flow rate due to the increased dispersion of the sample zone causing more reaction. An overall flow rate of 1.4 ml min^{-1} was chosen as a compromise between speed of analysis, signal and reagent consumption.

Sample volumes in the range 15-450 µl were studied and the signal increased with increasing sample volume. A volume of 225 µl was chosen for subsequent work as a compromise between sample volume and signal.

The signal obtained decreased with an increase in coil lengths above 50 cm due to dispersion of the sample zone,and below 50 cm the signal also decreased due to a lack of reaction time. A coil length of 200 cm was used for subsequent work as shorter coil lengths gave rise to irreproducible peaks due to pulsing in the reagent lines caused by the pump.

Hexamine acts as a pH buffer for the pyrocatechol violet and, when it was not present, only a very small signal was obtained (15 mV). Increasing the hexamine concentration produced an increase in signal up to a hexamine concentration of 25 g l^{-1}, further increases in concentration produced no further changes in the signal. A 50 g l^{-1} concentration was used so as to provide a degree of buffering for the analytical reaction.

The effect of pyrocatechol violet concentration was studied in the range 0.005 to 1.5 g l^{-1} and the signal increased with increasing pyrocatechol violet concentration up to 0.5 g l^{-1}, above which further increases in pyrocatechol violet concentration produced no further change in the signal. A concentration of 0.75 g l^{-1} was used for subsequent work.

6.3.1.2. Calibration

A calibration using this manifold was obtained using standards in the range 0 to 2 mg l^{-1} Al and was described by the equation,

signal (mV) = 740.3 x concentration (mg l^{-1}) + 29.5

with a correlation coefficient of 0.9994. The mean of six replicate injections, standard deviation and relative standard deviation for each standard are shown in table 6.1.. The limit of detection (2σ) was calculated from the blank as 5 μg l^{-1} Al.

6.3.1.3. Iron Interference

The most serious interference with this determination was that of iron (28), where both iron (II) and iron (III) interfered to the same extent. A calibration for iron (III) was obtained using iron standards in the range 0 to 5 mg l^{-1} and was described by the equation,

signal (mV) = 574.1 x concentration (mg l^{-1}) + 60.0

with a correlation coefficient of 0.9967.

The easiest way to remove iron is to complex it with 1,10-phenanthroline, after first converting it all to iron (II).

6.3.2. Aluminium Manifold with Removal of Iron

6.3.2.1. Optimisation

This manifold was optimised using a univariate approach and, 1 mg l^{-1} Al, 1 mg l^{-1} Fe standards and a blank solution were used throughout.

The effect of the overall flow rate on the signal obtained from the aluminium standard was studied in the range 1.2 to 3.2 ml min^{-1}, and the signal was found to

Al Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
2.00	1500	8.2	0.5
1.00	797.5	12.8	1.6
0.50	378.0	4.0	1.1
0.25	229.2	3.1	1.4
0.00	19.2	0.3	1.6

[a] n=6

Table 6.1. Calibration Data for Aluminium Manifold

increase with increasing flow rate due to the increase in dispersion causing a greater amount of reaction to occur. The flow rate was found to have no effect on the iron signal and an overall flow rate of 1.4 ml min^{-1} was chosen for subsequent work as the best compromise between speed of analysis, signal and reagent consumption.

Sample volumes in the range 15 to 450 µl were studied and the aluminium signal increased with increasing volume. The iron signal was unaffected and remained low. A volume of 225 µl was used for further work.

The effect of iron masking coil length was examined in the range 0 to 300 cm and, as the coil length was increased the iron signal decreased. The aluminium signal was also found to decrease slightly due to the increased dispersion of the sample zone at the longer coil lengths. A coil length of 200 cm was used so as to allow time for most of the iron to react.

Reaction coils were evaluated in the range 30 to 300 cm and the aluminium signal decreased with increasing coil length above 50 cm, whereas the iron signal remained unchanged. A coil length of 50 cm was used for subsequent work.

The composition of the iron masking reagent was optimised in two steps, first the hydroxylammonium chloride concentration was kept constant at 100 g l^{-1} whilst the 1,10-phenanthroline concentration was varied between 0 and 2 g l^{-1}. The signal from the aluminium standard decreased slightly with increasing 1,10-phenanthroline concentration but, the iron signal

decreased significantly up to 0.5 g l^{-1}, above which further increases in concentration produced little decrease in the iron signal. A concentration of 1 g l^{-1} 1,10-phenanthroline was chosen for subsequent work. The hydroxylammonium chloride concentration was studied over the range 0 to 400 g l^{-1} and the iron signal rapidly fell off at 1.0 g l^{-1}, further increases in hydroxylammonium chloride concentration produced a slight fall in the iron signal and, above 50 g l^{-1}, the iron signal remained constant at 15 mV. The aluminium signal decreased slightly with increasing concentration and a hydroxylammonium chloride concentration of 100 g l^{-1} was used for subsequent work.

The colour reagent composition was optimised for maximum response to the aluminium standard. The effect of pyrocatechol violet was studied in the range 0.01 to 3.0 g l^{-1} in a 100 g l^{-1} hexamine solution. The aluminium signal increased with increasing pyrocatechol violet concentration until 1 g l^{-1}, above which further increases in concentration produced no further increase in signal. A 1.5 g l^{-1} concentration was used in subsequent work. The hexamine concentration was varied over the range 0 to 500 g l^{-1} and the aluminium signal increased with increasing hexamine concentration until 100 g l^{-1}, above which further increases in the hexamine concentration produced only a slight increase in the obtained signal. A 200 g l^{-1} solution was chosen so as to offer some spare buffering capacity.

6.3.2.2. Calibration

A calibration of this manifold was obtained using aluminium standards in the range 0 to 2 mg l^{-1} Al which was described by the equation,

signal (mV) = 508.3 x concentration (mg l^{-1}) - 14.6

and had a correlation coefficient of 0.9996. The mean of six replicate injections, standard deviation and relative standard deviation for each standard are given in table 6.2.. The linear calibration range was 0 to 10 mg l^{-1} Al and the limit of detection (2σ) was calculated from the blank signal as 33 μg l^{-1} Al.

6.3.3. Reagent Injection Manifold

6.3.3.1. Optimisation

This manifold was optimised using a univariate approach using a 1 mg l^{-1} aluminium standard, 2 mg l^{-1} iron standard and a blank solution.

The effect of the injected reagent volume was studied over the range 7.5 to 300 μl and the sensitivity (defined as the difference between the 1 mg l^{-1} Al standard and the blank signals) was essentially constant over the whole range of volumes, although the peak heights increased with increasing volume. The ratio of the 1 mg l^{-1} Al standard signal to the 2 mg l^{-1} Fe signal also remained constant, with the aluminium signal being twice the iron signal. A reagent injection volume of 15 μl was used for subsequent work so as to conserve reagent.

The colour reagent mixing coil was chosen as 50 cm because this was the shortest coil length which would provide adequate mixing of the colour reagent with the

Al Concentration (mg l^{-1})	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
2.00	1010	10.0	1.0
1.00	483.4	7.6	1.6
0.50	230.0	2.9	1.3
0.25	110.0	0.9	0.8
0.00	0.0	-	0.0

[a] n=6

Table 6.2. Calibration Data for Aluminium Manifold with Remval of Iron

iron masking reagents. The flow rates of the reagents were chosen so that they mixed in the ratio 1 volume pyrocatechol violet solution : 1 volume hexamine : 2 volumes iron masking reagent and also so that the reagent loop was flushed out quickly by freshly mixed reagents. The overall flow rate (i.e. through the injection valve) was 1.4 ml min^{-1}.

The effect of the reaction coil length was studied in the range 0 to 300 cm and the sensitivity increased with increasing coil length. The iron signal remained constant throughout and a coil length of 300 cm was chosen for maximum sensitivity.

The sensitivity of the manifold to aluminium decreased with increasing flow rate (figure 6.5.) due to the shortened reaction time, although the 2 mg l^{-1} Fe signal remained constant, indicating that it has a faster rate of reaction with pyrocatechol violet than aluminium does. A compromise flow rate of 0.7 ml min^{-1} was used as it offered the best sensitivity with regard to analysis time.

The effect of pyrocatechol violet concentration on the sensitivity was studied over the range 0.01 to 2.0 g l^{-1} (figure 6.6). The sensitivity of the manifold to both aluminium and iron increased with increasing pyrocatechol violet concentration and a 1.5 g l^{-1} solution was chosen as offering good sensitivity.

Hexamine solutions were prepared in the range 0 to 400 g l^{-1} and their effect on the sensitivity was studied. Increasing the hexamine concentration above 100 g l^{-1}

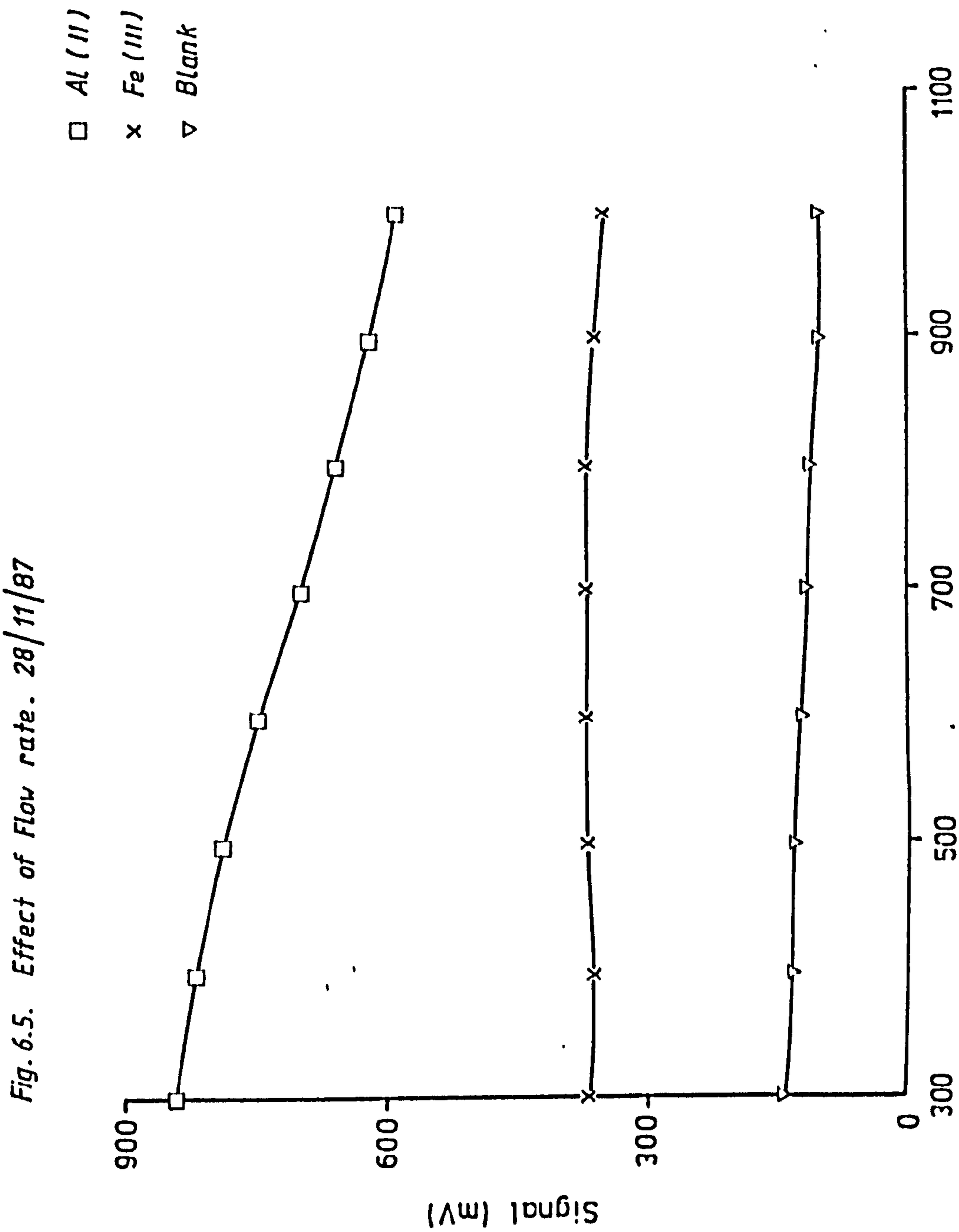


Fig. 6.5. Effect of Flow rate. 28/11/87

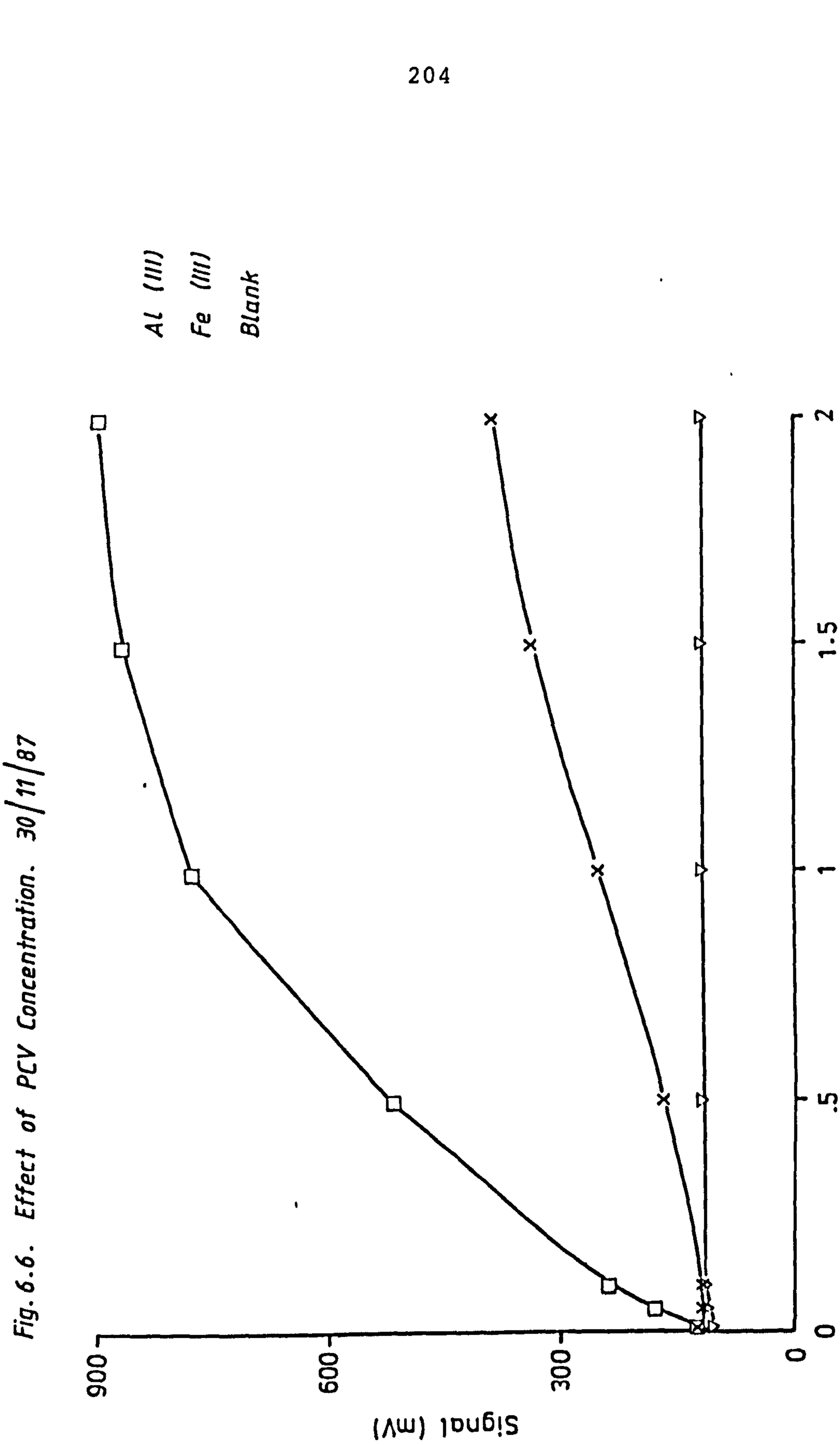


Fig. 6.6. Effect of PCV Concentration.

produced no further increases in sensitivity and a 200 g l^{-1} solution was used for subsequent work so as to provide a buffering capacity for the determination.

The composition of the iron masking reagent had no effect on the aluminium signal and was optimised in two stages. 1,10-phenanthroline solutions in the range 0 to 4.0 g l^{-1} were prepared in a 100 g l^{-1} hydroxylammonium chloride solution. Increasing the 1,10-phenanthroline concentration produced a decrease in the iron signal until 1 g l^{-1}, above which further increases in concentration produced no further decreases in signal. A concentration of 2 g l^{-1} was used for subsequent work so as to provide the capacity for dealing with iron concentrations higher than 2 mg l^{-1} Fe. Hydroxylammonium chloride solutions in the range 0 to 400 g l^{-1} were prepared in a 2 g l^{-1} 1,10-phenanthroline solution. The optimum concentration was 100 g l^{-1}, below this concentration the iron signal increased and above this the blank signal increased due to refractive index differences between the injected reagent zone and the sample stream.

6.3.3.2. Calibration

A calibration of this manifold was obtained using standards in the range 0 to 1000 μg l^{-1} Al and was described by the equation,

signal (mV) = 1.17 x concentration (μg l^{-1}) + 205.7

with a correlation coefficient of 0.9998. The mean of six replicate injections, standard deviation and relative standard deviation for each standard are shown in table

6.3.. The linear calibration range was 0 to 1000 $\mu g\ l^{-1}$ Al and the limit of detection (2σ) was calculated from the blank signal as 20 $\mu g\ l^{-1}$ Al

6.3.3.3. Iron Interference

The current manifold will tolerate iron at low levels; a 2 mg l^{-1} Fe solution gives a signal equivalent to 15% of the signal from a 1 mg l^{-1} Al solution. At lower iron levels the interference becomes negligible, and although it is hard to eradicate completely this interference, when the iron levels are low, as in natural waters, it is not a problem.

6.4. Design and Construction of an Automated Monitor

The block diagram of the automated monitor is the same as that for the ammonia monitor (figure 5.8). The computer and pump box were as previously described (section 5.4.).

6.4.1. Manifold Board

The manifold board contained the flow injection manifold and the solid state photometric detector. The manifold was constructed from teflon tubing (0.5 mm i.d.) on a blockboard base similar to that described previously (section 5.4.1.). The chemistry used in this manifold gave rise to a blank signal so two three-way solenoid valves (Lee, Westbrook, CN) were used to switch between a blank, the sample and the standard. The solenoid injection valve was as previously described and the reference channel of the detector had the sample stream flowing through it prior to passing through the injection valve.

Al Concentration ($\mu g\ l^{-1}$)	Mean Signal[a] (mV)	Standard Deviation	Relative Standard Deviation (%)
1000	1380	11.0	0.8
750	1083	7.6	0.7
500	778.3	7.0	0.9
250	501.4	5.6	1.1
100	318.8	3.9	1.2
50	255.0	2.6	1.0
0	221.0	2.9	1.3

[a] n=6

Table 6.3. Calibration Data for the Reagent Injection Manifold

An extra flow stream was added to the manifold so as to add 1 M hydrochloric acid to the sample stream to solubilise any acid soluble aluminium complexes present. The manifold is shown in figure 6.7..

6.4.2. Cube Eurobeeb Computer

The Cube Eurobeeb computer was as described in section 4.4.3.. The software (similar to that shown in appendix 1.) to control the monitor was written in BASIC and programmed onto an E.P.R.O.M. for stand alone operation. The program was activated on the hour and half hour, and, after a 2 minute delay in order to flush the system out and clear the reagent lines of old reagent, duplicate injections of reagent into the blank, followed by the sample and the 1 mg l^{-1} Al standard were made. The detector output was sampled every 300 ms and the value stored digitally in R.A.M.. After the six reagent injections had been made, the mean of each duplicate injection was ratioed (as described in section 4.4.3.) and the resultant aluminium concentration was displayed on the miniature LCD and printer.

6.5. Monitor Trials

6.5.1. Laboratory Trial

The aluminium monitor was set up in the laboratory using a 1 mg l^{-1} Al standard solution and a 0.3 mg l^{-1} Al solution as the sample so that the reagent lifetime and consumption could be evaluated, along with the precision and stability of the monitor.

It was found that the reagent consumption was 1.5 l of iron masking reagent, 0.75 l of hexamine and 0.75 l of

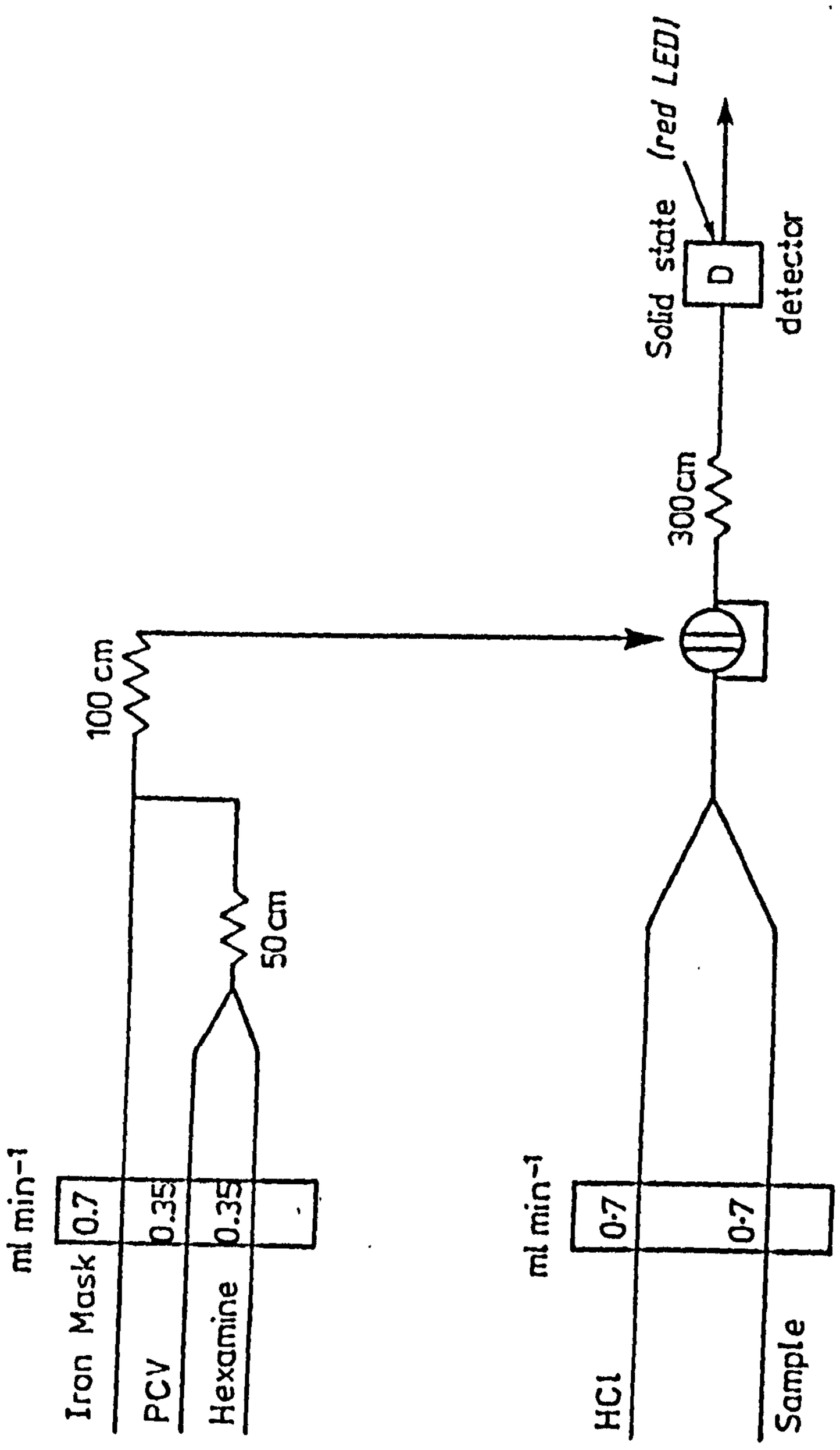


Fig. 6.7. Manifold used in Automated manifold.

pyrocatechol violet over a 7 day period at a rate of two determinations per hour. This was equivalent to an overall consumption of 9 ml of mixed reagent per determination. The consumption of the 1 mg l^{-1} Al standard was 1.4 l and the blank was 2.1 l. The consumption of 1 M hydrochloric acid was 5.1 l.

The relative standard deviation of the result obtained for the 0.3 mg l^{-1} Al was 3.1% (n=336) and subsequent trials confirmed these figures. The reagents were found to be stable for at least 4 weeks, as reproducible calibrations could still be achieved after this time.

6.5.2. Field Trials

An aluminium monitor has been installed at the Durleigh treatment works near Bridgewater in Somerset. Problems with the valves which switch the sample and standards have prevented the aquisition of any data at this time. This will be rectified by the purchase of some better valves which are more robust.

6.6. References

1. B.Mason, "Principles of Geochemistry", J.Wiley, New York, 1960, pages 41-57.

2. K.B.Krauskopf, "Introduction to Geochemistry", M^{C}Graw-Hill, New York, 1967, pages 601-606.

3. C.T.Driscoll, Intern. J. Environ. Anal. Chem., 16(1984)267.

4. W.S.Holden, "Water Treatment and Examination", J.A.Churchill, London, 1970, Chapter 30.

5. R.F.Packham, "Cationic Polyelectrolytes as Primary Coagulants", Water Research Association Technical Paper n^{O}. 100, W.R.A., Medmeham, June 1973.

6. D.R.Crapper McLachlan and U.De Boni, Neurotoxicology, 1(1980)3.

7. R.D.Terry, "Progress in Brain Research", Vol. 70, Ed. D.F.Swaab, E.Fliers, M.Mirman, W.A.Van Gool and F.Van Haaren, Elsevier, Amsterdam, 1985, Chapter 3.

8. L.L.Heston, A.R.Mastri, V.E.Anderson and J.White, Arch. Gen. Psychiatry, 38(1981)1085.

9.D.R.Crapper McLachlan and M.F.A.Van Berkum in Ref. 7, Chapter 25.

10.J.Gardinier and G.Mance, "United Kingdom Water Quality Standards arising from European Community Directives", W.R.C. Technical Report n^{O}. TR204, Water Research Centre, Stevenage, July 1984.

11. B.Welz, "Atomic Absorption Spectrometry", Second Edition, VCH, Weinheim, FRG, 1985.

12. "Inductively Coupled Plasmas in Analytical Atomic Spectroscopy", Ed. A.Montasser and D.W.Golightly, VCH, New York, 1987.

13. D.J.Hydes and P.S.Liss, Analyst, 101(1976)922.

14. Y.K.Agrawal and A.K.Nagar, J. Indian Chem. Soc., 59(1980)614.

15. P.C.Ioannou and P.A.Siskos, Talanta, 31(1984)253.

16. N.E.Fortier and J.S.Fritz, Talanta, 32(1985)1047.

17. C.M.G. Van Den Berg, K.Murphy and J.P.Riley, Anal. Chim. Acta, 188(1986)177.

18. N.J.Nicholson and A.H.Nield, "Automatic Determination of Aluminium in Water", W.R.A. Technical Paper n°. 68, Water Research Association, Medmeham, 1969.

19. O.Royset, Anal. Chem., 59(1987)899.

20. A.C.Edwards and M.S.Cresser, Talanta, 30(1983)702.

21. C.Wyganowski, S.Motomizu and K.Toei, Anal. Chim. Acta, 140(1982)313.

22. J.A.Kennedy and H.K.J.Powell, Anal. Chim. Acta, 184(1986)329.

23. A.Anton, Anal. Chem., 32(1960)725.

24. O.Royset, Anal. Chim. Acta, 178(1985)223.

25. Z.Marczenko and M.Jarosz, Analyst, 107((1982)1431.

26. W.K.Dougan and A.L.Wilson, Analyst, 99(1974)413.

27. "Acid Soluble Aluminium in Raw and Potable Waters by Spectrophotometry 1979", Methods for the Examination of Waters and Associated Materials, HMSO, London, 1979.

28. O.Royset, Anal. Chim. Acta, 185(1986)75.

29. T.Korenga, S.Motomizu and K.Toei, Analyst, 105(1980)328.

CHAPTER SEVEN

CONCLUSIONS AND SUGGESTIONS FOR FUTURE WORK

This project has demonstrated the applicability of flow injection analysis to continuous monitoring. Continuous monitors for water quality parameters can be easily constructed using simple flow injection components and offer distinct advantages over conventional water quality monitoring using electrochemical techniques.

Almost all the species of interest in water quality monitoring can be determined using flow injection analysis and thus could be adapted to continuous monitoring.

Some suggestions for future work are as follows :

(i) The design and construction of a single channel detector so as to remove the need for a reference line.

(ii) The construction of a multiparameter monitor utilising just one computer but several manifolds for complementary species such as nitrate and ammonia.

(iii) The writing of a better algorithm to find the peak heights which would ignore air bubbles etc..

(iv) The application of the continuous monitor to other fields such as process control.

There are many species of analytical interest that are currently not available on commercial monitors which could be developed by this technique, such as, iron, total organic carbon, other metals particularly the heavy metals etc..

Appendix 1.

Nitrate Field Monitor Control Software

Output channels : 0=inject solenoid

1=fill solenoid

2=three way valve

3=not used

4=reagent pump

5=sample pump

```
10 REM *** SET UP CUBE FOR AUTORUN ***
20 HIMEM=&2000:LOMEM=&E00
30 DIM MV(4)
40 OUTCH 0 TO 15
50 REM *** WAIT FOR 00 OR 30 ON REAL TIME CLOCK ***
60 DELAY 6000
70 ZZ$=MID$(CLOCK$,4,2)
80 ZZ=VAL(ZZ$)
90 NN=1
100 GOTO 150
110 ZZ$=MID$(CLOCK$,4,2)
120 NN=0
130 ZZ=VAL(ZZ$)
140 IF ZZ=00 OR ZZ=30 THEN 150 ELSE 110
150 TURNON 5
160 DELAY 5000
170 TURNON 4
180 DELAY 8000
190 REM *** SET UP A/D CONVERTER FOR 5 READINGS/SEC ***
200 SAMPLE 3000,200000,&4000,32
210 REM *** DO FOUR INJECTIONS ***
220 TURNON 0 : DELAY 100 : TURNOFF 0
230 TURNOFF 5
240 DELAY 2500
250 TURNON 1 : DELAY 100 : TURNOFF 1
260 DELAY 1500
270 TURNON 5
280 DELAY 8000
290 TURNON 0 : DELAY 100 : TURNOFF 0
300 TURNOFF 5
310 DELAY 2500
320 TURNON 1 : DELAY 100 : TURNOFF 1
330 DELAY 1500
340 TURNON 5 : TURNON 2 : REM *** SWITCH TO STANDARD ***
350 DELAY 8000
360 TURNON 0 : DELAY 100 : TURNOFF 0
370 TURNOFF 5
380 DELAY 2500
390 TURNON 1 : DELAY 100 : TURNOFF 1
```

```
400 DELAY 1500
410 TURNON 5
420 DELAY 8000
430 TURNON 0 : DELAY 100 : TURNOFF 0
440 TURNOFF 2,5
450 DELAY 2500
460 TURNON 1 : DELAY 100 : TURNOFF 1
470 DELAY 8000
480 REM *** TURNOFF ALL OUTPUT CHANNELS ***
490 REM *** EXCEPT REAGENT PUMP ***
500 TURNOFF 0 TO 3,5
510 DELAY 8000
520 TURNOFF 4
530 SAMPLE 0,0,0,0
540 REM *** FIND FOUR PEAKS ***
550 I=1 : ZA=1
560 REAING=SAMPLE I,32
570 FOR J=1 TO 4
580 I=I+1
590 PREVIOUS=REAING : REAING=SAMPLE I,32
600 IF REAING<PREVIOUS OR REAING=PREVIOUS THEN 580
610 MIL=PREVIOUS
620 I=I+1
630 PREVIOUS=REAING : REAING=PREVIOUS
640 IF REAING>PREVIOUS OR REAING=PREVIOUS THEN 620
650 MAL=PREVIOUS : MVL=(MAL-MIL)/8
660 IF J=1 THEN MAL1=MAL
670 IF MVL<30 THEN 580
680 MV(J)=MVL
690 IF J=2 THEN MV(1)=(MAL1-MIL)/8
700 MEXT J
710 REM *** CALCULATE NITRATE CONCENTRATION ***
720 SAM=(MV(1)+MV(2))/2 : REF=(MV(3)+MV(4))/2
    :NO3=(SAM/REF)*10
730 NO3=100*NO3 : NO3=INT(NO3) : NO3=NO3/100
740 REM *** OUTPUT RESULT TO PRINTER AND DISPLAY ***
750 *FX3,0
760 VDU2
770 TIMES$=LEFT$(CLOCK$,3)
780 TIMES$=TIMES$+ZZ$
790PRINT DATE$;"  ";TIMES$;"  ";NO3
800 VDU3
810 *FX3,7
820 REM *** OUTPUT RESULT TO S.I.P.S. ***
830 LEV=NO3/12
840 LEV=LEV*255
850 LEV=INT(LEV)
860 IF LEV>255 THEN LEV=255
870 ?&FE0C=?&FE0C AND 31 OR 192
880 ?&FE00=LEV
890 ?&FE0C=?&FE0C AND 31 OR 224
900 REM *** CHECK IF RESULT HAS CHANGED MORE THAN ***
910 REM ***        10% OVER LAST 30 MINUTES        ***
920 IF NN=1 THEN 950
930 NX=NO3 ; NX1=NX+(NX/10) : NX2=NX-(NX/10)
940 IF NZ>NX1 OR NZ<NX2 THEN 70
950 NZ=NO3
960 GOTO 110
```

Appendix 2.

Presentations and Publications from this Thesis

Presentations

(1) An Automated Spectrophotometric Field Monitor for the Determination of Nitrate.
Poster presentation, Strathclyde University, Glasgow, 7/07/87, at Research and Development Topics Meeting.

(2) An Automated Spectrophotometric Field Monitor for the Determination of Nitrate.
Poster presentation, La Villete, Paris, France, 10/09/87, at Euroanalysis VI.

(3) An Automated Spectrophotometric Field Monitor for the Determination of Nitrate.
Poster presentation, Las Vegas, U.S.A., 19/04/88, at Flow Analysis IV.

(4) An Automated Flow-injection Based Spectrophotometric Field Monitor for Water Quality Parameters.
Lecture presentation, Plymouth Polytecnic, Plymouth, 18/07/88, at Research and Development Topics Meeting.

Publications

(1) Spectrophotometric Field Monitor for Water Quality Parameters : The Determination of Phosphate. P.J.Worsfold, J.R.Clinch and H.Casey, Anal. Chim. Acta, 197(1987)43.

(2) An Automated Spectrophotometric Field Monitor for Water Quality Parameters : Determination of Nitrate. J.R.Clinch, P.J.Worsfold and H.Casey, Anal. Chim. Acta, 200(1987)523.

(3) Use of a Continuous Spectrophotometric Monitor for Nitrate Based Fertilisers in Hydroponic Cultivation. J.R.Clinch, P.J.Worsfold, H.Casey and S.M.Smith, Anal. Proc., 25(1988)71.

(4) An Automated Spectrophotometric Field Monitor for Water Quality Parameters : Determination of Ammonia. J.R.Clinch, P.J.Worsfold and F.W.Sweeting, Anal. Chim. Acta, in press (accepted for publication).

(5) Solid-state Photometric Detectors for Flow Injection Analysis.
M.Trojanowicz, P.J.Worsfold and J.R.Clinch, Trends Anal. Chem., in press (accepted for publication).