Dry reagent delivery system for field monitors and chemical sensors

This item was submitted to Loughborough University’s Institutional Repository by the/an author.

Additional Information:

• A Doctoral Thesis. Submitted in partial fulfilment of the requirements for the award of Doctor of Philosophy of Loughborough University

Metadata Record: https://dspace.lboro.ac.uk/2134/12374

Publisher: © John Matthew Lee

Please cite the published version.
This item was submitted to Loughborough University as a PhD thesis by the author and is made available in the Institutional Repository (https://dspace.lboro.ac.uk/) under the following Creative Commons Licence conditions.

![Creative Commons License](https://creativecommons.org/licenses/by-nc-nd/2.5/

** Attribution-NonCommercial-NoDerivs 2.5 **

You are free:

- to copy, distribute, display, and perform the work

Under the following conditions:

** Attribution.** You must attribute the work in the manner specified by the author or licensor.

** Noncommercial.** You may not use this work for commercial purposes.

** No Derivative Works.** You may not alter, transform, or build upon this work.

- For any reuse or distribution, you must make clear to others the license terms of this work.
- Any of these conditions can be waived if you get permission from the copyright holder.

Your fair use and other rights are in no way affected by the above.

This is a human-readable summary of the Legal Code (the full license).

Disclaimer:

For the full text of this licence, please go to:
http://creativecommons.org/licenses/by-nc-nd/2.5/
Dry Reagent delivery system for Field Monitors and Chemical Sensors.

by

John Matthew Lee

A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of

Doctor of Philosophy of the Loughborough University of Technology

September 1996

Supervisors:  Dr T.E.Edmonds
              Dr J.D.Lee,

© by John Matthew Lee 1996
# Table of contents

Table of contents. i

Acknowledgements viii

Abstract ix

Publications and other information. xi

Introduction.

<table>
<thead>
<tr>
<th>Sensor Technology</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Optical Sensors</td>
<td>1</td>
</tr>
<tr>
<td>Electrochemical Transducers</td>
<td>3</td>
</tr>
<tr>
<td>Voltammetric Transducers</td>
<td>4</td>
</tr>
<tr>
<td>Amperometric Transducers</td>
<td>5</td>
</tr>
<tr>
<td>Potentiometric Sensors</td>
<td>5</td>
</tr>
<tr>
<td>Other common transducers</td>
<td>6</td>
</tr>
<tr>
<td>Mass Transducers</td>
<td>6</td>
</tr>
<tr>
<td>Dry reagent chemistries (Transducerless Sensors)</td>
<td>7</td>
</tr>
<tr>
<td>References</td>
<td>9</td>
</tr>
</tbody>
</table>

| Sulphur dioxide                   | 11   |
| Effects on man                    | 11   |
| Effect on plant life and industrial materials | 12 |
| References                        | 13   |

| Oxides of Nitrogen                | 14   |
| Effects on humans                 | 15   |
| References                        | 16   |
Aluminium (Aluminium in the environment) 17
Aluminium in the diet 17
Aluminium in water 18
Neurotoxic effects of aluminium 18
Aluminium and its connection with neurodegenerative disorders 19
References 19
Molybdenum 21
Industrial uses of molybdenum 21
Molybdenum, the role in human health 22
Effects of molybdenum deficiency in plants 23
References 24

Silver 25
Silver in the environment 25
Effect of silver on human, animal and plant health 26
References 26

Existing test kits 27
Test kits for aluminium 28
Test kits for molybdenum 29
Test kits for silver 29
Test kits for nitrite 29
Test kits for sulphite 30
Colorimetry 30
References 31

1.0. Paper as a support medium 32
1.0a. Colorimetric comparisons 32
1.1. A dip test for sulphur dioxide 33
1.2. Reagents and equipment used 33
1.3. Determination of suitable methods 34
1.4. Paper test development 36
1.4a. Coating and drying papers 36
1.4b. Testing for sulphite with the dried papers 36
1.4c. Reflectance measurements and calibration 37
1.4d. Stability of the complex 38
1.4e. Reproducibility 38
1.4f. Effectiveness of test papers after storage 39
1.4g. Conclusions 39

Graphs and spectra for chapter 1 41
References 45

2.0. A dip test for nitrogen dioxide 47
2.1. Reagents and chemicals used 47
2.2. Determination of suitable methods 48
2.3. Nitrite dip test development 49
2.3a. Coating and drying papers 49
2.3b. Testing for nitrite with the dry papers 49
2.3c. Reflectance measurements and calibration 49
2.3d. Reproducibility 50
2.3e. Effectiveness of test papers after storage 51
2.3f. Conclusions 51

Graphs and Spectra for chapter 2 52
References 54

3.0. A paper test for aluminium 55
3.1. Reagents and equipment used 55
3.2. Determination of suitable methods 56
3.3. Quinalizarin as a reagent for aluminium 57
3.4. Buffer variation 57
3.5. Interferences 58
3.6. Transfer of reaction to paper 58
3.6a. Type of paper used 58
3.7. Laying down reagents on paper
3.8. Test results
3.9. Conclusions
   Graphs and Spectra for chapter 3

4.0. Xylenol Orange as a reagent for aluminium
   Graph and Spectra for chapter 4
4.1. Effect of interferences
   Spectra for chapter 4
4.2. Transfer of reaction to paper
4.3. Effect of interferences
4.4. Conclusions

5.0. Alizarin red S as a reagent for aluminium
5.1. Effect of Buffer variation
5.2. Interferences
5.3. Transfer of reaction to paper
5.4. Conclusions from Alizarin red S paper test
5.5. Conclusions on the determination of aluminium using a paper dip test
   Graphs and Spectra for chapter 5
5.6. References

6.0. Polymer as a reagent delivery system
   Visual comparisons
6.1. Reagents and materials used
6.2. Selection of a polymer matrix
6.3. The effect of polyvinyl pyrrolidone on the Alizarin red S aluminium reaction
   Graphs and Spectra for chapter 6
6.4. Polyvinyl pyrrolidone as support matrix for the Alizarin aluminium reaction
6.5. Paste and Soluble Polymer Matrix Test (SPMT) manufacture
   SPMT operation and results
6.6. PVP K30 paste, deionised water samples
Graph for chapter 6

6.7. PVP K30 paste, tap water samples

6.8. PVP K90 paste, tap water samples

Graphs and Spectra for chapter 6

6.9. Conclusions

6.10. Real water samples and comparison with standard method

6.11. Beacon stream water

6.12. Loughborough and Blackpool tap waters, Burton fountain water

6.13. Summary of results

Graphs and Spectra for chapter 6

6.14. Drying the PVP tests

6.15. Increasing the dissolution rate of the solid polymer

6.16. Addition of soluble and insoluble inorganic solids

6.17. Addition of decomposable inorganic solid

6.18. Addition of organic solvent

6.19. Investigation of foam structure

6.20. Foamed PVP Alizarin SPMT calibration

Graphs and Spectra for chapter 6

6.21. Sequenced chemical reactions using PVP foam layers

6.22. Dissolution of layers in specific order

6.23. Reagent molecule location within the polymer matrix

6.24. Reduction of SPMT dissolution time and transmittance improvement

6.25. Results of varying the PVP mass in the SPMT

Graph for chapter 6

6.26. Conclusions from transmittance results

6.27. Effect of ageing on the test

6.28. Increasing the mass of reagent contained within the PVP matrix

6.29. Liquid evaporation method

6.30. Conclusions
<table>
<thead>
<tr>
<th>Chapter</th>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.8</td>
<td>Dry reagent tubes</td>
<td>151</td>
</tr>
<tr>
<td>9.9</td>
<td>Experimental</td>
<td>151</td>
</tr>
<tr>
<td>9.10</td>
<td>Results and observations</td>
<td>152</td>
</tr>
<tr>
<td>9.11</td>
<td>Conclusions</td>
<td>152</td>
</tr>
<tr>
<td>9.12</td>
<td>References</td>
<td>153</td>
</tr>
<tr>
<td></td>
<td>Graph for chapter 9</td>
<td>154</td>
</tr>
<tr>
<td>10.0</td>
<td>Lead in the environment</td>
<td>155</td>
</tr>
<tr>
<td>10.1</td>
<td>Uses and pathways into the body</td>
<td>155</td>
</tr>
<tr>
<td>10.2</td>
<td>Health effects of lead</td>
<td>157</td>
</tr>
<tr>
<td>10.2a</td>
<td>References</td>
<td>159</td>
</tr>
<tr>
<td>10.3</td>
<td>Lead in blood, Project outline</td>
<td>161</td>
</tr>
<tr>
<td>10.4</td>
<td>Reagents and equipment used</td>
<td>161</td>
</tr>
<tr>
<td>10.5</td>
<td>Production of a thin film</td>
<td>162</td>
</tr>
<tr>
<td>10.6</td>
<td>Effect of blood on the polymer/acid system pH</td>
<td>165</td>
</tr>
<tr>
<td>10.7</td>
<td>Estimation of film thickness</td>
<td>166</td>
</tr>
<tr>
<td>10.8</td>
<td>Speed of film dissolution</td>
<td>167</td>
</tr>
<tr>
<td>10.9</td>
<td>Addition of chloride to the film</td>
<td>168</td>
</tr>
<tr>
<td>10.10</td>
<td>Other conductance experiments</td>
<td>168</td>
</tr>
<tr>
<td>10.11</td>
<td>Crystal Growth</td>
<td>169</td>
</tr>
<tr>
<td>10.12</td>
<td>Conclusions</td>
<td>172</td>
</tr>
<tr>
<td></td>
<td>Graphs and Spectra for chapter 10</td>
<td>173</td>
</tr>
<tr>
<td>11.0</td>
<td>Conclusions and Future prospects</td>
<td>177</td>
</tr>
</tbody>
</table>
Acknowledgements

I would like to extend my grateful thanks to the following people in the Chemistry Department at Loughborough University of Technology, whose help has been invaluable in this project:

My supervisors, Dr Tony Edmonds and Dr John Lee, for their support, experience, advice and assistance throughout this work; All the technical staff in the Analytical, and Medicinal chemistry sections for their help and technical assistance in the laboratories. John Spray for glass blowing and Alan Stevens for mechanical work. Also to Dr Ged Salt and Dr Arnold Fogg for their help and to my fellow researchers over the past three years who contributed to the pleasant working environment in the laboratory.

My grateful thanks also go to my mother and father for all their help and assistance and also to Leah Hyde-Dryden for the loan of computer equipment for the duration of my writing.

Finally I would like to express thanks to Enviromed plc for their research award and to Loughborough University of Technology for the Studentship contribution, which enabled me to complete this work.
Abstract

There are many methods available for a laboratory analyst to measure the amount of a target analyte within an environmental sample containing a wide variety of other species. However the vast majority of these methods require expensive instrumentation and the analyst to be trained in its operation and safety procedures.

The aim of the following work was to produce a method of analysing environmental pollutants within a complex matrix, without, or with minimal sample pretreatment, using standard colorimetric methods. Results of the analysis would be displayed clearly within a short time and with a high degree of accuracy, selectivity and sensitivity. The tests could be used by an untrained operator and would not contain significant amounts of toxic or irritating chemicals. All the reagents required for the determination and removal of interferences would be contained within the test in the order required by the procedure.

The tests would be capable of mass production and would have a shelf life of at least 9 months.

The following work describes the research carried out to meet the above criteria. The species of environmental interest investigated are aluminium, silver, molybdenum, sulphur dioxide and nitrogen dioxide. These species were chosen for reasons which will be mentioned in greater depth in the introduction.

The tests are, however, not developed with a view to replacing existing instrumental methods, but as means for an operator to determine whether their sample requires further analysis using instrumentation.

The aim of the test result is to provide the operator with the answer to questions such as does my tap water contain more than the recommended limit of aluminium? or does the effluent from my photographic laboratory contain an amount of silver which could lead to my prosecution?. The operator when given a measurement from the tests, by comparing with a colour chart, for example, 0.1µg ml⁻¹, may then decide if they need further analysis using for example, ICP MS techniques.

The development of both paper dip tests and a novel soluble polymer reagent delivery system are described, as well as capillary fill and dry reagent tube devices.
The successful soluble polymer matrix test (SPMT) is also applied to develop a stripping voltammetric sensor for the determination of lead in whole blood. It is hoped that the SPMT will negate the need for pretreatment of the blood sample before determination.
Publications and other information

Publications.


Sequential Chemical Reactions
No.95216607.3
Sensor Technology.

A Sensor may be described as a device capable of converting a chemical signal to an electrical or other physical signals. They are devices which provide information about the types, concentrations and chemical states of the species present within a sample. The ideal sensor can be inserted into a sample and be able to display the analysis results quickly with a high degree of selectivity, sensitivity, accuracy and reproducibility, no sample preparation is required and the sensor is cheap, rugged and easily mass produced.\textsuperscript{1}

A sensor consists of two key elements. An area where a piece of selective chemistry takes place involving the identification of a target analyte by a reaction such as redox or complex formation, or an immunoassay or enzymatic technique, connected to a transducer which converts the chemical signal to an electrical display.

Classifying sensors is very difficult as they can be grouped into the mode of their transduction or their area of application.

By mode of transduction sensors can be grouped into three main categories, optical, electrochemical; and others including mass and catalytic transducers.

Another slightly different type of sensor, namely a dry reagent transducerless sensor is also a common commercial sensor and one that is central to this project and will also be discussed.

Optical Sensors.

Optical sensors are devices used for the detection and determination of physical or chemical parameters through the measurement of changes in some optical property.

They can be based on absorption, reflection, emission or scattering by coupling an optical fibre to a suitable indicator phase where the analyte of interest reacts with a chromophore or fluorophore. Cylinders and thin films, as well as fibres can all be used as waveguides.\textsuperscript{2}
Optical fibres give several attractive features to a chemical sensor. The optical fibre allows optical signals to be transmitted through long distances without interference and low power loss due to the internal reflection of light. Optical sensors are electronically passive and are not subject to electrical and electromagnetic interferences. They are flexible, easily miniaturised, inexpensive and rugged. They can be corrosion resistant and capable of real-time monitoring of samples. They also have some disadvantages including, ambient light can interfere with the optical signal.\textsuperscript{3}

The optical fibre is electrically isolated at the transducing end allowing use in an \textit{in vivo} medical environment.

Optical signals contain a high density of information, as they can have differing wavelengths, phases, decay profiles, polarisations and intensities. The ability of a single fibre to carry a large number of signals simultaneously can be exploited to analyse several target analytes at the same time. For example different species respond to different excitation frequencies in fluorescence based sensors, for example, optical fibre sensors for \textit{in vivo} continuous measurement of pH, O\textsubscript{2} and CO\textsubscript{2} have been bundled into a single probe for use in medical diagnostics.\textsuperscript{4}

The basic instrumentation associated with optical fibre transducers consists of a light source (LED, laser, tungsten halogen bulb etc), a photodetector a monochromator and optical couplers.

Many optical sensors employ optical fibre transducers that are interfaced with immobilised chemical reagents by means of a polymeric membrane.\textsuperscript{5}

An important aspect of this technology has been measurement of pH.\textsuperscript{6}

As already mentioned optical sensors may be based on absorption reflection, luminescence or scattering.

Absorption measurements can be used when the medium supporting the selective chemistry, as well as the selective chemistry is optically transparent.

When the medium and/or the selective chemistry is opaque or transmits light only weakly, then reflectance measurement may be used.
Diffuse reflection, where the light penetrates the medium and subsequently reappears at the surface after partial absorption and scattering within the medium, is the only useful reflectance measured, as it contains chemical information. Fluorescence is an extremely sensitive technique. It is suited to optical sensing and compatible with a single optical measurement as the wavelength of the incident and luminescent radiation are different.  

An earlier use of a photometric sensor was the measurement of blood oxygen saturation based on the fact that oxygenated haemoglobin has a different absorption and reflection spectrum to fully reduced haemoglobin.  

Fluorescent techniques with fibre optics have been used in nuclear power stations, pollution monitoring and detection of trace impurities in waters. Fibre optic probes enable localised measurements to be made in hostile environments as the instrumentation can be up to 1 kilometre away. There are countless other applications of optical sensors in the literature.  

Electrochemical Transducers.

Electrochemical sensors may be conveniently divided into two categories, potentiometric sensors, including ion selective field effect transistors and ion selective electrodes. Faradaic sensors, otherwise known as amperometric and voltammetric sensors. These sensors are based on detecting the current flow caused by oxidising or reducing an analyte. They have been highly successful due to their high selectivity and sensitivity.
Voltammetric Transducers.

A voltammetric measurement is made by recording the cell current as a function of the applied potential, while the potential difference across the cell is scanned between two preset values.

The electroactive species that undergoes electron transfer with the electrode is often the target analyte.

Voltammetric sensors are often quite bulky due to the associated electronic circuitry needed to scan the potential.

When a changing potential is applied to an electrode immersed in an electrolyte solution containing a redox species, a current is observed. This current arises from electron transfer between the electrode and the redox species.

Variations on the standard voltammetric techniques include, cyclic voltammetry, linear sweep voltammetry, differential pulse polarography and square wave voltammetry. These are discussed elsewhere in depth.\textsuperscript{10}

Anodic stripping voltammetry is a powerful tool, especially in the determination of heavy metal ions in solution\textsuperscript{11} and is a technique which will be used in this project.

A negative potential is applied to a working electrode usually a mercury drop, glassy carbon or mercury film coated glassy carbon, for a period of typically one to two minutes. Target species are reduced to form a film or amalgam on the electrode. This is the preconcentration step, the potential is then scanned in the anodic direction and the reduced species are sequentially reoxidised back into the solution. The current at the working electrode is monitored during this stripping step (which takes 10-30 seconds). Peaks in the working electrode current indicate the reoxidation of each of the different species.
Amperometric Transducers.

Amperometric measurements are made by recording the current flow in the cell at a single applied potential. The electrode potential of the cell is usually pulsed to a region where the analyte is electroactive. The decaying current reflects the growth of the diffusion layer. The time taken to perform an analysis using an amperometric sensor is dictated by the rate at which the analyte diffuses to the electrode, rather than the electrode response. Amperometric devices are often covered with a membrane permeable only to the analyte. Poisoning of the electrodes by electroactive or surface active species is limited. This membrane is often constructed from polymers, organics and immobilized biological materials. The whole field of amperometric sensors has been revolutionised by micrometer sized sensors, usually in the form of disks, cylinders or rings. Miniaturising amperometric sensors leads to rapid sensing as their small size leads to rapid diffusion rates, and as the amount of analyte needed is markedly lower. These small electrodes have been increasingly applied to biological systems for *in vivo* monitoring of species such as catecholamines, ascorbic acid, uric acid and oxygen.

Potentiometric Sensors.

Potentiometric sensors rely on the relationship between the potential of an electrochemical cell and the concentration of the chemical species in the sample. They include ion selective electrodes and field effect transistors, as well as certain gas sensors and biosensors which contain a potentiometric sensor, usually a pH electrode as the transduction device. By far the most well known and successful potentiometric sensor is the glass pH electrode. Ion selective electrodes are commonplace and have been reviewed in other texts. Ion selective electrodes can behave like chemical sensors under controlled conditions, but they have many limitations. They may be seen as high impedance voltage sources and measurement of the generated potential requires the use of high
membrane with sensing agents dispersed in the plasticizer. The reason being to incorporate an internal reference electrodes such as Ag/AgCl, in order to maintain a stable potential at both the internal reference electrode and the sensing membrane. Alternative approaches use conducting polymers such as polypyrroles to provide a stable internal reference electrode.\textsuperscript{16} pH Glass is undoubtedly still the best ion selective material, and until better methods are found and manufacturing processes are able to produce many devices with identical, known calibration parameters, their use as sensors will be limited. The miniaturisation of potentiometric sensors has allowed the characterisation of thin films, metal corrosion and enzymic hydrolysis.\textsuperscript{17}

Other common Transducers.

There are a number of other commonly encountered types of transducers which are amply discussed elsewhere.\textsuperscript{16} They include mass and catalytic sensors.

Mass transducers.

The common mass transducer is the piezoelectric crystal, the role of which is increasing in analysis. They have been used as humidity gauges, thin film monitors and gas sensors, since the 1960’s.\textsuperscript{18} Acoustic bulk wave devices work on the principal that an oscillating field applied between a thin slab of crystal produces a resonance of given frequency, depending on the thickness and boundary conditions at the surface. Hence any addition of analyte which alters the mass and thickness of the crystal alters the frequency of resonance, the magnitude of which is proportional to the change in surface mass. Piezoelectric crystals are transformed into chemical sensors by developing surface coatings which selectively bind gases. The manner and selectivity with which surface binding is controlled is the critical element in producing a practical chemical sensor. A limiting factor in the development of these sensors is their poor selectivity for one analyte.
Dry Reagent Chemical Tests. (Transducerless sensors)

Dry Chemistry allows an untrained individual to measure the concentration of a target analyte using a single strip containing all the necessary reagents. Whereas the corresponding wet chemistry techniques require trained personnel, an equipped laboratory and time. The dry reagent chemistry carrier contains all the reagents required to conduct an analysis. Separation steps required by conventional analysis are integrated.

Dry phase chemistry can be traced back over 2000 years when the ancient Greeks developed a papyrus test to detect iron. 19 In the 1830's a filter paper impregnated with silver carbonate was used to detect uric acid. During the 1860's, Goeppelsroeder, a German student detected 0.0025 ppb of methylene blue by allowing 1ml of a 1:410 solution of the dye to rise by capillarity. He obtained a faint absorption band of methylene blue on the filter paper. 20

Another more familiar example of dry reagent chemistry is litmus paper, which dates back to the 19th century. By introducing litmus, a coloured extract from lichens, onto a paper matrix, the inventor provided a method for testing for alkalinity and the dry reagent could be stored for later use. 21

These findings led to analytical tests on single drops of liquids, which became known as 'spot tests'. The drop may be placed, for example, on suitable absorbent paper, where it spreads uniformly until the surface forces are balanced. These 'spot tests' provided the analyst with relatively straightforward methods for sensitive and selective detection of almost all cations and anions. Some excellent texts have been written on this subject, particularly by Fritz Fiegli. 22,23

Dry Format chemistry first had an impact on clinical analysis in the 1950's when Ames Clinistix marketed a strip for testing urinary glucose. This lead to more sophisticated strips being produced for testing blood constituents. Discrete multilayered coatings, developed by the photographic industry were adapted by Kodak to coat dry reagent chemistry formats for clinical testing. 24
Each zone of a multilayered coating provides a unique environment for sequential chemical reactions.

Dry reagent chemistries have been described for many blood metabolites including cholesterol, triglycerides, uric acid and calcium. Analysis of many of these metabolites by conventional methods required several steps. The integration of several steps into a single step analysis is exemplified by the whole blood-glucose analysis strip, which proved especially useful to diabetics who were able to obtain accurate blood sugar level results in a matter of minutes.

Glucose is detected by a glucose oxidase-peroxidase procedure. A 50μl aliquot of whole blood is applied to the surface of the carrier where plasma containing glucose is separated from red blood cells by the carrier matrix, either paper or alginate. After approximately 2 minutes the erythrocytes are wiped away and the colour of the strip is compared with a standard.25

The common features of most dry reagent chemistry strips monitored by diffuse reflectance methods include a support zone base, a reflective zone and finally a reagent zone. The support material often serves as a base for the reagent chemistry zone. The reflective zone is usually constructed with white pigments or reflective foils. Where paper constitutes the reagent zone, the paper itself acts as a reflective material.

A common method of constructing the strips is to screen print uniform layers of reagent onto the support matrix, distinct bands of reagents may be printed onto the matrix, allowing the solution containing the target analyte to encounter each band of reagents in the correct order of the test. Other common support media include agarose, gelatin, polyvinylalcohol and cellulose acetate, the porosity of which is controlled by the molecular weight and degree of cross linking.26

The aim of this project is to produce a novel reagent delivery system by investigating conventional and new support media, known organic analytical reagents will be used. Tests kits will hopefully be produced using sequential chemical reactions to detect low levels of common environmental pollutants within a multisample matrix. The reagent delivery system will then hopefully be applied to other analytical problems.
References.

22. FiegL, F., *Spot Tests in Inorganic Analysis*
Sulphur dioxide.

Sulphur dioxide is a gas under ambient conditions and can act as a reducing or oxidising agent. It has a molecular weight of 64.06, a density of 2.927g dm⁻³ under normal atmospheric conditions and a boiling point of -10.02.¹

Globally, sulphur compounds enter the atmosphere largely from human activity, approximately 120 million tonnes of sulphur per year enters the atmosphere, primarily sulphur dioxide pollution results from the combustion of fossil fuels (60%), petroleum refining (20%), ore smelting (12%), sulphuric acid manufacture, refuse burning and paper making.¹

Sulphur also enters the atmosphere from natural sources including volcanoes, biological decay and the reduction of sulphates in deep seas.²

Many factors including temperature, humidity, light intensity, atmospheric transport and surface characteristics of particulate matter may influence the atmospheric chemical reactions of sulphur dioxide.

The majority of sulphur dioxide in the atmosphere is converted to sulphur trioxide, sulphuric acid and sulphate salts particularly ammonium sulphate and ammonium hydrogen sulphate.

Effects on man.

In spite of its irritating, choking odour sulphur dioxide is somewhat less toxic than other pollutant gases such as chlorine, hydrogen cyanide and ozone.

Sulphur dioxide causes death in humans at concentrations of 500 parts per million (ppm), and has in the past been partially responsible for acute poisoning and deaths, however even in the worst air pollution incident with this gas levels only reached 38ppm.³

Combined exposure to sulphur dioxide and microscopic air particles appears to be more toxic and damaging to lung tissue than either exposure alone.⁴

At concentrations of sulphur dioxide greater than 100ppm the gas is largely absorbed by the upper respiratory tract, due to the high solubility and the moisture contained in
the tissues of this region. At concentrations of 1ppm or less over 95% of the sulphur
dioxide enters the lungs resulting in mild bronchial contractions.\textsuperscript{5}

Inhaled sulphur dioxide is distributed throughout the body and it is possible that some
is bound to blood protein or sulphuric acid may be formed by oxidation in the lungs.
The high acidity and hygroscopic nature of sulphuric acid may result in hydrolysis of
cells and denaturation of proteins by protonation of amino acids carboxyl groups.
The threshold limit value set by the Health and Safety Executive, for industrial
exposure to sulphur dioxide in the UK is 5mg m\textsuperscript{-3} (2ppm) for an 8 hour shift, however
workers have experienced discomfort at levels of 3mg m\textsuperscript{-3}. The average approximate
sulphur dioxide concentration in urban areas rarely exceeds 30 parts per billion.\textsuperscript{6} The
pungent odour may begin to become detected around 0.5ppm.

**Effect on plant life and industrial materials.**

Atmospheric sulphur dioxide is rather more harmful to plants than man. Chronic
exposure to high levels causes chlorosis, a bleaching of the green leaf tissue,
concentrations as low as 0.3ppm can result in white bleaching and blotching between
leaf veins, and on grasses.\textsuperscript{7} Exposure of crops to 0.15ppm of sulphur dioxide has been
shown to cut yields by upto 50%.\textsuperscript{8}

Plant injury increases with the humidity of the atmosphere.

An obvious visible effect of sulphur dioxide damage is the corrosion of city buildings,
deposition of sulphur dioxide and sulphate particles leads to reaction with the calcium
carbonate in stone forming soluble calcium sulphate\textsuperscript{9} which may be washed away by
rainwater. Calcium sulphate combined with soot particles causes the blackening of city
buildings and monuments. Acid attack also leads to stone decay through salt creation
which then crystallises, expanding or contracting to produce fatigue.

Metals and paints also suffer corrosion and discoloration.\textsuperscript{10}

In recent years more effort has been made to prevent pollutant emissions, techniques
such as lime and limestone scrubbing can remove up to 90% of industrially produced
sulphur dioxide and often stack height is increased to disperse the pollutant before it
reaches the ground.
References.

Oxides of Nitrogen.

Nitric oxide and nitrogen dioxide are produced by both natural phenomena such as lightening, volcanic eruptions and bacterial action in soil as well as man made activities.

Estimation of natural, annual global emissions have been put at 1100 million tonnes.¹ This eclipses the amount produced by man activities, estimated at approximately 53 tonnes.

Natural emission, however, are distributed over the entire globe, and hence resulting air concentrations are almost negligible.

The major anthropogenic emissions of nitrogen oxides is the combustion of fossil fuels from power stations and transport media, producing mainly nitric oxide.

Nitric oxide is reactive and is readily oxidised to nitrogen dioxide in the atmosphere. Nitrogen dioxide absorbs strongly in the UV region between 300 and 400µm and is decomposed by sunlight to yield nitric oxide and ozone.²

\[ hν \]

\[ 3\text{NO}_2 \quad \rightarrow \quad 3\text{NO} + \text{O}_3 \]

An equilibrium reaction which may be reversed according to the time of day, latitude and atmospheric variables.³

In rural areas the daytime concentrations of nitric oxide are only a small fraction of the nitrogen dioxide concentrations.

The reactivity of nitrogen dioxide to photodissociation is seen by the fact that in direct sunlight its half life is only 1 to 2 minutes.

Other sources include the, manufacture of explosives and nitric acid⁴ and bacterial degradation of silage material.

Exposure to nitrogen oxides in the home can often be unexpectedly high. Sources such as gas stoves produce 1.1ppm concentrations at breathable height, and smoking, where between 160 to 500ppm of nitric oxide equivalent is inhaled from one cigarette.⁵

Natural background concentrations of nitrogen dioxide over urban areas is in the range 20 to 90µg m⁻³. Over rural areas these concentrations are approximately two fold lower.⁶
Nitrogen dioxide concentrations do not show consistent seasonal behaviour throughout all urban areas and are not necessarily highest during the months of maximum photochemical activity.\(^7\)

**Effects on humans.**

Nitrogen dioxide is far more toxic than nitric oxide,\(^8\) although nitric oxide does have an adverse effect on lung function by attaching to haemoglobin and reducing oxygen transport efficiency. The WHO threshold limiting value is 150\(\mu g\) m\(^{-3}\) for 24 hour exposure.\(^9\)

The lowest levels at which odour can be detected is approximately 200\(\mu g\) m\(^{-3}\) (0.08ppm).\(^10\)

Exposure to nitrogen dioxide levels of 0.7 to 2.0ppm for 10 minutes results in increased inspiratory and expiratory flow resistance,\(^11\) which becomes pronounced beyond 2ppm.

Concentrations of 4 to 5ppm produced an increase in airway resistance and a decrease in the arterial pressure of oxygen and carbon dioxide diffusion capacity.\(^12\)

Exposure to very high concentrations of nitrogen oxides has been occupationally reported. It has been estimated that exposure to nitrogen dioxide levels of 300 to 500ppm results in fatal pulmonary oedema or asphyxia and levels of 25 to 75ppm can cause bronchitis and pneumonia.

Farmers exposed to silo gases and miners using explosives continuously have been acutely affected, some fatally. Products from explosives has shown levels of nitrogen dioxide as high as 160ppm.\(^13,14\)

In comparison with large numbers of epidemiological studies of populations exposed to sulphur oxides and particulates, there have been fewer investigations where nitrogen dioxide has been considered as primary environmental factor to community exposure.

A study to evaluate the effects of nitrogen dioxide on the incidence of acute respiratory disease in children and their parents living near a source of exposure to nitrogen dioxide showed an excess rate of illness, however contribution from other pollutants such as sulphuric acid aerosols made it impossible to attribute the cause to nitrogen dioxide.\(^15\)
Nitrogen dioxide is less harmful to plants than other major air pollutants such as sulphur dioxide, and nitric oxide is even less phytotoxic. Concentrations of nitrogen dioxide of approximately 10ppm cause a decrease in the level of photosynthesis, and higher concentrations can result in leaf spotting.16

References.

9. World Health Organisation, Urban air pollution in megacities in of the world, Blackwells, 1992
Aluminium

Aluminium in the environment.

Aluminium is the third most abundant element in the Earth's crust, after oxygen and silicon. Hence aluminium is often found bonded to silicates in nature. For reasons of its chemical nature, existing as a highly charged small cation it has been effectively excluded from normal biochemical and metabolic processes. The low solubility of aluminium silicates, phosphates and oxides render the aluminium chemically unavailable. These are no active or specific pathways for the uptake and retention of aluminium by man, hence medicines such as antacids and phosphate excluders have been based on aluminium compounds. Aluminium is a non essential part of the diet.

There have been proved certain factors which can affect the uptake of aluminium by the body. Increased calcium levels have been shown to reduce the retention of aluminium in the bone of rats. Citrate is another factor that can influence aluminium absorption in tissues, due to the strong complex it forms with aluminium. Orange juice greatly enhances aluminium absorption in the gut and should not therefore be taken in conjunction with antacid preparations.

As aluminium is ubiquitous and abundant it has found a range of uses in engineering, construction and consumer goods, indeed in 1986 aluminium consumption reached 22 million tonnes. However it is difficult to analyse low concentrations of aluminium in the environment and this difficulty resulted in the late discovery of aluminium as a health threat in 1970 when links were made between aluminium levels in tap water used for renal dialysis equipment and the accumulation of the element in the brain tissue with the possibility of the onset of dialysis dementia.

Aluminium in the diet.

Most people consume 2 to 25mg of aluminium daily, mainly from natural sources such as water and food. Aluminium is also found in food additives such as acid raising agents and food dye and colours. Aluminium can also enter the diet from cooking utensils and pharmaceutical preparations. The latter being a major source of
aluminium, in the form of antacids and antidiarrhoeal agents. The aluminium intake from a course of antacids can reach 5000mg day\(^{-1}\).

Aluminium in the food supply comes mainly from natural sources. Most foods with the exception of tea leaves and certain herbs contain less than 5\(\mu\)g g\(^{-1}\) of aluminium.

As most of the aluminium in tea leaves is insoluble the daily aluminium intake for individuals ranges between 1 and 10\(\mu\)g. The amount of aluminium in vegetables can vary greatly due to variety and soil conditions. The aluminium content in soil on average is estimated at 7.1\%.\(^3\)

The effect of soil acidity on plant growth and aluminium’s role in the soil, particularly related to nutrient deficiencies has been extensively studied.\(^4\)\(^-\)\(^7\)

**Aluminium in water.**

The amount of aluminium in surface and groundwater usually varies between 0.012 and 2.5mg dm\(^{-3}\). When the pH of water is less than 5 the soluble aluminium in water tends to increase. The drinking water standard set by the EEC for aluminium is 200\(\mu\)g dm\(^{-3}\), this is a figure based on aesthetic considerations.

Sulphate of aluminium has long been used as a coagulant for removing colloidal matter from potable water, little soluble aluminium is found in the treated water as flocculent aluminium hydroxide and free carbon dioxide are formed.\(^8\)

Golding\(^9\) in 1991 reported on the outcome of pregnancy following the addition of a vast excess of aluminium sulphate to the north Cornwall water supply, and concluded that among the small number surveyed (88) it was not possible to say that high doses of aluminium sulphate are harmless in pregnancy but there was no evidence that major problems were apparent at birth.

**Neurotoxic effects of Aluminium.**

In the 1960s long term artificial kidney treatment began. As mentioned the idea that aluminium might be poisonous did not become widely accepted until after 1970.\(^10\)

A syndrome, which became known as Dialysis Encephalopathy, began to be recognised in dialysis patients and its clinical manifestation explored. Four factors were found to
be responsible for these symptoms. However the main source of concern was largely due to the heavy aluminium contamination of haemodialysis fluid made from tap water. The symptoms of aluminium intoxication, such as speech disturbance, facial distortion and irregular limb seizures, accompanied by memory loss and dementia, were arrested by introducing water treatment such as filtration, softening, carbon adsorption and deionisation.

Aluminium and its connection with Neurodegenerative disorders.

Neurodegenerative disorders are conditions in which there is slow progressive loss of specific populations of neurones in the central nervous system giving rise to characteristic functional deficits, such as memory loss, speech disturbance, tremor etc, leading to eventual death. Aluminium has been implicated in the pathogenesis of four neurodegenerative conditions, but by far the most important neurodegenerative condition linked with aluminium is Alzheimer's disease a condition effecting 5% of the population aged 70 years or over in the UK. There has been shown to be an age related increase in brain aluminium content.

Aluminium is bound by transferrin which is then internalised by tissue cells. Using a $^{67}$Ga marker for aluminium transport it was shown that the highest level of uptake occurs in the cerebral cortex, hippocampus, septum and amygdalla, are areas which contain the highest density of transferrin receptors and also the areas selectively vulnerable in Alzheimer's disease. Since the density of transferrin receptors remains unaltered this is a possible mechanism for aluminium deposition.

References.

2. Greger, J.L., Ciba Foundation Symposium, 1992, 169, 26
Molybdenum.

Molybdenum is known to be an essential trace element in minute quantities in living systems.\(^1\)

Molybdenum occurs naturally as the disulphide mineral, it has several relatively stable nuclei close to the mass number 98, and it is more abundant than its immediate neighbours in the periodic table, but 10000 times less abundant than iron.\(^2\)

Molybdenum may exist in a variety of oxidation states between 0 and +6, however the common oxidation states available to biological systems are Mo(V), Mo(IV) and Mo(III) and in particular Mo(VI).

The soluble form in which molybdenum is available, the oxyanion molybdate is probably unique among essential trace metals in biology, but is a common species for non-metals such as \(\text{SO}_4^{2-}\). It is possibly for this reason that molybdenum is essential to plant growth as it may be absorbed from the soil into bacteria and plants in as similar way to non metal anions.

Industrial uses of Molybdenum.

Over 50 years ago molybdates were first identified and used as corrosion inhibitors. However they have become commercially significant in the past twenty years, mainly due to the fact that they are environmentally acceptable, being non toxic or polluting, and have been substituted in place of chromates and nitrites. In recent listings of carcinogenity of metal ions molybdenum compounds were placed in the lowest potentially carcinogenic category.\(^3\) Fresh water aquatic life has been reported as being generally insensitive to molybdenum.\(^4\)

It is also cost effective as low concentrations may be used and combination with other species also maintains effectiveness.

Simple alkali metal molybdates with the general formula \(\text{M}_2\text{MoO}_4\) are readily soluble and are mostly used for corrosion inhibition, however group 2 metals have also been used as corrosion inhibiting paint pigments.

Molybdates have found use as corrosion inhibitors as they are very weak oxidants, hence not oxidising the iron(II) ion.
The mechanism of corrosion inhibition involves the formation of an impervious surface by adsorption of molybdate onto the porous oxide steel surface, probably by means of ion exchange, this mechanism is described in other text. Molybdates have also been used as corrosion inhibitors in engine coolants, paints and coatings, cooling waters and hydraulic fluids.

Molybdenum The role in human health.

Molybdenum was first discovered in animal tissues in 1932 and under normal conditions the concentration is approximately 0.1 μg g⁻¹. The chemical state of the molybdenum in animal tissue is largely unknown although it is thought to be bound to molybdoenzymes or to unknown storage proteins. Molybdenum is also present in human blood at levels of 5 μg dm⁻³ however this may vary greatly for differing molybdenum exposure. It is thought that blood molybdenum, bound to proteins may be related to haematologic cellular development. The first molybdenum protein characterised was xanthine oxidase, containing two non-haem Fe/S centres and one Mo centre. The enzyme generally exists in the liver and catalyses the oxidative hydroxylation of some purines, pteridines and other heterocyclic aromatic molecules at its Mo centre, reducing it from the (VI) state to the (IV) state. However its exact biological function has not been specified, but animals deficient in the enzyme suffer symptoms of mild myopathy and eventual deposition of xanthine crystals in muscles and renal calyces.

The levels of the enzyme in the liver have been found to be related to the levels of protein, molybdenum, riboflavin and vitamin E in the diet. Another enzyme present in animals which contains a molybdenum centre is sulphite oxidase, the role of which is associated with the oxidation of sulphite to sulphate, preventing the accumulation of toxic sulphate metabolites, the enzyme may be essential for providing sulphate specific tissues. Deficiency of this enzyme has given rise to mild seizure, mental retardation and dislocated ocular lenses.
Complete molybdenum deficiency has never been achieved in animals, however attempted experiments have shown most animals do not normally show any ill effects.

Effects of molybdenum deficiency in plants.

Soil molybdenum content is related to the weathering of the parent rock, the concentration of molybdenum in soil ranges between 0.5 and 5.0μg g⁻¹, levels lower than 0.5μg g⁻¹ would result in deficiency.

Whiptail is the classic feature of molybdenum deficiency and was assigned as such over 50 years ago.¹⁶ The crops most commonly affected include those demanding a high soil pH for growth, such as broccoli and cauliflower, where the growth may be rosetted and there is severe restriction of the lamina and the younger leaves develop in an irregular way, and the leaf tissue barely developing beyond a narrow edge along the midrib.

Further symptoms and affected plant species have been described.¹⁷

Molybdenum is unique among trace elements in that it is more available under alkaline soil conditions than acidic conditions, molybdenum uptake increases approximately ten fold on raising the pH from 5.0 to 7.0.

It was therefore noticed that adding lime to soil greatly increased the capacity for molybdenum uptake,¹⁹ however it is also advisable to apply sodium molybdate.

Deficiencies of phosphorus and sulphur can also limit the uptake of molybdenum as can excess manganese in soils.
References.

2. Williams, R.J.P., *Biological Role of Molybdenum*, Amax co, USA, 1978
4. Bioassay report for Climax molybdenum company of Michigan, Ann Arbor, USA, 1973
18. Williams, J.H., *Plant and Soil* 1956, 7, 327
Silver.

Silver occurs in nature as the native metal, natural alloys and various minerals particularly argentiferous galena from which it may be obtained by smelting. Silver exhibits oxidation states of +1, +2 and +3 with the +1 state being the most stable. Silver has a melting point of 960.5°C and has high electrical and thermal conductivity.\(^1\)

Silver in the Environment.

The average silver content in rocks and soils is about 0.1\(\mu\)g g\(^{-1}\),\(^2\) ambient air concentrations of silver range from 0.17ng m\(^{-3}\) in rural areas to 7.0ng m\(^{-3}\) in urban areas, (the threshold limit being 10ng m\(^{-3}\))\(^1\).

Natural fresh waters contain an average of 0.2ng ml\(^{-1}\) silver and seawater contains an average of 0.25ng ml\(^{-1}\).\(^2\) The maximum allowable concentration for silver in domestic water supplies is 10ng ml\(^{-1}\) although levels rarely exceed 2ng ml\(^{-1}\).

Silver is emitted to the environment in a variety of forms. Smelting often produces silver carbonate and chloride to the air. Burning fossil fuels, refuse and photographic waste to recover silver produces emissions containing the vaporised silver halides.

Another source of silver emissions to the air is cloud seeding from ground based generators or planes.\(^3\)

Silver from the coating, electroplating\(^4\) and photographic industry is often disposed of to municipal sewers as chlorides, thiosulphates and hydrous oxides indeed the chief source of water pollution by silver is that lost annually as silver thiosulphate complexes in photographic developing solution discarded into sewers.
Effect of Silver on Human, Animal and Plant health.1

Silver and its compounds pose little environmental health problems, despite having very low threshold values. Argyria 6 in humans has only resulted from medicinal applications and occupational exposure. The human body rarely contains more than 10μg g⁻¹ dry weight, the worst case of argyria resulted in a wet weight of 1300μg g⁻¹. Generalised argyria is a slate grey pigmentation of skin and hair caused by silver deposition in the tissues and a blue halo around the cornea and conjunctiva appear in acute cases. Silver nitrate is responsible for over half the cases of Argyria, indeed ingestion of 10g is usually fatal,7 following acute effects such as tissue necrosis, haemorrhage, gastro-enteritis and pulmonary edema. Silver is toxic to bacteria and has been used as an antimicrobial in medicine and water purification.8 Silver serves no useful purpose to plant life and is very toxic to lower plant life,

References.

1. Smith, C.I., and Carson, B.L., in Trace metals in the environment, Vol 2 Silver, Ann Arbor. USA, 1976 -
4. US Environmental protection agency, National emissions inventory of sources and emissions of silver, Washington,1975
6. Data Hazard sheets. BDH ltd, 1992
Existing Test kits.

Test kits are by no means a new innovation, and manufacturers have long realised the need for field testing and analysis of commonly found pollutants. Test kits that are currently available are aimed at those people who require the knowledge of an analysis without having access to the instrumentation found in the analytical laboratory, and also at those situations where it is not practical for a sample to be removed to the laboratory as immediate analysis is needed.

Many of the test kits on the market therefore, I believe, appear to be designed for either the trained analyst working in the field or industrially experienced personnel with an understanding of the principles behind the kits reactions.

However there should be a market for test kits that the 'average person in the street' can use with ease to solve problems they may have such as, is this soil suitable for growing certain crops, does my drinking water contain excess pollutants during drought periods, will the emissions from my laser printer or photocopier have adverse effects on my health etc. By providing answers to these type of questions manufacturers and suppliers, for example water authorities, will become increasingly responsible for producing better quality products and become more accountable to their customers.

The test kits available on today's market from companies such as Camlab ltd and Palintest ltd generally consists of the following: a series of bottled reagents, glassware i.e. flasks, cells test tubes etc., reagents, a colour chart, a photometer and a set of instructions, all housed in a portable plastic case.

A cross section view through the case showing the contents is a daunting sight to anyone not having enjoyed their science classes at school, and is therefore not acceptable to untrained personnel. The reagents may also be unpleasant to handle without some knowledge of health and safety procedure. The test kits are often quite expensive, especially when a portable analytical instrument is required. However with an experienced user the tests can be relied upon to produce accurate, sensitive and selective results.

The species investigated during the course of this work, aluminium, molybdenum, silver, sulphite and nitrite, with a view to producing simple test procedures, already
have available test kits, although these are limited in the case of silver. The available kits for detecting these species are briefly discussed below.

Test kits for Aluminium:

Camlab ltd produce a test kit for aluminium detection in the range 0 to 0.25μg ml⁻¹ with a claimed increment of 0.01μg ml⁻¹. The test is based on the Eriochrome cyanine R method, the standard method cited for aluminium determination in waters.¹

The Camlab test retails at £455* less vat and contains enough reagents for approximately 50 tests, this would equate to approximately £10 per determination.

A similar test is available from palintest ltd although detection with their kit is in the range 0 to 0.5μg ml⁻¹. The reagents are not particularly expensive once a photometer such as the 7000 series has been purchased.

Lovibond ltd also provide an aluminium test kit for this detection range although the reagents used are not mentioned in the literature. Indeed this manufacturer states that test kits can be made to order depending on the target analyte.

Chememetrics water analysis test kits supplied through Galgo ltd consists of slightly more innovative technology. Auto dilution ampoules are used allowing the analyst to test concentrated samples without preliminary dilutions. The ampoules are held horizontally while the tip of the capillary is touched to the sample filling the pipette, which is then immersed in a diluent, the tip of the ampoule is broken and the vacuum pulls the sample and diluent through the capillary where it is mixed with the reagent in the ampoule. The colour is then compared with a colour chart.

Self filling ampoules using similar vacuum style techniques are also described in their literature along with hand held titration cells, an evacuated glass ampoule containing premeasured liquid titrant with an attached flexible valve assembly, the devices work on a reverse titration principle, whereby the sample is added to the titrant until the appearance of a colour change signals an equivalence point. When this point is reached the test result is read directly opposite the liquid level from a scale on the side. Unfortunately no test for aluminium is described within the companies literature.
Tests for Molybdenum:
Camlab Ltd produce 4 tests kits for molybdenum depending on the concentration range under investigation. In a complete range test 0 to 50μg ml⁻¹, the kit uses mercaptoacetic acid with a colour disc comparator. With lower concentration ranges a ternary complex is formed, the colour intensity of which is again compared with a colour comparator. The standard kit retails at over £150* and contains enough reagents for approximately 100 tests. Of other companies only Palintest Ltd appear to produce a standard kit for molybdenum testing, one for the range 0 to 15μg ml⁻¹ and one for the range 0 to 100μg ml⁻¹, the reagents used are not stated, again a standard Palintest photometer is required.

Tests for Silver:
Of the manufacturers investigated only Camlab Ltd appear to have a test kit available for the determination of silver. The test is capable of detection in the range 0 to 0.7μg ml⁻¹ with a 0.02μg ml⁻¹ increment. However the reagents used are not mentioned. The dithizone reagent is used for the spectrophotometric determination in the standard methods of analysis, although this reagent forms complexes with many metal ions, including lead. The Camlab test kit retails in excess of £500*.

Tests for Nitrite:
Testing for nitrite has not appeared to pose to many problems for test kit manufacturers, the majority of kits using a diazotisation reaction followed by use of a photometer, or a simple comparison with a colour disc, with concentrations of nitrite below 0.5μg ml⁻¹. For greater concentrations, up to 2mg ml⁻¹ a photometer is not generally required, a ferric or ceric sulphate procedure is used, with the nitrite acting as a reducing agent on the ferric ion. again the final colour of the ferrous sulphate is compared with a colour disc comparator. However it is not uncommon for the user to provide their own deionised water. These tests requiring a colour disc comparator only tend to be much cheaper (less than £25*).
Tests for Sulphite.

As with nitrite there are a number of test kits available for the determination of sulphite. The majority of these kits are based on the Iodimetric method, also described in the literature of Standard methods\(^1\), the sulphite is titrated with iodide-iodate reagent using starch indicator.

For example Camlab Ltd manufacturers two tests for sulphite both using the iodimetric method, below 10\(\mu\)g ml\(^{-1}\) a drop count titration kit is supplied. For the range 10 to 800\(\mu\)g ml\(^{-1}\) a digital titration kit is required, this apparatus tends to increase the price of the test kit. The Camlab Ltd test retails at over £200.*

* 1995/96 prices are quoted

Colorimetry.

The majority of tests which have been developed in this work are based on colorimetry. This term can be described as any method by which an unknown colour is evaluated in terms of a known colour.

Colorimetry may be classed as visual or indirect. Visual colorimetry is when the unknown colour is presented beside a comparison field into which may be introduced any one of a range of known colours from which the operator may visually choose the colour matching the unknown. Indirect colorimetry can be described as when the light leaving the unknown specimen is split into its component spectral parts by means of a prism or diffraction grating, and the amount of each component part is separately measured by a photometer.

Colorimetric analysis is defined as the technique for visual identification and comparison of coloured solutions. It has become a generic term for all types of analysis involving coloured solutions.

Identification of a substance by the colour of its solution is known as qualitative analysis. Determination of its concentration in a solution by comparison of the intensity of its colour to a colour intensity standard is termed quantitative analysis.
When the human eye is used as the detector, quantitative colorimetric methods have relatively poor precision. The eye is able to recognise a colour change i.e. red to blue, good quantitative analysis however, is not possible with a high degree of precision.

The eye is a non linear detector and therefore quantitation is best carried out by comparison with a close colour or by interpolation between two close colours, whereas a photometer (wavelength selection by filter) or a spectrometer (wavelength selection by monochromator) give linear responses. On the other hand the eye is a multiwavelength detector and can detect a dramatic colour change (such as green to yellow), whereas an instrument with a single wavelength response such as a photometer or spectrometer might see very little change at the monitored wavelength.

The eye and a spectrometer are therefore not similar devices and responses that are clearly seen by one may not be so obvious with the other.

The ability to discriminate light on the basis of wavelength composition is one which is only found in humans and certain other animal species. It is known that humans possess three types of cone receptor, each selectively absorbing light from a certain region of the visible spectrum. An adult with normal vision can see colours in that part of the visible spectrum between approximately 370 and 670nm.

References:

1.0. Paper as a Support Medium.

The first substance selected as a support medium was paper. Paper was chosen as it is inexpensive, readily available and may act as both a supportive and reflective medium.

Three types of filter paper were used for the following work, qualitative, ashless and hardened ashless. In addition to filter paper cellulose acetate electrophoresis paper was also tried, however no success was had with this material.

Before transferring any method to paper, the wet method was first subject to stringent tests. The criteria for selection of a test to be investigated on paper were, non toxicity of reagents, reagents soluble in water, high degrees of sensitivity, selectivity and accuracy, complexes formed need to be stable for long periods of time and the final paper test requires little or no sample preparation.

Three environmentally interesting species were selected with a view to producing a paper indicator test, these species were sulphur dioxide, nitrogen dioxide and aluminium.

1.0a. Colorimetric Comparisons.

All Colorimetric comparisons discussed in this section of work (Paper as a support medium), were carried out on five members of the research laboratory. Three of these people were male and two female. For a greater view of colour perception, two of the subjects were of Chinese origin, one was of African origin and two were white.

For a test to be classed as visibly distinguishable from a standard, or other test, all five subjects on the panel were required to see the difference.
1.1. A dip test for sulphur dioxide.

1.2. Reagents and equipment used.

All chemicals were supplied by Fisons, Loughborough, and were of Analar grade unless otherwise stated.

Ammonium acetate
Buffer - pH 7 phosphate
Deionised water
4,7-diphenyl 1,10 phenanthroline (SAF)
Disodium EDTA (Lancaster Synthesis)
5,5 dithiobis-2-nitrobenzoic acid (Ellman's reagent) (SAF)
Ethanol
Ferric ammonium chloride
Ferric ammonium sulphate
Formaldehyde
Hydrochloric acid (conc)
Mercuric chloride
Potassium chloride
Pararosaniline (Lancaster Synthesis)
1,10 Phenanthroline (SAF)
Potassium fluoride
Propylene carbonate
Sodium hydroxide
Sodium metabisulphite
Sulphuric acid (conc)
2,4,6 tri(2-pyridyl) 1,3,5-triazine (TPTZ) (SAF)

Qualitative filter paper, fast (1), medium(40), slow grades(540) (Whatman)
Ashless filter paper, medium (41) (Whatman)
Hardened ashless filter paper, (541) slow (Whatman)
UV Spectrometer, Philips UNICAM 8700
Spectrometer, Spex Industries dm3000
Water bath (Griffin Instruments)

1.3. Determination of suitable methods.

There have been many methods for determining sulphur dioxide and sulphite, (when sulphur dioxide is dissolved in water, sulphurous acid is formed, which is then partially dissociated to hydrogen ions and sulphite ions) either colorimetrically or spectrophotometrically in the literature.¹⁻³⁷

Five of these methods were selected for further investigation following a literature search.

All of these methods were carried out as described in the literature. The first selected method was that of West and Gaeke². This method used tetrachloromercurate(II) as the trapping reagent for sulphite.

\[
2H^+ + [\text{HgCl}_2]^{2-} + \text{SO}_3^{2-} \rightarrow [\text{Hg(SO}_3)_2]^{2-} + 4\text{Cl}^-
\]

The method had advantages which included, Beer's law was obeyed up to 25 µg ml⁻¹ of sulphite and there was a good visible difference between the colour of solutions containing sulphite concentrations in the region of 7 µg ml⁻¹ and 40 µg ml⁻¹. A plot of absorbance against sulphite concentration produced a straight line over the region below 25 µg ml⁻¹, with a sensitivity of 0.222 and intercept of 0.041. However the toxicity of tetrachloromercurate(II), the requirement of formaldehyde and hydrochloric acid and the need for 30 minutes for final colour development meant the disadvantages were too great to proceed with this method further, for use as an indicator strip.

Methods involving the reduction of iron(III) to iron(II) by sulphite ions and the subsequent determination of iron(II) by 1,10 Phenanthroline²⁵, 4,7-diphenyl 1,10-phenanthroline²⁹ and 2,4,6 Tri(2,pyridyl) 1,3,5-Triazine) (TPTZ)³⁰ were tried. Little success was had with 4,7-diphenyl 1,10-phenanthroline, the reagent was very difficult to get into solution and a 0.005M solution required a 1:1 ethanol:water solvent, with
three hours of shaking on an ultrasonic water bath. Following iron(III) reduction and
determination with this dye a murky violet solution with no absorbance at 593nm, was
obtained, whereas straightforward addition of iron(II) to the reagent resulted in a
bright red complex with absorbance at 593nm.

The 1,10 Phenanthroline method provided good visible differences between
concentrations of sulphite, however for maximum colour formation a temperature of
50°C was required. The colour required 2 hours for maximum development but
appeared stable for 24 hours, and Beer's law was obeyed between 3 and 25µg ml⁻¹. A
plot of absorbance at 50°C against sulphite concentration produced a straight line, the
sensitivity of which was 0.008, and the intercept was 0.017.

The method using TPTZ also obeyed Beer's law in the sulphite concentration range
investigated, 3 to 25µg ml⁻¹, and the colour of the solutions varied from pale violet in
the lower concentrations to dark purple in higher concentrations. However for
maximum sensitivity the complex, due to its greater solubility in organic solvents, had
to be extracted into propylene carbonate before spectrophotometric determination, at
593nm. A plot of absorbance against sulphite concentration produced a straight line
with a sensitivity of 0.021, and an intercept of 0.022.

5,5 dithiobis-2-nitrobenzoic acid (Ellman's reagent) provided a straightforward
method for the determination of sulphite. Beer's law was obeyed between 0.4 and
6.0µg ml⁻¹ and the solutions varied in colour between pale and dark yellow with
increasing concentration. Maximum colour development was complete in 2 minutes
and the complex formed was stable for up to 24 hours, only cyanide and sulphide ions
are said to interfere in the literature. The reagents used are simple and non toxic.

The reaction was carried out in a pH 7.0 buffer, (as a pH between 6 to 8 is required),
and an EDTA solution. The absorbance of the complex formed was measured at
412nm. A plot of absorbance at 412nm against sulphite concentration, 0.8 to 6.0µg
ml⁻¹ produced a straight line, the sensitivity of which was 0.288, and the intercept
0.052. This method was selected for further investigation and transfer to paper.
1.4. Paper Test Development.

1.4a. Coating and Drying papers.

Strips of filter paper (Qualitative, Ashless and Hardened Ashless) 8cm in length and 2cm wide were soaked in a 0.01M solution of Ellman's reagent made up in a pH 7.0 phosphate buffer. Batches of each type of paper were soaked for different lengths of time varying from 30 seconds to 30 minutes, before they were removed.

Three methods were tried to dry the different types of papers, one batch was hung on a string line in a fume cupboard, the second was dried flat on a wire rack and the third batch was dried using a hot air gun.

It was found that the type of paper and the drying conditions had no effect on the test results, as the complex formed on each type of paper was of similar reflectance. Hence all tests were subsequently done using qualitative filter paper soaked for thirty seconds and dried with a hot air gun, in order to maximise the number of tests that could be carried out.

1.4b. Testing for sulphite with the dried papers.

Sulphite solutions were made up such that a 10µl aliquot would contain concentrations 0.5, 2.5 and 5.0µg ml⁻¹. 10µl aliquots of each solution were dropped onto the plane of the test paper. The test papers were slightly off white in colour. Within 2 minutes these areas of the paper were yellow in colour, the intensity of this colour increased with increasing sulphite concentration. The colour of the yellow paper spots was visibly similar to that of the corresponding wet method colour. After two hours the yellow paper spots had decreased in colour intensity.
1.4c Reflectance measurements and calibration.

Attempts were made to calibrate the paper tests with a greater degree of accuracy than by using the eye.

Reflectance measurements were attempted using the Spex Industries dm3000 spectrometer, with a fibre optic probe attachment.

The end of the probe was placed flat against the face of the paper for measurements. To reduce interference from specular reflectance a light tight box was constructed from black plastic with a sealable hole through which the probe was inserted. This box was then placed on top of the paper.

Scans were performed by increasing the wavelength of the incident light from 300 to 650nm. The diffuse reflectance patterns were monitored over this range and these were compared with the corresponding absorbance spectra.

The following solutions and papers were scanned.

i. A 0.001M solution of Ellman's reagent in pH 7.0 phosphate buffer. \textit{fig 1a}. 
ii. A 0.0001M solution of Ellman's reagent following addition of a 1µg ml\(^{-1}\) sulphite solution. \textit{fig 1b}. (tenfold dilution to enable peak to be measured by spectrometer) 
iii. An untreated qualitative filter paper. \textit{fig 1c}. 
iv. A qualitative paper soaked in a pH 7.0 solution of Ellman's reagent. \textit{fig 1d}. 
v. A 1.0µg ml\(^{-1}\) sulphite treated test paper. \textit{fig 1e}. 

It can be seen from the reflectance spectra (\textit{figs 1c, d, e}) that there are common peaks at 422nm, 468nm and 483nm. The intensity values at these peaks may be seen in \textit{table 1}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|}
\hline
 & 422nm & 468nm & 483nm \\
\hline
Paper. fig1c & 152250 & 254470 & 212290 \\
Ellman's fig1d & 103250 & 158710 & 130790 \\
Sulphite. fig3 & 110210 & 471960 & 435030 \\
\hline
\end{tabular}
\caption{Reflectance intensities (arbitrary units) at common wavelengths.}
\end{table}
It can be seen that the all the peak values are reduced markedly on addition of Ellman's reagent and then increased on addition of sulphite solution. The change in the major peak values at the 422nm and 468nm wavelengths may be observed in fig 1f.

1.4d. Stability of the complex.

Reflectance measurements were made on sulphite reacted papers after two minutes, as above and also after 3, 6, 9 and 24 hours. It was noticed that the reflectance at 422nm decreased considerably over 24 hours, the yellow spot was now barely visible.

1.4e. Reproducibility.

A batch of ten papers was taken and a 10µl aliquot of solution containing 1µg of sulphite was pipetted onto each. The papers were scanned as before. The results are shown in table 1b overleaf, a Dixon's Q test applied to the results indicates that point 8 is an outlier: rejecting this point, a mean of 12044 is obtained with an average deviation of 1829.
Table 1b. Reproducibility of reflectance measurements made at 422nm on sulphite reacted papers.

<table>
<thead>
<tr>
<th>test number</th>
<th>reflectance at 422nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>11021</td>
</tr>
<tr>
<td>2</td>
<td>9097</td>
</tr>
<tr>
<td>3</td>
<td>11205</td>
</tr>
<tr>
<td>4</td>
<td>11342</td>
</tr>
<tr>
<td>5</td>
<td>12984</td>
</tr>
<tr>
<td>6</td>
<td>9644</td>
</tr>
<tr>
<td>7</td>
<td>14983</td>
</tr>
<tr>
<td>8</td>
<td>23056</td>
</tr>
<tr>
<td>9</td>
<td>14376</td>
</tr>
<tr>
<td>10</td>
<td>13743</td>
</tr>
</tbody>
</table>

1.4f. Effectiveness of test papers after storage.

A batch of test papers soaked in reagent were stored in a vacuum dessicator for a period of one month. On removing the papers and dropping on 10μl aliquots containing 1μg of sulphite yellow spots were obtained of similar visible intensity to that corresponding colour from the wet test. It is hoped that the effectiveness is therefore not diminished over the period of one month. Unfortunately no reflectance measurements could be made after this time period due to the reflectance instrument no longer being available.

1.4g. Conclusions.

A paper test has been produced which is able to detect levels of sulphite in aqueous solution at levels as low as 0.5μg ml⁻¹ by the naked eye. The tests are free from common interferences, according to the literature, and good reproducibility of results is obtained, the tests seem able to be stored for at least one month without loss of effectiveness, however further clarification of this fact is required.
There appears to be only a subtle difference between the reflectance spectra obtained for the filter paper, reagent soaked filter paper and the sulphite reacted paper, common peaks in all three spectra were observed at 422nm, 468nm and 483nm. The filter paper and reagent treated paper were both similar in colour, white and yellow/white respectively, whereas the sulphite paper was clearly yellow in the area where reaction had taken place. It would be expected that the reflectance spectra would be a 'reverse' image of the absorbance spectra for the reagent and the sulphite reacted papers, as the colour of the reagent and complex should reflect light at all wavelengths where they do not absorb light. It can be seen in figs 1a and b that maximum absorbance is observed at 324nm for the reagent solution only, and 412nm for the complex. In figs 1d and e, low reflectance is observed in the region below 400nm for the reagent soaked paper, and below 440nm for the sulphite reacted paper and there does appear to be some correlation between absorbance and reflectance, however at higher wavelengths, reflectance is again decreased, a pattern not seen in the absorbance spectra.

The similarity between the reflectance spectra obtained and there seemingly low correlation with the absorbance spectra could be explained by specular reflectance from external light sources, masking the diffuse reflectance patterns. Attempts were made to eliminate the external light sources using a box cover for the probe end, however this does not appear to have eliminated enough stray light.
Absorbance at 324nm  1.528 units

Fig 1a. An absorbance scan of a 0.001M, pH 7.0, solution of Ellman’s reagent. Blank: pH 7.0 buffer.

Absorbance at 412nm  0.526 units

Fig 1b. An absorbance scan of a 0.0001M, pH 7.0 solution of Ellman's reagent following reaction with a 1µg ml⁻¹ sulphite solution. Blank: Reagent blank.
Fig 1c. A scan of reflectance against wavelength for an untreated qualitative filter paper. Reflectance intensity at 490 nm: 1.8984e+05 units.

Fig 1d. A scan of reflectance against wavelength for a qualitative filter paper treated with a pH 7.0 solution of 0.01M Ellman's reagent. Reflectance intensity at 490 nm: 1.2246e+05 units.
Fig 1e. A scan of reflectance against wavelength for an Ellman's reagent treated qualitative filter paper after reaction with a 1.0 μg ml⁻¹ sulphite solution. Reflectance intensity at 490 nm: 2.1375e⁻05 units.
Fig 1f. Reflectance change for sulphite test

Refer to section 1.4c. table 1.
References.

2.0. A Dip test for nitrogen dioxide.

2.1. Reagents and equipment used.

All chemicals were supplied by Fisons, Loughborough and were of Analar grade unless otherwise stated.

Acetic acid (glacial)
4-aminobenzoic acid (SAF)
8-anilinonapthalene sulfonic acid (ammonium salt) (SAF)
Hydrochloric acid (conc)
Potassium hydrogen phthalate
Resorcinol
Sodium nitrite
Sulphamic acid
Sulphuric acid (conc)

UV Spectrophotometer, Philips UVIKON
Spex Industries dm3000 spectrometer
Qualitative filter paper, fast (1), medium (40), slow grades (540) (Whatman)
Ashless filter paper, medium (41) (Whatman)
Hardened ashless paper, slow (541) (Whatman)
UV spectrometer, Philips UNICAM 8700
2.2. Determination of suitable methods.

As with sulphur dioxide there are many colorimetric and spectrophotometric methods available for the determination of nitrogen dioxide and nitrites (nitrogen dioxide dissolves in water to produce nitric and nitrous acid, nitrous acid is partially dissociated to nitrite ions and hydrogen ions.)\(^1\)\(^{-25}\).

Two of these methods were selected from literature and carried out as described. The first method attempted involved the use of 1\%w/v solutions of 4-aminobenzoic acid in 3\%v/v hydrochloric acid and 8-anilinonapthalene sulphonic acid (ammonium salt) in glacial acetic acid.\(^23\) This method involved the formation of a violet complex, from a dark green solution, the absorbance of the complex was read at 565nm. The visible intensity of the complex did not vary substantially with increasing concentration of nitrite. Beer's law was obeyed between 4 and 20\(\mu\)g \(\text{ml}^{-1}\). A response curve of absorbance against nitrite concentration, produced a straight line between 4 and 20\(\mu\)g \(\text{ml}^{-1}\), the sensitivity of which was 0.218, and the intercept 0.288.

Resorcinol solution\(^23\) gave a complex which could be distinguished more easily from the starting solution, with nitrite, colourless to yellow with increasing concentration, the absorbance of the complex was measured at 350nm. A 2\%w/v resorcinol solution was used in acidic solution. The test worked best with 0.5M potassium hydrogen phthalate solution as acid, however 1M and 5M hydrochloric, 5M sulphuric acid and 1M sulphamic acid were also tried.

Beer's law appeared to be obeyed over the concentration range 2 to 20\(\mu\)g \(\text{ml}^{-1}\). Approximately 60 minutes was required for full colour development, and the colour was stable for several hours, a plot of absorbance against nitrite concentration, 0 to 20\(\mu\)g \(\text{ml}^{-1}\), produced a straight line with sensitivity 0.087, and intercept 0.026.

As this method appeared simple and required only non toxic reagents it was pursued further using a paper technique.
2.3. Nitrite dip test development.

2.3a. Coating and drying papers.

A 2%w/v Resorcinol solution was made up in a 0.5M potassium hydrogen phthalate. Strips of filter papers (Qualitative, Ashless and Hardened Ashless) 2cm wide and 8cm in length, were soaked in 1 ml of this solution. The papers were allowed to soak for 30 seconds before being removed and dried as before, either suspended from a line, flat on a wire rack or hot air dried. It was found that drying the papers with a hot air gun was the method that gave most uniform coverage of reagent.

As with the sulphite test the type of paper used did not effect the results.

2.3b. Testing for nitrite with the dry papers.

Solutions of sodium nitrite were made up such that a 10ml aliquot would contain, 1.25, 2.5, 5.0 or 10µg of nitrite. Drops of these solutions of 10µl in volume, were pipetted onto the test papers. Within 60 minutes a yellow colour developed, the intensity of which increased with increasing concentration. Below concentrations of 0.125 µg ml⁻¹, no colour could be seen.

2.3c. Reflectance measurements and calibration.

With the same equipment used for the sulphite experiments, the papers were scanned for reflectance between 350 and 600nm. The blank filter paper, the reagent papers and the complex spots were scanned. The reflectance spectra were compared with the corresponding absorbance scans of a 2% resorcinol solution in 0.5M phthalate, fig 2a, and a 1µg ml⁻¹ nitrite solution after reaction with the resorcinol, fig 2b. Reflectance patterns produced for the blank paper and the reagent paper were similar, fig 2c. The reflectance of the nitrite complex colour increased very slightly with increasing concentration, fig 2d. The reflectance was measured at 366 and 442nm and is shown in table 2a.

Table 2a. Reflectance intensities (arbitrary units) at common wavelengths, (multiply by 10⁶) over.
From these results it seen that there is little difference between reflectance measurements at 442nm. However reflectance increase is seen between 0.25 and 1μg ml⁻¹ at 366nm.

On rescanning the 1μg ml⁻¹ spot, after 24 hours the fading of the intense yellow colour corresponded with a decrease in reflectance intensity.

2.3d. Reproducibility.

A batch of ten reagent dried papers was taken and a 10μl aliquot of solution containing 0.5μg of nitrite was pipetted onto each paper, and the coloured spots were scanned as before. The results are shown in table 2b, below.

Table 2b. Reflectance values of ten 0.5μg ml⁻¹ nitrite tests at 442nm.

<table>
<thead>
<tr>
<th>test number</th>
<th>reflectance 442nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16.903</td>
</tr>
<tr>
<td>2</td>
<td>16.309</td>
</tr>
<tr>
<td>3</td>
<td>16.298</td>
</tr>
<tr>
<td>4</td>
<td>16.009</td>
</tr>
<tr>
<td>5</td>
<td>16.753</td>
</tr>
<tr>
<td>6</td>
<td>16.871</td>
</tr>
<tr>
<td>7</td>
<td>18.126</td>
</tr>
<tr>
<td>8</td>
<td>16.436</td>
</tr>
<tr>
<td>9</td>
<td>16.768</td>
</tr>
<tr>
<td>10</td>
<td>16.987</td>
</tr>
</tbody>
</table>

A Dixon's Q test applied to point 7 shows it not to be an outlier, and hence it is not rejected. The mean of the points is 16.7, with a standard deviation of 0.6.
2.3e. Effectiveness of test papers after storage.

A batch of reagent dried paper were stored in a vacuum desiccator for a period of six weeks. The nitrite test was carried out after this period of time, using 0.5μg ml⁻¹ nitrite solution. The visible colour of the paper spot was comparable to that obtained with the wet test. A similar degree of visual correlation with the previous tests was obtained.

No reflectance instrument was available at this time.

2.3f. Conclusions.

A paper test has been produced which is capable of determining nitrite visually at levels as low as 0.125μg ml⁻¹. The complex formed absorbs light at approximately 350nm. Although theoretically on the limit of wavelengths seen by the human eye, the complex could be seen easily. The test requires 60 minutes for the complex to reach full colour formation. Good reproducibility is obtained for the ten paper tests investigated.

As with the sulphite test there is little difference in the shapes of the reflectance spectra obtained for the blank paper, reagent soaked paper and the nitrite reacted paper, again this is due to specular reflectance from external light sources masking the diffuse reflectance patterns.

However comparison of the reflectance and absorbance spectra, figs 2b and d show some good correlation, as mentioned the complex should reflect light at all wavelengths where it does not absorb light. The absorbance spectrum shows light absorbance in the region of 330nm to 360nm, with a gradual decrease to zero absorbance apart from a very small unreacted resorcinol peak at 593nm.

The reflectance spectra show low reflectance in the region below 360nm, with a gradual increase in reflectance to 480nm followed by a very slow decrease in reflectance.

The reflectance spectrum of the resorcinol paper, fig 2c, shows less correlation with its corresponding absorbance spectrum, fig 2a.
Absorbance at 593nm: 0.052 units

Fig 2a. An absorbance scan of a 2% w/v resorcinol in 0.5M phthalate solution. Blank: 0.5M phthalate solution.

Fig 2c. Scans of reflectance against wavelength for an untreated qualitative filter paper, a resorcinol treated paper and a nitrite reacted resorcinol treated paper. Reflectance intensity at 484 nm: 1.5205e+07 units.
Fig 2b. An absorbance scan of a 1 ug ml⁻¹ nitrite solution after reaction with a 2% w/v resorcinol solution in 0.5M phthalate.
Blank: reagent blank.

Fig 2d. Scans of reflectance against wavelength for series of nitrite reacted resorcinol treated papers, concentration range 0.125 to 1 µg ml⁻¹.
Reflectance at 484 nm : 1.6308e-07 units.
References.


54
3.0. A paper test for aluminium.

3.1. Reagents and equipment used.

All reagents used were of Analar grade and supplied by Fisons, Loughborough, unless otherwise indicated.

Acetic acid (glacial)
Alizarin red S (1,2-dihydroxyanthraquinone-3-sulphonic acid) (BDH ltd)
Aluminium nitrate
Ascorbic acid
Calcium chloride
Disodium EDTA (Lancaster synthesis)
Ethanol UV grade
Ferric ammonium sulphate
Furoic acid
Hydrochloric acid (conc)
Magnesium chloride
Nitric acid (conc)
Phenyl acetic acid
Potassium fluoride
Potassium hydrogen phthalate
Quinalizarin (1,2,5,8-Tetrahydroxyanthraquinone) (SAF)
Sodium acetate
Sodium chloride
Sodium citrate
Sodium hydroxide
Sodium nitrite
Sodium oxalate
Sodium sulphite
'Tween' 20
Universal indicator
Xylenol Orange (3,3'-Bis(N,N-di(carboxymethyl)aminomethyl)-o-cresolsulphonphthalein) (BDH ltd)

Kontron UVIKON 810 Spectrometer
Cardy portable pH meter.
Philips UNICAM spectrometer 8700
Filter paper (qualitative, ashless, hardened ashless) slow, medium and fast grades (1,40,41,541)
Silica TLC plates
3.2. Determination of suitable methods.

There are many methods available for the spectroscopic and colorimetric determination of aluminium. These methods are often based on the formation of strongly coloured lakes between the aluminium and a suitable organic reagent. These reagents are often compounds with a phenolic OH group ortho or para to such complexing groups as =O, -OH, and -N= etc.

Many of these reactions are not ideal and often require close control of pH and interfering ions particularly iron(III). Colour is often slow to develop at room temperature and a heating step is often required.

Many reviews and studies for the colorimetric determination of aluminium have been written, and many organic reagents have been suggested.

Reagents that have been used for the determination of aluminium include Aluminon, (Aurinetricarboxylate) which produces a red lake from a yellow solution, the absorbance of which is measured at 525nm.

Haematoxylin, has been used as a colorimetric reagent forming a blue lake with aluminium, however iron(III) interferes badly and the method is not suitable for spectroscopic determinations.

Eriochrome Cyanine R, yields a violet lake with aluminium in acetate buffer, Beer's law is obeyed below 2.5μg ml⁻¹, however magnesium and iron(III) both interfere.

8-Hydroxyquinoline is a popular method based on the extraction of the aluminium hydroxyquinolate complex in chloroform and the subsequent spectrophotometric determination of the yellow solution at 325nm.

Pyrocatechol violet and catechol violet both allow determination of aluminium in water samples.

Three reagents were selected from the literature in order to determine aluminium in water samples with high degrees of selectivity, sensitivity and accuracy. These reagents were Quinalizarin, Xylenol Orange and Alizarin red S. These were selected for their non-toxicity and apparent simplicity in determining aluminium.

All three reagents were developed as a paper test.
3.3. Quinalizarin as a reagent for aluminium.

The first organic reagent selected was quinalizarin (1,2,5,8-tetrahydroxyanthraquinone). Two texts were found which gave methods for the determination of aluminium$^{13,14}$ and both were attempted. Greater success was had with the following. 

To a 10ml aluminium test solution was added 0.5ml of an acetic acid/acetate buffer of pH 5.6 and 0.3ml of a 0.1% alcoholic quinalizarin solution. A 0.1% w/v quinalizarin solution was scanned for absorbance between 350 and 650nm, maximum absorbance of 498nm was shown with a shoulder peak at 513nm. Calibrations were carried out according to the method given. Solutions of Aluminium nitrate were made up such that the aluminium concentrations were in the range 0 to 50µg ml$^{-1}$. The blank solution was brick red/orange in colour and the aluminium tests were cherry red in colour, the intensity of which increased with increasing aluminium concentration. The complex could be seen in lake form, and was visible down to 0.5µg ml$^{-1}$ concentrations.

All tests were scanned for absorbance between 350 and 650nm against a buffer blank. The blank showed maximum absorbance at 478nm, the test solutions above 10µg ml$^{-1}$ had a maximum absorbance at 530nm. Below 10µg ml$^{-1}$ the maximum absorbance gradually shifted to 500nm in the 0.5µg ml$^{-1}$ test. This peak maximum shift is often seen in these types of reaction when a water blank is used. The colour of the final complex becomes less intense at lower concentrations and hence when combined with the dye colour appears as a colour which absorbs at a wavelength close to that of the dye. Response curves were plotted and are shown, figs 3, 0 to 50µg ml$^{-1}$ aluminium, and 3a, 0 to 5µg ml$^{-1}$ aluminium. In fig 3 it is seen that the plot appears to comprise of one linear portion between 0 and 10µg ml$^{-1}$, and one linear area between 10µg ml$^{-1}$ and 50µg ml$^{-1}$. Fig 3a shows further detail of the linear area between 0.5 and 5.0µg ml$^{-1}$. The high blank absorbance could be due to stray light, the sensitivity of the linear portions of the curves are indicated.

3.4. Buffer variation.

For any method to be developed as a paper dip test, reagents that may be dried as solid need to be used to form a distinct band on the paper. In this respect acetic acid/acetate buffers are unsuitable. The experiment was repeated with a phthalate buffer of pH 4.0 and
a phthalate/sodium hydroxide buffer\textsuperscript{19} (forming sodium potassium hydrogen phthalate) of pH 5.82.
The pH 4.0 phthalate buffer produced pink lakes with low absorbances in the 500 - 530nm region. This was also true of a pH 4.0 acetate/acetic acid buffer.
Both the pH 5.6 acetate/acetic acid and the 5.82 phthalate buffers produced similar traces when a neutral 5.0µg ml\(^{-1}\) aluminium solution was scanned between 350 and 650nm. This absorbance of the solution in acetate buffer at 530nm was measured at 0.737, the phthalate buffered solution gave an absorbance of 0.755 at 530nm.

3.5. Interferences.
Tests were carried out on the effect of other metal ions on the aluminium-quinalizarin reaction. Magnesium ions were of particular concern as quinalizarin has also been used as a test for this ion. Calcium and iron(III) ions were also investigated. 50µg ml\(^{-1}\) solutions of the ions were used in the same manner as aluminium ions. In all cases the colour of the solution remained brick red/orange and no absorbance was observed between 480 and 530nm. This was the case when the buffer pH was varied between 4.45 and 5.95. Control of pH seemed the most obvious method of dealing with interferences from divalent ions, as these ions require a high pH for complexing with quinalizarin whereas iron(III) ions require a pH lower than 3.0.

3.6. Transfer of reaction to paper.
3.6a. Type of paper used.

Three types of paper were tried, Qualitative, Ashless and Hardened ashless, grades fast, medium and slow. Qualitative paper was discarded as when a brick red/orange spot of quinalizarin was dropped onto the paper a violet halo immediately appeared around the dye spot characteristic of quinalizarin in solution of pH greater than 6.5. A moistened piece of qualitative filter, cut into a 1cm square and inserted onto the portable pH meter electrode area, indicated this paper had a pH of 6.95. Ashless and Hardened ashless papers had a pH of approximately 6.0.

3.7. Laying down reagents on paper.
Filter papers were cut into strips 1.5cm wide and 7.0cm long. In order that the paper test may be conducted in a similar fashion to thin layer chromatography, whereby the base of
the strip is dipped into the sample solution which is then drawn up the strip. The pH 5.82 buffer was painted onto the bottom 1 cm of the strip and dried with a hot air gun. A spot of 0.1% ethanolic quinalizarin approximately 2.5 mm in diameter, (depending on the grade of the paper), was dried onto the paper 0.5 cm above the buffer band. The spot was concentrated by spotting and drying the quinalizarin five times in order to improve the sensitivity of the test by intensifying the complex colour over a small area. The solvent front was allowed to move to within 1 cm of the top of the paper before the paper strip was removed and dried with a hot air gun.

3.8. Test Results.

Solutions of 50 μg ml⁻¹ of aluminium, magnesium, deionised water and tap water were allowed to run up the paper. When the papers were compared the 50 μg ml⁻¹ aluminium and the magnesium tests both showed a violet halo around the dye spot. The aluminium spot was more intense. The tap water test also showed some purple coloration as did the deionised water, however the latter was barely visible. It was hoped that this violet halo would only be produced with the aluminium test. Below 10 μg ml⁻¹ of aluminium and magnesium no violet colour due to metal ions could be seen.

The portable pH meter was employed to check the pH of the strips at 1 cm intervals to confirm that the buffer was working. A pH of 5.6 was obtained along the whole length of the strip. Acid washing the papers before use with dilute hydrochloric acid, in the hope of keeping the pH on the paper acidic, also made no improvement to the results.

As the pH seemed consistent on the paper, divalent metal ion were expected to be the source of the interference, and therefore were further investigated. Various complexing agents such as citrate, tartrate, phosphate and EDTA were used in an attempt to remove such ions, however none of these proved specific for removing divalent ions at the pH required for the test. An Amberlite IR 120 Na⁺ cation exchange resin also proved unsuccessful.

It was also thought that it may be possible to use a crown ether to remove the magnesium ion due to the different ionic radii. Benzo-15-crown-5 ether appeared ideal, having capture sites similar in size to the magnesium ion radius, however it was insoluble in water.

In an attempt to investigate the filter papers for metal ion content, in particular magnesium, atomic absorption spectroscopy was employed with an air/acetylene flame and a wavelength set at 285.2 nm. Magnesium standards, 0.1 to 1.0 μg ml⁻¹ were used to produce a calibration curve. 10 strips of ashless filter paper were soaked in 2 ml of 0.1M
nitric acid for 30 minutes, before the resulting solution was determined to give a value of approximately 0.1μg ml⁻¹ magnesium per strip: a value that would not affect the paper test.

It would have been appropriate to carry out aluminium determinations to clarify the usefulness of the dip tests, and indeed the tests which will be subsequently described, however a nitrous oxide - acetylene flame was not available for this purpose.

The literature¹³ also states that fluoride ions also give a violet complex with quinalizarin with concentrations greater than 3μg ml⁻¹ at pH 5.6. However this was thought to be of lesser importance as concentrations of fluoride in tap water rarely exceed 1μg ml⁻¹, and no attempt was made to remove fluoride ions.

3.9. Conclusions.

From the wet spectroscopic method it could be concluded that magnesium ions pose no interference problems. However this does not appear to be the case when the reaction is transferred to a paper medium.

It may also be possible that the sodium potassium hydrogen phthalate formed from the buffer components, is not holding the strip at the required pH.
Fig 3, top, sensitivity between 10 and 50 μg ml⁻¹ is 0.0115 μg⁻¹ ml, from the linear regression 
y = 0.0115x + 0.7975

Fig 3a, bottom, sensitivity between 0.5 and 5.0 μg ml⁻¹ is 0.023 μg⁻¹ ml, from the linear regression 
y = 0.023x + 0.593.

Refer to section 3.3. Quinalizarin as a reagent for aluminium.
4.0. Xylenol Orange as a reagent for aluminium.

3,3'-Bis(N,N-di(carboxymethyl)aminomethyl)-o-cresolsulphonphthalein (Xylenol Orange) was used to determine aluminium. The method suggested\textsuperscript{15} is as follows: 0.0375g of xylenol orange is dissolved in 2ml of water, mixed with 75ml of ethanol and made up to 100ml with deionised water. Two drops of `Tween' 20 surfactant are added. This yellow/orange solution when scanned for absorbance between 350 and 650nm showed a peak at 433nm with a shoulder at 303nm.

A 0.2M acetic acid/acetate buffer of pH 3.80 was suggested in the literature, however with a view to producing a paper test a phthalate buffer of pH 4.0 was also used. To a 1ml aliquot of buffer was added 50µl of the xylenol orange solution and an aliquot of 1mg ml\textsuperscript{-1} aluminium solution such that the final concentration was 50µg ml\textsuperscript{-1}. This solution immediately became orange pink. When scanned for absorbance this solution showed peaks at 302nm, 464nm and 551nm. The first two peaks probably due to unreacted reagent as on rescanning 60 minutes later the solution had become deep pink and only the 551nm peak remained.

A calibration was attempted using both buffer types and aluminium concentrations in the range 0.25 to 5.0µg ml\textsuperscript{-1}. The absorbance was measured at 555nm after 10, 90, 180 and 240 minutes against a reagent blank. Curves of the shape seen in fig 4 were obtained. The sensitivity of both response curves is indicated. Visually the solutions in acetate buffer reached their final colour quicker than the phthalate tests, also seen in the calibration plots. However the phthalate buffered tests showed similar visual colour distinction between the lower aluminium concentrations.

To investigate the time required for full colour development 10 and 0.25µg ml\textsuperscript{-1} aluminium solutions were used with the phthalate buffer and the absorbance between 400 and 600nm measured every 15 minutes for 4 hours. The period between 45 and 90 minutes is shown in fig 4a. It can be seen that the dye peak at 445nm gradually reduces whilst the complex peak at 555nm gradually increases. Visually the 10µg ml\textsuperscript{-1} solutions took 30 to 45 minutes to change from orange/pink to pink, and the 0.25µg ml\textsuperscript{-1} solution took 225 minutes.
Response to aluminium of xylenol orange in two different buffers

![Graph showing response to aluminium of xylenol orange in two different buffers](image)

Fig 4. Xylenol orange response to aluminium 10 minutes after addition, refer to section 4.0

Sensitivity of the curve obtained with acetate buffer, $0.125\mu g/\text{ml}$, from the linear regression $y = 0.1246x + 0.0857$.

Sensitivity of the curve obtained with phthalate buffer, $0.071\mu g/\text{ml}$, from the linear regression $y = 0.071x - 0.0008$.

It would appear that the reaction is almost twice as sensitive with acetate buffer.

Both curves appear to be subject to interference.
Fig 4a.

Complex formation between the times of 45 and 90 minutes, after addition of aluminium to the phthalate buffered Xylenol orange solution.

---

*Refer to section 4.0. Xylenol orange as a reagent for aluminium.*
4.1. Effect of interferences.

A 50µg ml\(^{-1}\) solution of aluminium was replaced with a 50µg ml\(^{-1}\) iron(III) solution, made up using ferric ammonium sulphate. The iron formed a violet/grey coloured complex with a UV/Vis peak at 569.6nm.

The WHO guidelines for drinking water impurities state that the maximum allowable concentration of iron(III) in tap water is 1µg ml\(^{-1}\). At this concentration the iron(III) produced a grey complex, just visible above the orange colour of the dye.

Various reagents were tried in an attempt to remove the iron(III) interferences, the first of which was EDTA, 275µg ml\(^{-1}\) of which was required to remove a 50µg ml\(^{-1}\) iron(III) interference. This solution, however, was also effective in removing the aluminium and below concentrations of 25µg ml\(^{-1}\) no complex colour could be seen visibly or by UV/Vis spectroscopy.

Sulphite and nitrite were both unsuccessful in reducing iron(III) to iron(II), and hence removing the interference. However, a 500µg ml\(^{-1}\) solution of ascorbic acid seemed effective in masking the interference of a 50µg ml\(^{-1}\) iron(III) solution. When this concentration of ascorbic acid was introduced into aluminium concentrations of 10 and 0.25µg ml\(^{-1}\), there appeared little visual difference compared to those without the ascorbic acid. However the complex absorbance was approximately 2.5% lower with ascorbic acid, and the 0.25µg ml\(^{-1}\) test required 6 hours before colour was registered. 

**fig 4b.** It can be seen from the blank trace in **fig 4b** that there is some contamination from environmental aluminium.

Fluoride also interferes with the test, reducing the absorbance of the complex. To a series of buffered tests containing 10µg ml\(^{-1}\) of aluminium was added aliquots of 1mg ml\(^{-1}\) potassium fluoride, such that the fluoride concentration was in the range 0 to 2.5µg ml\(^{-1}\). These solutions were scanned for absorbance as before after 90 minutes, and an average of the absorbance reduction indicated fluoride reduced the absorbance of the aluminium-dye complex by 10%
Fig 4b  The effect of ascorbic acid addition on the aluminium xylenol orange reaction.

A.A : ascorbic acid

refer to section 4.1. Effect of interferences.
4.2. Transfer of reaction to paper.

Ashless filter paper was used for all the xylenol orange experiments, as this seemed to give reasonable results with the quinalizarin test. A 10µl spot of the xylenol orange solution was dropped onto the filter paper strip. When these strips were dipped into solutions of buffered aluminium concentration range 0 to 50μg ml⁻¹ the yellow dye spot changed to a pink colour almost immediately, with concentrations greater than 5μg ml⁻¹, the 1 and 2μg ml⁻¹ tests also showed a faint pink tinge on the edge of the yellow spot. Using corresponding iron(III) solutions, in concentrations greater than 5μg ml⁻¹, a yellow spot with a violet base was seen. In mixed solutions this violet band was still present around the pink spot.

In an attempt to increase the sensitivity of the test by concentrating the dye in a small area, a 1% w/v solution of xylenol orange was made up in a 1:1 ethanol:water mix. Spots were repeatedly dried onto a small area, using slow grade paper to prevent dye spreading. The dye was dried on with a hot air gun. Below this spot was dried a band of pH 4.0 phthalate buffer approximately 1cm in width.

When these strips were dipped into aluminium solutions a different situation was observed. The majority of the dye did not dissolve with the solvent front, and the aliquot that did produced a thin yellow dye streak that moved up the paper, with the solvent front. Without ethanol present, no colour change could be seen in those tests with an aluminium concentration less than 5μg ml⁻¹. Those in greater concentration produced a pink edge to the yellow streak. However with aluminium concentrations greater than 1μg ml⁻¹ it was noted on drying that the immobilised dye spot had turned orange, this was not the case when tap water and deionised water blanks were used, where this spot remained yellow.

Having seen that ethanol appeared to be a key to colour development, attempts were made to lay down a species on the paper that would form ethanol in situ on reaction with the acidic buffer. Ethyl acetate was dried on the papers above the buffer band in the hope that addition of the acid buffer may result in alcohol formation. The tests were repeated using aluminium solutions and a tap water blank. When the solvent front had moved 60% up the paper strip it was removed and dried with a hot air gun. The 10 and 5μg ml⁻¹ tests showed the yellow streak with a pink edge, as before,

67
however no real improvement had been made on the previous papers. The detection limit still appeared to be 1 to 2μg ml⁻¹.

4.3. Effect of Interferences.

Iron(III) solutions also produced similar results to aluminium, violet bands were observed around the yellow streak. The orange colour of the immobilised dye spot was also seen on drying in concentrations greater than 5μg ml⁻¹.

As with the wet method ascorbic acid was used for iron(III) inhibition. However with the paper test greater quantities were needed, possibly due to an amount of ascorbic acid not dissolving before the solvent front reached the xylenol orange. 380μg ml⁻¹ of ascorbic acid were needed to remove interference from a 25μg ml⁻¹ iron(III) concentration.

When this amount of ascorbic acid was dried onto the paper above the buffer band, the detection limit for aluminium still remained approximately 1 to 2μg ml⁻¹.

4.4. Conclusions.

Attempts have been made to develop a simple paper test for the detection of aluminium using the dye xylenol orange, a phthalate buffer of pH 4.0 and ascorbic acid. It appears from the results obtained that concentrations of aluminium greater than 2μg ml⁻¹ may be seen by this method, although further investigation would be needed to confirm these observations. Iron does not seem to interfere in concentrations less than 25μg ml⁻¹.

However as aluminium levels in tap water are required to be lower than 0.2μg ml⁻¹ by law, this test would need to be ten times more sensitive to detect useful levels of aluminium.
5.0. Alizarin red S as a reagent for aluminium.

Alizarin red S, (1,2-dihydroxyanthraquinone-3-sulphonic acid) has been used in the literature as a reagent for aluminium.\textsuperscript{16-18} The method of determination was adapted in a similar manner to the xylene orange method. Using an acetate/acetic acid buffer of pH 4.50 as suggested in the literature\textsuperscript{16}, and a 1\%w/v aqueous solution of alizarin red S, concentrations of aluminium in the range 0.5 to 25\(\mu\)g ml\(^{-1}\) were determined both visibly and spectrophotometrically, by scanning for absorbance between 400 and 600nm. Those concentrations greater than 1\(\mu\)g ml\(^{-1}\) registered a colour change of yellow to orange, immediately on addition of aluminium. Concentrations less than 1\(\mu\)g ml\(^{-1}\) took 5 to 10 minutes to develop a colour change, yellow to yellow/orange, after 15 minutes it was barely possible to distinguish between the colour of these lower concentrations. The solutions were scanned for absorbance against a buffer blank. The alizarin red S in buffer registered a peak at 422nm, while the complex showed a peak at 478nm. When scanned against a reagent blank this complex peak shifted towards 500nm. A response curve was drawn, fig 5.

5.1. Effect of Buffer variation.

With a view to transferring the reaction from wet to paper medium, the experiment was repeated with a phthalate buffer of pH 4.0. All the tests were of a much less intense colour than those in acetate buffer, the 0.5\(\mu\)g ml\(^{-1}\) test showed no colour change and the absorbance obtained for the 25\(\mu\)g ml\(^{-1}\) test was almost equivalent to that obtained for 2.5\(\mu\)g ml\(^{-1}\) with acetate. After 75 minutes there was little difference visibly between the two types of test. To check this observation further, a solution of deionised water was adjusted to pH 4.5 using sodium hydroxide and dilute hydrochloric acid, the experiment was repeated using this solution and a set of results were observed similar to those seen in acetate buffer. It was thought possible that a complex was forming between aluminium and phthalate, or that phthalate was in some manner destroying the aluminium-alizarin complex.
This theory was investigated using UV spectrometry. Phthalate only produced a peak at 278nm, a 200μg ml⁻¹ aluminium solution showed a peak at 302nm. A combination of these two solutions when scanned for absorbance showed the two characteristic peaks obtained previously, and no other peak. A similar situation was observed when phthalate was substituted with acetate.

A pH 4.0 unbuffered solution of 1%w/v alizarin and a 10μg ml⁻¹ aluminium solution, orange in colour showed an absorbance at 499nm, when scanned against a reagent blank. On addition of solid potassium hydrogen phthalate to this solution it changed from orange to yellow in colour, whereas the pH remained at 4.0. When this solution was scanned for absorbance, the peak at 499nm was of a much lower amplitude than previously seen.

When this experiment was repeated with addition of solid sodium acetate, and pH adjustment to 4.0 with hydrochloric acid, no colour change was observed and the 499nm absorbance was of similar amplitude to that obtained without the addition, decreasing only slightly due to the dilution factor.

From these observations it was concluded that phthalate was having a detrimental effect, disrupting the aluminium alizarin complex, possibly due to an ion association effect between the charged complex and buffer. It was therefore necessary to find another solid acid to replace the phthalate as a buffer.

Two species were found in the literature: a sodium furoate/furoic acid buffer, and a phenyl acetic acid/phenyl acetate buffer.

A 10μg ml⁻¹ aluminium solution and a 1%w/v aqueous alizarin solution were used with each buffer as before. The furoic/furoate buffer gave no colour change with this concentration of aluminium. UV absorbance experiments were carried out to investigate the effect of furoic acid on the aluminium ion and the aluminium-alizarin complex as with phthalate.

No evidence was obtained that the acid had any effect on either of the two species.

Phenyl acetic acid/acid buffer gave a similar colour change with a 10μg ml⁻¹ aluminium solution, as that seen with the acetate buffer. This was reflected in the absorbance values at 493nm, which were also similar.
A calibration was attempted using this buffer and aluminium concentrations 0.05μg ml\(^{-1}\) to 1μg ml\(^{-1}\), corresponding to levels often encountered in the environment. A response curve was obtained, fig 5.

Both the response curves obtained are of a shape showing a steep linear area at low concentrations with a gradual fall in absorbance as concentration increases. Indicating a higher degree of sensitivity at low concentrations. It can also be seen that the absorbances obtained with the acetate buffer are slightly greater than those obtained using the phenylacetic acid buffer.

### 5.2. Interferences.
**Iron(III)**

A 10μg ml\(^{-1}\) concentration of iron(III), made up using ferric ammonium sulphate, when substituted for aluminium produced a grey/violet complex, when scanned for absorbance, this complex produced a peak at 560nm. The literature\(^{17}\) states that up to 1μg ml\(^{-1}\) iron(III) interference can be removed with sodium thiosulphate, however EDTA was tried. A 125μg ml\(^{-1}\) concentration of EDTA proved effective in removing a 5μg ml\(^{-1}\) concentration of iron(III), whilst still enabling a 0.05μg ml\(^{-1}\) aluminium solution to just be detected. The levels of iron(III) encountered in tap water rarely exceed 1μg ml\(^{-1}\), and at this concentration, no interference was observed.

**Calcium.**

Calcium ions are stated to enhance the aluminium-alizarin complex, by forming a calcium-aluminium-alizarin complex, increasing the colour intensity of the former complex.\(^{18}\) Concentrations of calcium(II), made up from calcium chloride, either 100 or 250μg ml\(^{-1}\), were added with a 1μg ml\(^{-1}\) concentration of aluminium to a buffered alizarin solution. The solutions containing the 250μg ml\(^{-1}\) of calcium registered their final colour slightly quicker than those solutions containing 100 or 0μg ml\(^{-1}\) calcium(II), however the final absorbance readings at 495nm were similar.

This was perhaps unsurprising, as a 250μg ml\(^{-1}\) concentration of calcium, when added to a solution of buffered alizarin changed the colour from yellow to orange, this complex had an absorbance peak at 522nm. When the aqueous dye solution was
added to a buffered solution of calcium ions (greater than 250µg ml⁻¹) this orange colour was intensified, and on leaving the solution for 15 minutes, a red precipitate settled out. Experiments were carried out to investigate the following:

The importance of the order of addition of reagents;
The importance of the solution in which the alizarin was made up in;
Which species could be used to remove calcium ion interference.

A 1%w/v alizarin solution was made up in pH 4.6 phenyl acetic acid/sodium acetate buffer.

Using 10µl aliquots of this and the previously used aqueous dye solution, in 2 ml of buffer the following results were obtained. Absorbance was measured at 523nm.

<table>
<thead>
<tr>
<th>100µg ml⁻¹ Ca added before 10µl of alizarin</th>
<th>colour</th>
<th>absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alizarin in water</td>
<td>orange/yellow</td>
<td>0.022</td>
</tr>
<tr>
<td>Alizarin in buffer</td>
<td>yellow</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>100µg ml⁻¹ Ca added after 10µl of alizarin</th>
<th>colour</th>
<th>absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alizarin in water</td>
<td>yellow</td>
<td>0.017</td>
</tr>
<tr>
<td>Alizarin in buffer</td>
<td>yellow</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>250µg ml⁻¹ Ca added after 10µl of alizarin</th>
<th>colour</th>
<th>absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alizarin in water</td>
<td>yellow with red ppte</td>
<td>0.00</td>
</tr>
<tr>
<td>Alizarin in buffer</td>
<td>yellow</td>
<td>0.00</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>250µg ml⁻¹ Ca added after 10µl of alizarin.</th>
<th>colour</th>
<th>absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alizarin in water</td>
<td>yellow/orange</td>
<td>0.212</td>
</tr>
<tr>
<td>Alizarin in buffer</td>
<td>yellow/orange</td>
<td>0.161</td>
</tr>
</tbody>
</table>

From these observations it would appear that using alizarin solution made up in buffer, added before the calcium ions is the most effective way of preventing major interference from this species.
A 500μg ml\(^{-1}\) solution of sulphate was made up in deionised water to be used as a complexing agent for the calcium ions, in the hope that at this concentration, calcium would complex preferentially with sulphate rather than alizarin. The set of experiments above were repeated using this concentration of sulphate, which was also added to a 0.5μg ml\(^{-1}\) aluminium test, this was compared to a control with no sulphate.

The only solution to register any absorbance, 0.012, was the 250μg ml\(^{-1}\) calcium solution with 10μl of aqueous alizarin added previously. The solution however, remained yellow.

The absorbances of the aluminium tests when measured at 491 nm were identical within experimental error, and they were both orange in colour.

It was therefore concluded that a 500μg ml\(^{-1}\) solution of sulphate was capable of masking the interference of 250μg ml\(^{-1}\) calcium, whilst having no effect on the aluminium alizarin complex. This also appeared to negate the need for reagents to be added in a specific order, i.e. alizarin before the calcium.

5.3. Transfer of reaction to paper.

Ashless filter paper, grade 40 was used for all experiments, as this medium had given the most success in previous work.

Batches of this filter paper were cut into strips 2cm wide and 7cm in length.

A phthalate buffer of pH 4.0, and a phenyl acetic acid/sodium acetate buffer of pH 4.6, were both used for paper experiments, even though the phthalate proved to be much less effective in the wet method than the phenyl acetate buffer, the colour changes it produced after 75 minutes were comparable with the phenyl acetate buffer.

Batches of paper strips were soaked in phthalate buffer and dried with a hot air gun. This process was repeated three times. Aqueous alizarin solution was applied to the paper by spotting, approximately 1.5 cm from the base using a microcapillary tube. A band of EDTA, 125μg ml\(^{-1}\), was painted below the dye spot and dried with a hot air gun.
The strips were then placed in solutions of deionised water spiked with either aluminium, iron(III) or a mixture of both. The following was stated to be observed.

(again by the panel of observers mentioned early)

<table>
<thead>
<tr>
<th>ion/conc(\mu g) ml(^{-1})</th>
<th>colour after 15 minutes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>aluminium 10.0</td>
<td>yellow dye spot, orange base</td>
</tr>
<tr>
<td>aluminium 1.0</td>
<td>yellow dye spot, faint orange base</td>
</tr>
<tr>
<td>aluminium 0.5</td>
<td>yellow spot, (some orange colour after 75 minutes)</td>
</tr>
<tr>
<td>iron(III) 10</td>
<td>yellow spot, violet base</td>
</tr>
<tr>
<td>iron(III) 0.5</td>
<td>yellow spot</td>
</tr>
<tr>
<td>aluminium 10, iron(III) 10</td>
<td>yellow spot, purple base</td>
</tr>
<tr>
<td>aluminium 0.5, iron(III) 0.5</td>
<td>yellow spot</td>
</tr>
</tbody>
</table>

The experiment was repeated with tap water spiked with metal ions. As the solvent front reached the alizarin spot, it immediately changed from yellow to red. The pH of the tap water was measured at 7.82, however universal indicator showed that the pH of the strip following use was between 4 and 5. From wet experiments it was not thought that calcium ions, in the concentration found in tap waters would be capable of causing such a dramatic colour change. However as the dye was effectively being added after the calcium ions some red coloured precipitate could have been expected. A band of sulphate 250\(\mu g\) ml\(^{-1}\), added to the paper above the EDTA band resulted in no improvement. A 500\(\mu g\) ml\(^{-1}\) band eliminated the red colour however no orange complex could now be seen when 10\(\mu g\) ml\(^{-1}\) concentrations of aluminium were tried. Batches of paper strips were soaked in the phenyl acetic acid buffer, and the experiments repeated as with the phthalate soaked papers, with bands of EDTA and sulphate. The strips were dipped into solutions of deionised water spiked with aluminium, concentrations, 0.5, 1.0 and 10\(\mu g\) ml\(^{-1}\), when the solvent front had almost reached the top of the strip, it was removed and allowed to dry, after 30 minutes there was no colour change in any of the tests.
5.4. Conclusions from Alizarin red S paper test.

A method for the determination of aluminium at low concentration levels has been developed from previous methods, iron(III) in concentrations lower than $1\mu g\, ml^{-1}$ does not appear to interfere with the test, greater concentrations up to $5\mu g\, ml^{-1}$ can be removed with EDTA. Methods have been investigated which allow for calcium ions to be eliminated from the test. The method was so designed to work in a dry reagent environment, using a phenylacetic acid buffer. However when the method was adapted as a filter paper indicator test, no success was obtained in determining aluminium without interference.

Some success was obtained when phthalate buffer was used for the paper test, however calcium ion interference remained a problem, and the test did not meet criteria set for sensitivity.

5.5. Conclusions on the determination of aluminium using a paper dip test.

Aluminium ions have a very low mobility within paper, and it is known that water travels ahead of solutes when aqueous solutions rise in filter paper. It is known that alkali and alkaline earth metals rise almost as high as the solvent and are independent of the concentrations with respect to height and ascent, whereas heavy metals and aluminium ions do not rise nearly as high as water, and the height decreases with increasing dilution. Cations of a similar nature rise to approximately the same height particular aluminium and iron(III).

However extensive differences in the height of ascent that can be used for analytical purposes are often observed in the case of dyes and organic compounds, but rarely inorganic salts.
Alizarin red S response to aluminium in two different buffers of identical pH

![Graph showing Alizarin red S response to Aluminium concentrations in two different buffers.](image)

Fig 5. The response of Alizarin to aluminium concentrations in two different buffers.

Refer to sections 5.0 and 5.1.
5.6. References.

20. Handbook of Chemistry and Physics, 76th ed, CRC press
6.0. Polymer as a reagent delivery system.

Although some success was had with the production of tests for the detection of sulphite, nitrite and aluminium using a paper matrix, severe limitations were encountered as previously discussed such as the inability of aluminium to move greatly through a paper matrix, and the problems with interferences. A new matrix was required which would still allow the reactions to be sequenced, but would overcome the problems mentioned. Water soluble polymers were chosen as this matrix. They were chosen for the reasons that they were readily available, have good solubilities in aqueous systems, are inexpensive and non toxic in the small quantities that would be required.

The method of use of the polymeric material was to form a paste with the raw powder using an aqueous solution of reagents required for the test. It was important that the polymer was able to form a good paste of uniform viscosity in order that equal masses of polymer may contain equal amounts of reagents, and material may also be dried and redissolved within a known time, and that layers of the polymer may be formed containing different reagents.

Visual Comparisons.

As mentioned at the start of the paper test section, all visual comparisons and colour evaluations were carried out on a panel of independent observers. During this section of work 5 people were still used, however only one was female. One observer was of Chinese origin, the others were white.
6.1. Reagents and materials used.

All reagents were supplied by Fisons, Loughborough and were of Analar grade unless otherwise stated.

- Alginic acid sodium salt (Lancaster synthesis Ltd)
- Alizarin Red S (as before)
- Aluminium nitrate
- Aluminon (SAF Ltd)
- Ammonium acetate
- Ammonium ferric sulphate
- Ascorbic acid
- Buffer tablets phosphate pH 7.0
- Dichloromethane
- N,N' Diethyl-p-phenyldiamine (DPD no.1) (Palintest Ltd)
- Disodium EDTA
- Gelatin
- Hexane
- Hydrochloric acid 5M
- Mercaptoacetic acid (BDH Ltd)
- Nitrogen gas
- Oxygen gas
- Pentane
- Polyvinyl alcohol Mw 15,000 (SAF Ltd)
- Polyvinyl Pyrrolidone K25 Mw 24,000 (SAF Ltd)
- Polyvinyl Pyrrolidone K30 Mw 40,000 (SAF Ltd)
- Polyvinyl Pyrrolidone K90 Mw 120,000 (SAF Ltd)
- Phenyl acetic acid
- Potassium permanganate
- Sodium chloride
- Sodium hydroxide
- Sodium hydrogen carbonate
- Sodium sulphate
Starch
Tartrazine
Tiron (SAF Ltd)
Philips X-Ray diffractometer, Hilton Brooks motor drive, Sietronics software package
Philips UNICAM 8700 UV spectrometer
Gallenkamp oven

6.2. Selection of a polymer matrix.

The required polymer type material needed to be water soluble, inexpensive and non toxic. Five materials were selected.

**Algicic acid sodium salt/Gelatin.**

Algicic acid sodium salt and Gelatin were dissolved in hot water and small amounts poured into the bottom of large sample tubes such that the final depth was approximately 0.5cm. The tubes were lightly stoppered and left either on the laboratory bench or in the fridge overnight. The material left in the fridge had set to some degree of hardness, the material left at room temperature was still 'jelly like' in texture. The alginate solid showed some bacterial growth and was hence discarded at this stage. The gelatin was very difficult to redissolve and was also yellow/brown in colour, this material was also discarded at this stage.

**Polyvinyl alcohol (PVA).**

Polyvinyl alcohol with a molecular weight 15,000 was dissolved with some difficulty to form a sticky granular like white paste. A pH 3.7 phenyl acetic acid buffer was used as the solvent. The pH of the polymer solution was 6.0 and this was only lowered slightly on increasing the acidity of the buffer to a maximum. Addition of this polymer solution to a dried spot of alizarin red s aqueous solution immediately turned the dye purple due to the higher pH.
The PVA was discarded due to its pH and difficulty in forming a good paste.

**Polyvinyl pyrrolidone (PVP).**

Polyvinyl pyrrolidone of molecular weights 25,000 (K25), 40,000 (K30) and 120,000 (K90) were used. The white solids were dissolved in water to form a white 'frothy' paste. The polymers dissolved more easily as the molecular weight decreased. The K90 powder had a desiccated appearance and was difficult to get into a smooth paste form.

Solutions of all three polymers were acidic in nature, with pH approximately, 2.90 to 3.0.

The pastes were made again using all three polymers and a phenylacetic acid buffer of pH 4.90. Solutions of these pastes showed that the different polymers had different buffering capabilities.

The K90 PVP showed a pH of 4.20, the K30 PVP, a pH of 3.90 and the K25 PVP, a pH of 3.20.

Increasing the amount of sodium hydroxide solution in the buffer, changing its pH to 5.90, managed to increase the pH of polymer solutions to approximately, 4.00 (K25), 4.50 (K30) and 5.00 (K90).

With this information the polymer chosen for initial investigation was polyvinyl pyrrolidone with a Mw of 40,000 (PVP(K30)).

**6.3. The effect of polyvinyl pyrrolidone on the alizarin red S aluminium reaction.**

According to previous work, PVP is said to enhance the formation of colour in the aluminium alizarin red S reaction. The experiment was carried out according to the literature, with the exception that a phenyl acetic acid/sodium hydroxide buffer was used.

To 4ml of buffer, pH 4.6, was added 1.6ml of a 5% K30 PVP solution (the pH of the buffer was unaffected). A 10µl aliquot of a 1% w/v aqueous alizarin solution was added.
Aliquots of 1mg ml⁻¹ aluminium solution were added and the volume made up to 10ml, such that the final aluminium concentrations ranged from 0.05 to 2.0μg ml⁻¹.

The test was carried out without the PVP, as a control.

The complex colour formed within 60 seconds in all tests containing PVP. This colour was pink/orange in the 0.5 to 2.0μg ml⁻¹ tests and orange in the 0.1 and 0.05μg ml⁻¹ tests.

It was possible to visibly distinguish between the 0.1 and 0.05μg ml⁻¹ tests.

Without PVP addition the colour took 2 minutes to form in the 0.5 to 2.0μg ml⁻¹ tests and approximately 10 minutes in the lower concentration tests, where it was only possible to distinguish the 0.1 and 0.05μg ml⁻¹ tests by a trained eye.

Both blanks were yellow in appearance.

Both sets of tests were scanned for absorbance between 400 and 600nm.

The average maximum absorbance was seen at 500nm in the PVP tests and 493nm in the tests without PVP, to demonstrate this bathochromic shift on addition of PVP, the spectra of the corresponding 0.05μg ml⁻¹ tests are shown as fig 6. The blank scan (reagent blank) indicates the possibility of some contamination.

Response curves were plotted on the same axes to compare the two sets of conditions. fig 6a.

Once again both response curves indicate a very sensitive test, with linear areas at low concentrations, and smaller absorbance increases at higher concentrations.

Adding PVP to the alizarin reaction does appear to enhance the complex colour, whilst increasing the sensitivity. The absorbance of the 0.05μg ml⁻¹, PVP added test, is similar in absorbance to the 2.0μg ml⁻¹ test without PVP.
Addition of surface active species is known to be a common method of increasing the sensitivity of spectrophotometric determinations. The addition of a surface active species to a metal ligand system may cause hyperchromic effects and bathochromatic shifts in absorbance. Such species have been applied to the determination of trace elements.

Formation of micelles is thought to occur between binary complexes and ionic surfactants. The effects produced by non ionic surfactants is not clear.

In the alizarin red S aluminium reaction a stoichiometry of 1:1 is normally observed, however at a PVP concentration of 0.8%, this stoichiometry is reported to be 3.0. The difference is thought to be due to changes in the co-ordination sphere of aluminium, the PVP possibly causing a rearrangement of the water molecules around the metal ion increasing its co-ordination capacity.\textsuperscript{1}

The authors also report that addition of sodium sulphate slightly increases the absorbance of the complex.

Reference.

Fig 6.
The absorbance spectra of 0.05µg ml⁻¹ tests.

sample a. no PVP addition absorbance 0.097
sample b. PVP addition absorbance 0.161

refer to section 6.3. Effect of PVP on the alizarin red S aluminium reaction.
Fig 6a. refer to section 6.3 effect of PVP on the aluminium alizarin reaction.

It can be seen that the PVP has sensitised the reaction, comparing the original phenylacetic acid buffered reaction to that containing PVP.
6.4. Polyvinyl pyrrolidone as support matrix for the alizarin aluminium reaction.

Polyvinyl pyrrolidone provided a good paste, the acidity of which could be controlled with the phenylacetic acid/sodium hydroxide buffer, and hence it was used as the support medium for the aluminium alizarin reaction. Plastic microcuvettes, with a volume of 2.5ml, were initially used as the containing vessels, as they could easily be inserted into the UV spectrometer, had a cell depth of 3cm for good visual observation, and the microcuvettes were inexpensive and could be washed and reused at least twice before the plastic became scratched.

6.5. Paste and Soluble Polymer Matrix Test (SPMT) manufacture.

A 1%w/v aqueous solution of alizarin red S was used. Aliquots, 5μl in volume, of this dye were micropipetted into the base of a large number of microcuvettes. The dye was left to dry, in a time of one hour on the laboratory bench, as a yellow stain in the base of the microcuvette.

The PVP paste was made by mixing approximately 0.4g of K30 PVP, with 240μl of pH 5.95 phenyl acetic acid/sodium hydroxide buffer and 40μl of 5000μg ml⁻¹ sodium sulphate solution. The pH of this paste was 4.20. This paste was then placed into the cuvette on top of the alizarin stain. The cuvettes were then stored in the fridge overnight, or covered and kept out on the laboratory bench.

The viscosity of the paste was critical, as paste of low viscosity would allow the dye to diffuse through the paste, causing it to turn yellow in colour. Addition of tap water to this sample would allow calcium ions to reach the dye before the sulphate and buffer had chance to work, the SPMT would then become pink instead of the yellow expected. The ideal viscosity was one in which the paste would sit in the bulk area of the cuvette, separated from the dye in the narrow region. The dye would then harden slightly and contact between the dye and paste would not result in diffusion.
SPMT operation and results.

6.6. PVP K30 paste, deionised water samples.

All SPMTs were stored for 24 hours before use. On using the tests the paste was of a viscosity similar to 'toffee', and a small glass rod could just be forced into this paste. To 10 SPMTs was added 0.5ml of deionised water. The water was left for 5 minutes before it was stirred into the paste with a small glass rod of diameter 1mm. When the SPMT had reached a uniform viscosity, it was scanned for absorbance against a deionised water blank between 250 and 600nm, a small peak appeared to be visible at 422nm as expected and all blank samples were scanned at this wavelength for absorbance.

All the deionised water tests were pale yellow in colour. The mean absorbance of the ten tests at 422nm was 0.305, with a standard deviation of 0.061. (RSD 20%) The remaining 30 SPMTs were taken and 0.5ml of aluminium spiked deionised water of concentrations 0.05, 0.5 and 2μg ml⁻¹ added to 10 tests respectively. These tests were then scanned for absorbance between 400 and 600nm against the reagent blank which had an absorbance at 422nm closest to 0.305, after 30 minutes from addition of solution.

The SPMTs all showed a maximum absorbance at 496nm. Fig 6b shows the absorbance of a 0.5μg ml⁻¹ test. Please note that all spectra in this and forthcoming sections are blank subtracted.

The table below shows the mean absorbance and standard deviation of the SPMTs.

<table>
<thead>
<tr>
<th>Aluminium conc. μg ml⁻¹</th>
<th>mean absorbance at 496nm</th>
<th>standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>0.05</td>
<td>0.052</td>
<td>0.012</td>
</tr>
<tr>
<td>0.50</td>
<td>0.091</td>
<td>0.018</td>
</tr>
<tr>
<td>2.00</td>
<td>0.099</td>
<td>0.021</td>
</tr>
</tbody>
</table>
The colour of the SPMTs was orange with the 2μg ml\(^{-1}\) concentration, orange/yellow with the 0.5μg ml\(^{-1}\) concentration and dark yellow with the 0.05μg ml\(^{-1}\) concentration.
Fig 6b

Absorbance spectrum of a 0.5μg ml⁻¹ aluminium sample, after addition to PVP K30 alizarin test.

reference: reagent blank

refer to section 6.6. PVP K30 paste, deionised water samples.
6.7. PVP K30 paste, tap water samples.

The experiment was repeated using tap water as the blank solution and aluminium spiked solutions of tap water. SPMTs stored overnight in the fridge and SPMTs stored at laboratory temperature were compared.

The aluminium concentrations were 0.05, 0.1 and 2.0 μg ml⁻¹. 0.05 and 0.1 μg ml⁻¹ were chosen as realistic levels of aluminium in water samples, 2.0 μg ml⁻¹ was chosen as a token higher concentration. Three aluminium concentrations were chosen to allow a greater number of tests to be repeated at one concentration, to get through a large number of tests it was not really practical to carry out tests on more concentrations.

The tap water blank showed a maximum absorbance at 422 nm, when scanned against a tap water blank. The aluminium tests showed a maximum absorbance at 494 nm, when scanned against the reagent blank with an absorbance equal to the mean absorbance of all the reagent blanks. The colour of the tests after thorough mixing is indicated below.

The final test colour appeared to be identical regardless of how the test were stored. A small amount of dried alizarin remained as a yellow/brown stain at the base of all tests.

<table>
<thead>
<tr>
<th>Aluminium conc&lt;sup&gt;n&lt;/sup&gt; μg ml⁻¹</th>
<th>test colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>yellow</td>
</tr>
<tr>
<td>0.05</td>
<td>dark yellow</td>
</tr>
<tr>
<td>0.10</td>
<td>yellow/orange</td>
</tr>
<tr>
<td>2.00</td>
<td>orange/pink</td>
</tr>
</tbody>
</table>

The mean absorbances after 30 minutes, and standard deviations of the fridge stored SPMTs are shown in the table below. Batches of 8 SPMTs were used for the calculations. A Dixon Q test indicated that no results were outliers.
<table>
<thead>
<tr>
<th>Aluminium conc$^n$ $\mu g$ ml$^{-1}$</th>
<th>mean absorbance at 494nm</th>
<th>standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.001</td>
<td>0.007</td>
</tr>
<tr>
<td>0.05</td>
<td>0.079</td>
<td>0.017</td>
</tr>
<tr>
<td>0.10</td>
<td>0.087</td>
<td>0.010</td>
</tr>
<tr>
<td>2.00</td>
<td>0.104</td>
<td>0.013</td>
</tr>
</tbody>
</table>

An absorbance scan between 300 and 600 nm of the tap water test, 0.05 $\mu g$ ml$^{-1}$ test and the 2.0 $\mu g$ ml$^{-1}$ test after 60 minutes from sample addition are shown as fig 6c. A response plot was drawn, see fig 6d. The error bars indicate overlap in absorbance between the aluminium concentrations. A situation not observed with the deionised water samples, it is fair to assume these samples are not free from contamination.

After 4 hours the fridge stored SPMTs were re-examined. The blank test was still yellow in colour. The 0.05 $\mu g$ ml$^{-1}$ tests were yellow with orange diffusion in the bottom 1 cm of the cuvette, the 0.1 $\mu g$ ml$^{-1}$ tests were orange with some yellow colour towards the top of the cuvette and the 2.0 $\mu g$ ml$^{-1}$ tests were pink with yellow tint at the top of the solution. It was now easier to visually distinguish between the 0.05 and 0.1 $\mu g$ ml$^{-1}$ tests.

6.8. PVP K90 paste, tap water samples.

PVP K30 was replaced by the K90 molecular weight type. Paste was made up using this polymer such that the final viscosity was similar to that obtained with the K30 PVP. Additional solvent was required, and the paste appeared to be of a more plastic nature than previously.

The SPMTs were stored on the laboratory bench overnight. 0.5 ml aliquots of the same tap water and aluminium spiked tap water samples were added to the sets of 10 SPMTs and they were stirred for the same length of time with the small glass rod, until they had reached uniform consistency.
The tests were scanned for absorbance between 300 and 600nm against a tap water reference after 30 minutes and 4 hours.

It was just possible to distinguish the blank from the 0.05µg ml⁻¹ test as the 0.05µgml⁻¹ test was slightly more yellow. The 0.1µg ml⁻¹ test was yellow/orange and the 2.0µgml⁻¹ was orange.

The SPMTs were scanned for absorbance at 500nm against a reagent blank after 30 minutes. The mean absorbance of the blank SPMT was subtracted from the aluminium SPMT mean absorbances. see table below.

<table>
<thead>
<tr>
<th>Aluminium concn µg ml⁻¹</th>
<th>mean absorbance at 500nm</th>
<th>standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>0.00</td>
<td>0.002</td>
</tr>
<tr>
<td>0.05</td>
<td>0.165</td>
<td>0.072</td>
</tr>
<tr>
<td>0.10</td>
<td>0.281</td>
<td>0.060</td>
</tr>
<tr>
<td>2.00</td>
<td>0.441</td>
<td>0.053</td>
</tr>
</tbody>
</table>

A response plot was drawn up, fig 6e, once again overlap between absorbance is seen. Probably due to contamination, even though all precautions were taken to try and avoid contamination, such as covering the cuvettes.
Fig 6c

Absorbance spectra of 0.05\(\mu\)g ml\(^{-1}\), 2.00\(\mu\)g ml\(^{-1}\) aluminium and tap water samples after addition to a PVP K30 alizarin, fridge stored tests and a 60 minute wait.

*reference*: tap water

refer to section 6.7. PVP K30 paste, tap water samples
Fig 6d  Response of SPMTs to aluminium spiked tap water.

Error bars shown, calculated from average standard deviation, 95% confidence limit.

This curve is intended only to give an idea of the response of the SPMTs to the samples, a more accurate calibration would require more data.

Refer to section 6.7
Fig 6e, the response of PVP K90 SPMTs to aluminium spiked tap water samples.

Errors bars calculated from the mean of one standard deviation, only to give a guide to the errors experienced in the test.

The sensitivity of the initial region is $2.81 \mu g^{-1} ml$, from the linear regression $y = 2.81x + 0.0817$.

Refer to section 6.8.
6.9. Conclusions.

The SPMTs so far have been successful to a certain extent in colorimetrically and spectrophotometrically determining levels of aluminium in the region of 0.05μg ml⁻¹. The optimum time for spectrophotometric determination of the test is 30 minutes, however colour formation takes place within a few minutes of stirring the sample into the tests.

The tests have been made and left for twenty four hours, either on the laboratory bench or in the fridge, and there appears to be no advantage in either method.

The polymer is not set to full hardness when left at these temperatures for this length of time, and has a viscosity similar to 'toffee' when used. PVP K30 makes a better paste than PVP K90, however PVP K90 is more easily buffered.

6.10. Real water samples and comparison with standard method.

The SPMTs produced so far were now tried with real water samples, the samples used were:

i. Beacon stream water
ii. Blackpool tap water
iii. Loughborough tap water.
iv. Burton fountain water.

The PVP method was compared with a standard spectrophotometric method, that which uses the dye Aluminon.¹

A method used by water authorities before the advent of instrumental, automated sampling. It is not ideal to use this method, however it was not possible to set up the atomic absorption spectrometer to measure aluminium, as previously mentioned. The ICP MS equipment was also out of operation. Due to no money being available it was also impractical to obtain a pre-analysed matrix from a water authority.

The aluminon method followed was described as.

*To a sample of aluminium solution, add 2.0ml of 5M hydrochloric acid, 1.0ml mercaptoacetic acid 4.5%w/v, 3.0ml of filtered starch solution and 5.0ml of 3.5M
ammonium acetate solution. dilute to approximately 45.0ml and add 2.0ml of a
0.396w/v aqueous aluminon solution and make volume up to 50.0ml.
Mix and heat in a boiling water bath for 10 minutes. Cool to room temperature and
scan for absorbance at 525nm against a water blank.


A 500ml sample of river water was collected from Beacon Hill, Loughborough in a 0.5
litre, clean plastic sample bottle, and stored in the fridge overnight.
SPMTs were made from a paste using a 2:1 mix of PVP K30:K90, the paste pH was
4.17, a phenylacetic acid/sodium hydroxide buffer of pH 5.19 was used and
5000µg ml⁻¹ sodium sulphate was added to remove calcium interferences.
Samples of aluminium spiked deionised water, deionised water and river water, 1.0ml
in volume, were added to sets of 8 SPMTs.
The SPMTs were stirred until the paste appeared to be of uniform viscosity and
colour.
They were then scanned for absorbance at 502nm against a reagent blank after a 30
minute wait. 502nm was chosen as this was the maximum absorbance of the 2.0µgml⁻¹
aluminium sample.
The mean absorbances of the tests and their colour are shown in the table below

<table>
<thead>
<tr>
<th>Aluminium concmüg ml⁻¹</th>
<th>colour</th>
<th>mean absorbance at 502nm (30 mins)</th>
<th>st.dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>yellow</td>
<td>0.006</td>
<td>0.004</td>
</tr>
<tr>
<td>0.05</td>
<td>yellow</td>
<td>0.266</td>
<td>0.016</td>
</tr>
<tr>
<td>0.10</td>
<td>yellow/orange</td>
<td>0.396</td>
<td>0.034</td>
</tr>
<tr>
<td>0.50</td>
<td>orange/yellow</td>
<td>0.534</td>
<td>0.079</td>
</tr>
<tr>
<td>2.00</td>
<td>orange/pink</td>
<td>0.702</td>
<td>0.098</td>
</tr>
<tr>
<td>river water</td>
<td>yellow</td>
<td>0.096</td>
<td>0.018</td>
</tr>
</tbody>
</table>
From the absorbance results above a response curve was drawn, fig 6f, and using this curve, the approximate aluminium concentration in the river water sample was estimated to be 0.02µg ml⁻¹(±20%).

The aluminium content of the river water sample was estimated by the aluminon method, carried out as stated, with the exception of the reaction being scaled down from 50ml to 10ml.

The absorbances at 525nm (Sandell) and the corresponding colours are shown below.

<table>
<thead>
<tr>
<th>Aluminium conc⁰</th>
<th>absorbance at 525nm</th>
<th>colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>µg ml⁻¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.00</td>
<td>0.631</td>
<td>pale red</td>
</tr>
<tr>
<td>0.05</td>
<td>0.684</td>
<td>pale red</td>
</tr>
<tr>
<td>0.10</td>
<td>0.714</td>
<td>pale red</td>
</tr>
<tr>
<td>0.25</td>
<td>0.902</td>
<td>red</td>
</tr>
<tr>
<td>0.50</td>
<td>1.080</td>
<td>red</td>
</tr>
<tr>
<td>1.00</td>
<td>1.402</td>
<td>red</td>
</tr>
<tr>
<td>2.50</td>
<td>2.168</td>
<td>cherry red lake</td>
</tr>
<tr>
<td>river water</td>
<td>0.642</td>
<td>pale red</td>
</tr>
</tbody>
</table>

Using these absorbance values a response curve, fig 6g, was plotted.

Using this curve, the absorbance value of the river water sample was used to estimate the aluminium concentration as 0.03µg ml⁻¹, due to the relatively high absorbance of the blank, and the possibility of contamination, this figure again should be taken as ±20%.

6.12. Loughborough and Blackpool Tap waters, Burton fountain water.

The experiments described above were repeated with samples of tap water from Loughborough and Blackpool, and Burton fountain water.

Using the aluminon method the following absorbances were obtained.
Using the response curve, the aluminium concentrations contained in the samples were estimated.

<table>
<thead>
<tr>
<th>sample</th>
<th>aluminium content (µg ml(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blackpool tap water</td>
<td>0.01</td>
</tr>
<tr>
<td>Loughborough tap water</td>
<td>0.01</td>
</tr>
<tr>
<td>Burton fountain water</td>
<td>0.06</td>
</tr>
</tbody>
</table>

As before these results are near the limit of detection for the test and will be subject to contamination.

The same samples were used with the soluble polymer matrix tests. The tap water samples when scanned for absorbance at 502 nm gave mean absorbance values which were less than or equal to that obtained with the deionised water blank, and therefore the aluminium content of these samples was taken as 0 µg ml\(^{-1}\) when determined by the SPMTs.

The Burton fountain water gave a mean absorbance of 0.364 when scanned for absorbance at 502 nm against a reagent blank.

From the response curve obtained, fig 6f, an approximate value for aluminium content in Burton fountain water as 0.08 µg ml\(^{-1}\). (+/-20%)

<table>
<thead>
<tr>
<th>Sample</th>
<th>aluminium content $\mu g$ ml$^{-1}$ aluminon method</th>
<th>aluminium content $\mu g$ ml$^{-1}$ PVP method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beacon river</td>
<td>0.03</td>
<td>0.02</td>
</tr>
<tr>
<td>Loughborough TW</td>
<td>0.01</td>
<td>non detected</td>
</tr>
<tr>
<td>Blackpool TW</td>
<td>0.01</td>
<td>non detected</td>
</tr>
<tr>
<td>Burton Fountain</td>
<td>0.06</td>
<td>0.08</td>
</tr>
</tbody>
</table>

All results should be taken as $+/-$ 20%. It would have been advantageous to be able to check these results using atomic absorption spectrometry.
Fig 6f  Response of SPMTs to aluminium, for natural water aluminium content estimation.

Error bars calculated from average standard deviation, 95% confidence limit.

Refer to section 6.11
Fig 6g, Aluminon calibration curve for the estimation of natural water aluminium content.

Note the high blank absorbance, indicating contamination of samples from external sources.

The sensitivity of the curve is $0.757 \mu g^{-1} \text{ml}$, calculated from the linear regression:

$$y = 0.7567x + 0.66.$$

Refer to section 6.11.

The SPMTs using PVP as the support medium were so far successful, to some extent, in terms of sensitivity and selectivity, when the reaction had been carried out in a microcuvette, and the PVP had been allowed only 24 hours to harden. The reaction was transferred to large sample tubes, and hence a larger mass of PVP was used, initially approximately 1.5g. SPMTs were made up and allowed to dry on the laboratory bench. It was important that the SPMTs were allowed to dry in order that all tests contained the same mass of reagent in equal conditions, eliminating water was essential for this criteria to be met. After 1 week, the tests were still not completely dry and a small glass rod could be forced into the polymer to the base of the tube.

In an attempt to increase the rate of drying of the SPMTs they were placed in an oven at a temperature of 60°C. After a period of 48 hours they were set to full hardness, 5ml of deionised water was added to the cooled SPMTs, which were stoppered and shaken vigorously by hand for a period of 20 minutes. The tests failed to dissolve fully after this time, over 50% of solid polymer was still present in the tube.

6.15. Increasing the dissolution rate of the solid polymer.

A criterion for a successful test of this nature required the reagents to be dissolved in under 3 minutes. After the PVP was set hard by heating, this criterion was some distance from being met. Investigation was required in order to improve the rate of dissolution. Vastly increasing the surface area of the PVP structure appeared to be the most appropriate method.
6.16. Addition of insoluble and soluble inorganic solids.

An insoluble solid was needed which would drop out of the solid mass of PVP, as the immediate polymer around it dissolved, on addition of water, leaving holes in the structure, and hence provide the increase in surface area available to the solvent. It was hoped that the solid sand would provide a surface abrasion effect on shaking. Fine grade Sand, approximately 50μm, was the insoluble solid chosen. The sand was mixed with the PVP in a ratio 1:5 w/w, using deionised water as the solvent. The paste was then dried at 60°C in the oven for 24 hours. During the drying process the majority of the sand sunk to the base of the sample tube. Addition of 5ml of deionised water to the solid, followed by a 10 minute shake resulted in little improvement on the extent of dissolution.

The insoluble sand was replaced with soluble sodium chloride, it was thought that this solid would dissolve out of the bulk polymer to leave a large number of gaps in the solid increasing the surface area. A 1:5 w/w ratio of sodium chloride to PVP was chosen, deionised water was used as the solvent. After drying at 65°C for 24 hours the test was shaken with 5ml of deionised water, again no improvement was made on the speed of dissolution.


Following the lack of success with sand and sodium chloride, sodium hydrogen carbonate, a solid which decomposes to form carbon dioxide on heating was used as a blowing or foaming agent. The carbon dioxide produced would be trapped within the PVP solid producing a foam with a very large surface area. A solid carbonate would also be left in the solid, this would dissolve out to leave gaps in the polymer foam increasing the surface area further, however the presence of carbonate in the aluminium alizarin test may cause serious pH problems due to the carbonate buffering capacity. Pastes were made up using sodium hydrogen carbonate of weight percentages in the range 10 to 0.25%. The tests were left to dry in the oven at 60°C for 24 hours, after
only minutes of being heated in the oven, all tests were observed to froth. After drying the solid had a white appearance and contained many small holes. 5ml of deionised water was added to the dry SPMTs and they were shaken until they dissolved.

All the tests dissolved at a similar rate, in a time between 5 and 10 minutes. Many tests contained a centre of solid polymer, which appeared to slow down the dissolution process.

A paste was made up using the phenyl acetic acid/sodium hydroxide buffer as a solvent and 0.25% sodium hydrogen carbonate such that the final pH of the paste was 4.30. On drying and dissolving the solid formed, a final pH of 4.6 was obtained.


As success was obtained with an inorganic foaming agent, organic foaming agents were tried. The solvents which were used required a boiling point in the region of 50 to 80°C in order that they may be removed by gentle heating in a ventilated oven set at a temperature of 40 to 65°C.

The solvents that were chosen, and their boiling points were.

<table>
<thead>
<tr>
<th>solvent</th>
<th>boiling point °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>n-Hexane</td>
<td>68</td>
</tr>
<tr>
<td>Pentane/dichloromethane 1:4</td>
<td>36/40</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78</td>
</tr>
<tr>
<td>Acetone</td>
<td>59</td>
</tr>
</tbody>
</table>

The first solvent tried was n-hexane, using approximately 1g of PVP powder, enough hexane was added to just cause the powder to become wet in appearance, buffer was then added to this mixture to form a paste, a 1:1 mix of hexane:buffer was also used to form a paste. Both pastes were placed in an oven set at 62°C and left for 24 hours, after several minutes in the oven froth could clearly be seen in the tubes.

After 24 hours the tubes were removed and 5ml of deionised water added to each SPMT, the 1:1 hexane:buffer test appeared to be of a uniform foam appearance and took approximately 4 to 5 minutes to dissolve, whilst the SPMT containing less hexane
had a solid bulk of unfoamed polymer below the foam which did not dissolve in under 5 minutes.

A 1:1 organic solvent ratio was hence used as the PVP paste forming agent.

The 1:1 solvent ratio was repeated using ethanol, oven temperature 65°C, acetone, oven temperature 50°C and a 1:4 mix of n-pentane and dichloromethane, oven temperature 60°C.

The best foam of these three solvents was produced by ethanol, however this foam was still much inferior to that produced with n-hexane.

It was important not to heat vast amounts of tests together, and to use a ventilated oven to avoid a build up of potentially explosive gases. Fume cupboards and other safety precautions were taken when using hexane, pentane and dichloromethane.


To investigate the possibility of PVP structure changes on producing the foam using a water miscible organic solvent, X-Ray diffraction was employed.

Three samples were crushed to a fine powder using a pestle and mortar, these samples were:

- PVP K30 powder
- PVP K30 phenyl acetic acid buffer paste oven dried to solid at 65°C.
- PVP K30 phenyl acetic acid buffer/ethanol (1:1) paste oven dried to solid at 65°C.

The samples produced the diffraction patterns seen in fig 6h, there appeared to be little difference in the three patterns and therefore it was concluded that no difference in structure was produced on addition of ethanol.

6.20. Foamed PVP Alizarin SPMT calibration.

Aliquots of 1%w/v alizarin red S were dried onto the base of large sample tubes using an oven set at a temperature of 60°C. PVP paste was made using a 1:1 mix of n-hexane and phenyl acetic acid/sodium hydroxide buffer containing a mass of sodium sulphate such that the final sulphate concentration was 1mg ml⁻¹ and the final buffer pH was 5.95.

The SPMTs were allowed to dry in the ventilated oven at 60°C for 24 hours. On removing the SPMTs from the oven all contained excellent uniform foam, the alizarin stain appeared to be dark brown in colour. 5ml of deionised water was added to a SPMT which was then shaken until the solid had fully dissolved. A shaking time of approximately 5 minutes was required. This was repeated in triplicate. The yellow solution produced had a pH of 3.97, and when scanned for absorbance between 300 and 600nm, against a deionised water reference, gave the expected peak at 422nm.
Aluminium spiked deionised water samples were added in 5ml aliquots to the prepared SPMTs. Each aluminium concentration was carried out in triplicate. The colours produced after shaking until solid dissolution and the respective mean absorbances at 500nm are shown below.

<table>
<thead>
<tr>
<th>Aluminium conc\textsuperscript{H} µg ml\textsuperscript{-1}</th>
<th>colour</th>
<th>mean absorbance at 500nm</th>
<th>std. dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td>yellow</td>
<td>0.000</td>
<td>0.002</td>
</tr>
<tr>
<td>0.10</td>
<td>yellow</td>
<td>0.008</td>
<td>0.004</td>
</tr>
<tr>
<td>0.50</td>
<td>yellow/orange</td>
<td>0.063</td>
<td>0.011</td>
</tr>
<tr>
<td>1.00</td>
<td>orange</td>
<td>0.134</td>
<td>0.017</td>
</tr>
<tr>
<td>5.00</td>
<td>orange/pink</td>
<td>0.281</td>
<td>0.023</td>
</tr>
</tbody>
</table>

Using these absorbances a response curve was plotted, fig 6i.

The sensitivity of the tests, in terms of complex formation, appears to be slightly reduced from the previous test type involving viscous PVP, probably due to the lowered pH.

The response curve shows a linear portion between 0 and 1.0µg ml\textsuperscript{-1}. 
Fig 6h  X-ray diffraction patterns, showing the PVP K30, after solid formation with water and ethanol. Compared to that of the raw powder.
Fig 6i, Response of the foamed SPMTs to aluminium concentrations.

The sensitivity of the initial linear area of the curve is calculated to be $0.136 \mu g \cdot ml^{-1}$, from the linear regression $y = 0.136x - 0.00314$.

Error bars calculated from one standard deviation.

Refer to section 6.20.

The possibility of building up layers of PVP foam, each layer containing the necessary reagents for a specific sequence of a reaction was investigated. To investigate whether layers of foam PVP could be built upon without significant diffusion between the distinct regions, experiments with coloured foam layers were carried out.

PVP paste was made using a 1:1 mix of water:n-hexane and approximately 1g of this paste was dried in an oven at 60°C for 24 hours. A similar paste was made containing the dye tartrazine and was therefore orange in colour. To the dry polymer solid was added approximately 1g of the orange paste and this was dried as before for 24 hours. Ten of these SPMTs were made up. On examination of the ten SPMTs 8 had mixed layers, and orange paste could be seen on the base of the tube, two tests did not appear to have mixed to this extent.

To improve the layer building ability of the SPMT, the two coloured PVP foams were separated by a thin layer of PVP paste made with water only, this paste was of a low to medium viscosity and was able to be painted onto the first layer of foam. On drying this layer was then covered with the orange foam which was then dried as before. Ten SPMTs produced using this method showed no mixing between layers, all had two distinct coloured bands.

6.22. Dissolution of layers in specific order.

In order to check that the foam layers dissolved in the required order, and sample solution did not seep down the side of the tube or through fissures in the foam the following experiment was carried out.

Three PVP foam layers were built up each one separated by a painted layer of PVP only. The base layer contained the dye Tiron, the second layer contained EDTA, and the top layer contained 10μg ml⁻¹ iron(III).

A 10ml aliquot of pH 7.0 phosphate buffer was pipetted onto the foam surface containing iron(III), and the SPMT was stoppered and shaken gently until all three layers were dissolved.

The SPMT dissolved to leave a colourless solution.
A blank containing no EDTA in the second layer was also shaken with 10ml of pH 7.0 buffer, this SPMT produced a bright red solution, characteristic of an iron(III) tiron complex.

A blank consisting of only iron(III) and EDTA produced a colourless solution. The red solution when scanned for absorbance showed a iron(III) tiron complex peak at 472nm, whereas this peak was not shown when the colourless solutions were scanned.

It was therefore concluded that this method was successful in producing distinct layer of reagents, for sequenced chemical reactions. If this were not the case a red iron(III) tiron complex would be seen when the SPMT containing the iron(III) and tiron separated by EDTA was dissolved. However no complex was formed.

6.23. Reagent molecule location within the polymer matrix.

Blowing the PVP paste with the organic solvent n-hexane has so far been successful in lowering the dried test dissolution rate to a more acceptable level i.e. approximately 5 minutes, however following discussion with Palintest ltd, a manufacturer of test kits and indicator tablets, it was realised that further improvements needed to be made on this time for the SPMT to be commercially viable.

It was important to know how the reagent molecules present in the body of polymer solid were bound to the matrix. If the reagent molecules were situated in gaps in the polymer matrix chain, they may dissolve out quickly irrespective of whether the mass of polymer dissolved, and hence the speed of total dissolution would be unimportant. Alternately the reagent molecules might be coated and bound by the polymer molecules and may only escape the polymer matrix when it is dissolved, and therefore the speed of dissolution becomes an important factor.

A PVP paste was made using 1g of PVP and a 1:1 mix of n-hexane and water. A very small mass of tartrazine was added to the paste to just colour it yellow/orange. The test was repeated using potassium (VII) manganate in place of tartrazine.

Both SPMTs were oven dried at 60°C for 24 hours. To the dry SPMTs was added 10ml of deionised water and the tests were shaken for a period of 1 minute in an
ultrasonic bath. The SPMTs were removed and examined for colour differences. Both showed coloured masses of polymer remaining, the colour of the polymer mass remained unchanged throughout the dissolution process. It could therefore be concluded that the reagents do not escape the polymer matrix until the PVP itself dissolves.

6.24. Reduction of SPMT dissolution time and transmittance improvement of the dissolved SPMT.

In order to increase the speed with which the tests dissolved, the mass of PVP used in SPMT manufacture was lowered from approximately 1g to 0.5g. PVP powder of masses 0.5g, 1.0g and 1.5g were weighed out. Pastes were made using these masses and various volumes of solvent made up with 1:1 mixes of n-hexane and water, such that the pastes were all of differing viscosities before drying. All the SPMTs were then dried in the large sample tubes in an oven at 60°C for 24 hours.

Following further discussion with Palintest Ltd, we were informed that for the SPMT to be a commercial success, along with rapid dissolution, the transmittance i.e. the optical clarity of the final test result is required to be greater than 90%, (for optimum spectrophotometric determination), when 10.0 ml of sample solution is added. The SPMTs previously made and calibrated contained approximately 1.0g of PVP and had a transmittance value of 77%. This appeared to be caused by excessive frothing of the solution whilst shaking the tube, leaving the final solution with a cloudy appearance. Microscopic bubbles produced as the PVP dissolved by agitation took over 5 minutes to disperse. The SPMT, to be successful, needed the lowest time possible between sample addition and determination. Hence the solution needed to be clear within 2 to 3 minutes after agitation was complete. It was hoped that lowering the mass of PVP in the SPMT would lower the dissolution time as well as increasing the optical clarity of the final solution.
6.25. Results of varying the PVP mass in the SPMT.

The dry SPMTs made from various PVP masses were all of a similar appearance, all the tubes contained well foamed PVP.

10.0ml aliquots of deionised water were pipetted into the sample tubes in turn and the tests were shaken until the PVP had fully dissolved, this time was noted.

The solution was then scanned for transmittance between 450 and 700nm, 1 minute and 3 minutes after agitation was complete. A deionised water reference was used as a 100% transmittance blank. The average transmittance between 450 and 700nm was noted after each time.

The results of both the transmittance and dissolution experiments are shown in the tables following.

*Table 1.* Averaged time of dissolution for sets of three SPMTs, made from pastes using various masses of PVP and volumes of 1:1 water:n-hexane solvent. All tests dissolved in 10ml of water.

<table>
<thead>
<tr>
<th>mass of PVP and volume of solvent in SPMT</th>
<th>time to dissolve (average of three tests) minutes:seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5g in 0.50ml</td>
<td>3:20</td>
</tr>
<tr>
<td>0.5g in 0.55ml</td>
<td>3:30</td>
</tr>
<tr>
<td>0.5g in 0.60ml</td>
<td>3:20</td>
</tr>
<tr>
<td>0.5g in 0.75ml</td>
<td>2:50</td>
</tr>
<tr>
<td>0.5g in 1.00ml</td>
<td>2:58</td>
</tr>
<tr>
<td>1.0g in 1.50ml</td>
<td>4:32</td>
</tr>
<tr>
<td>1.5g in 2.25ml</td>
<td>&gt;6.00</td>
</tr>
</tbody>
</table>

*Table 2.* Over. averaged transmittances of the above tests. After 1 and 3 minutes from agitation completion. Deionised water reference.
<table>
<thead>
<tr>
<th>Mass of PVP and volume of solvent in test</th>
<th>%T (1 minute)</th>
<th>%T (3 minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5g in 0.50ml</td>
<td>85.0</td>
<td>96.8</td>
</tr>
<tr>
<td>0.5g in 0.55ml</td>
<td>91.9</td>
<td>97.7</td>
</tr>
<tr>
<td>0.5g in 0.60ml</td>
<td>94.0</td>
<td>96.5</td>
</tr>
<tr>
<td>0.5g in 0.75ml</td>
<td>94.5</td>
<td>98.4</td>
</tr>
<tr>
<td>0.5g in 1.00ml</td>
<td>92.9</td>
<td>95.8</td>
</tr>
<tr>
<td>1.0g in 1.50ml</td>
<td>75.1</td>
<td>91.2</td>
</tr>
<tr>
<td>1.5g in 2.25ml</td>
<td>48.8</td>
<td>72.4</td>
</tr>
</tbody>
</table>

A plot of %Transmittance against mass of PVP was plotted. Fig 6j. The critical transmittance of 90% is indicated.
Fig 6j

%Transmittance of PVP SPMTs after dissolution in 10ml water

refer to section 6.25. Results of varying the PVP mass in the SPMT.

From the transmittance results it may be concluded that a 0.5g mass of PVP, when blown with n-hexane to form a foam, dissolves in approximately 3 minutes and produces a solution of an optical clarity, after standing for 1 minute, with a %Transmittance greater than 90%.

The 1g PVP foamed tests, dissolve in approximately 4.5 minutes, and show a %Transmittance greater than 90 after standing for 3 minutes.

Foamed PVP layers may be built up containing specific reagents for an ordered reaction. The layers dissolve in the required order.

Combining these results with previous findings we were able to produce an SPMT for aluminium detection at sensitivity levels of approximately 0.01μg ml⁻¹, using Alizarin. Interference from calcium ions is eliminated by complexing with sulphate ions and levels of iron(III) greater than 1μg ml⁻¹ may be removed with EDTA. Although this has not been subject to a separate analytical study. The tests dissolve in under 3 minutes, to give a resulting solution, the optical clarity of which shows a %Transmittance greater than 90.

The SPMTs need to be made individually as attempts to mass produce the PVP paste using a 1:1 buffer:n-hexane solution results in the hexane separating out of the mix on standing, forming a liquid layer above the paste, due to hexane and water being immiscible.

The paste is also too viscous to be distributed into tests using a liquid production line.

6.27. Effect of ageing on the test.

SPMTs stored for a period of 4 months after manufacture, in a sealed sample tube, and out of daylight, were visibly similar to those stored for 2 days only.

Addition of deionised water and an aluminium spiked deionised water sample of concentration 0.5μg ml⁻¹, to these tests in 10ml volumes, produced yellow and orange coloured solutions with visible and spectrophotometrical similarity.

No SPMTs have been stored for longer periods of time than 4 months.
It could be assumed that the factor dictating the length of time that the SPMTs may be stored without loss of effectiveness, is the degradation of the alizarin due to oxidation. An attempt was made to monitor the time taken for oxygen to penetrate the PVP foam layer, using DPD no. 1, a solid white reagent which turns pink on oxidation. A 100mg mass of DPD no. 1 was placed in the base of a large sample tube and the PVP paste was added to the tube until the base was covered. Unfortunately on contact with the PVP paste, the DPD no. 1 immediately turned pink. No useful information could be obtained as the DPD no. 1 needed to be kept moist for the colour change process to work, and indeed the dry reagent when stored in a sealed sample tube filled with oxygen, failed to change from white to pink in colour.

It is hoped that as the SPMTs are still effective after 4 months, they will still be of similar effectiveness after storage for 1 year. The reagents in the test are not readily decomposed or degraded in this period of time.

6.28. Increasing the mass of reagent contained within the PVP matrix.

In order to investigate the maximum mass of reagents that could be retained in the PVP matrix, a mass ratio of 5:1, (PVP:reagent) being the aim, without the reagent being visible on the surface of the PVP, a new method of PVP layer production was developed which would also allow the SPMTs to be produced from a liquid line. This is described below.

6.29. Liquid evaporation method.

Calcium chloride was used as the reagent contained in the PVP due to its high solubility. Various masses of calcium chloride were dissolved in 5.0ml of deionised water and 0.5g of PVP K30 powder was dissolved in this 5.0ml. The masses of calcium chloride used were 0.2, 0.1, 0.05 and 0.01g. An SPMT using only 0.25g of PVP and 0.05g of calcium chloride was also made up. An SPMT containing 0.5g of PVP only in 5.0ml of water was used as a control.
The 5.0ml volumes were evaporated to dryness at a temperature of 55°C in an oven, over a period of 48 hours.

The solid appeared to be a very pale yellow in colour and was uniform in its depth of approximately 0.20cm, some solid had formed a ring around the base of the tube above the main body of polymer which also exhibited some fissures on the smooth surface. Both the control and the calcium chloride SPMT were similar in appearance and no reagent could be seen within PVP matrix.

The test containing 0.25g of PVP was approximately half as thick as the 0.5g test.

Using the oven to evaporate the 5.0ml solutions proved far more effective than using a rotary evaporator, which did not produce a solid of uniform depth, and failed to evaporate to complete dryness.

10ml volumes of deionised water were added to all the SPMTs which were then shaken and examined after 2 and 3 minutes.

The results are shown below.

<table>
<thead>
<tr>
<th>mass of PVP</th>
<th>mass of CaCl₂</th>
<th>extent of dissolution after 2 minutes</th>
<th>extent of dissolution after 3 minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.01</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>0.50</td>
<td>0.05</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>0.50</td>
<td>0.10</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>0.50</td>
<td>0.50</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>0.25</td>
<td>0.05</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>0.50</td>
<td>0.00</td>
<td>50%</td>
<td>70%</td>
</tr>
</tbody>
</table>

Following dissolution all solutions were colourless.

Addition of small masses of a salt such as calcium chloride, appear to slightly accelerate the dissolution process.

In an attempt to further reduce the time taken for the SPMTs to dissolve, they were removed from the oven when their volume had been reduced to approximately 0.75ml, and either ethanol or acetone was stirred into the paste, before they were evaporated to dryness.
Using the solvent failed to cause the PVP to foam, and the time taken for the tests to dissolve was not reduced.

Ethanol and acetone were used as hexane being immiscible with water remained on top of the paste and evaporated without being incorporated into the paste.

In an attempt to reduce the time taken for the PVP/reagent solutions to evaporate the oven temperature was increased to 72°C. Tests were evaporated to dryness in under 16 hours however they became pale yellow in appearance. On being dissolved with deionised water they remained pale yellow.

PVP may be used to contain reagents, for example those used in a colorimetric determination of a given cation, up to a mass ratio of 2.5:1, depending on reagent solubility. The reagents are not seen to form crystals on the surface of the polymer. The SPMTs are produced by evaporating aqueous solvent from a bulk liquid, at an optimum temperature of 55°C for a period of 48 hours.

The SPMTs produced, contain a mass of PVP no greater than 0.5g therefore giving an optical transmittance greater than 90%, after a standing time of 1 minute on completion of dissolution. Those containing 0.5g of PVP are dissolved completely in 10ml of water in a time under three minutes, those containing 0.25g of PVP require less than 2 minutes to dissolve completely. No blowing agents are required.

The soluble polymer matrix tests may be easily mass produced in commercially acceptable quantities, using a liquid production line system, however they may only be applied to those reactions requiring addition of reagents in a non specific order, as it is not possible to produce distinct layers by this evaporation method.

The soluble polymer matrix tests may be individually manufactured, if reactions require reagents to be layed down in specific orders, as described earlier.
7.0. Other species investigated with a view to producing a soluble polymer matrix test.

With a view to developing a soluble polymer matrix test (SPMT) for other species as well as aluminium, other species of environmental interest were investigated. The first of these species was molybdenum.

7.1. Reagents and equipment used.

All reagents were supplied by Fisons, Loughborough and were of Analar grade unless otherwise stated.

- Ammonium acetate
- Ammonium molybdate tetrahydrate
- Catechol (BDH Ltd)
- Diphenylcarbazone
- Dipotassium hydrogen orthophosphate
- Disodium EDTA (Lancaster synthesis Ltd)
- Ferric ammonium sulphate
- Ferrous ammonium sulphate
- Gallic acid
- Hydrochloric acid (0.2M)
- Iso butyl alcohol
- Phenyl acetic acid
- Polyvinyl pyrrolidone K30 (SAF Ltd)
- Potassium dihydrogen orthophosphate
- Pyrogallol (BDH Ltd)
- Sodium acetate
- Sodium hydroxide
- Sodium metabisulphite
- Sulphuric acid (0.1N)
- Tiron (disodium-1,2-dihydroxybenzene-3,5-disulphonate) (SAF Ltd)
- Xylenol Orange (BDH Ltd)

- Gallenkamp flask shaker
- Plastic microcuvettes
- Philips UNICAM 8700 UV spectrophotometer
7.2. Spectrophotometric and colorimetric methods of determining Molybdenum.

There are a large number of spectrophotometric methods described for the determination of molybdenum, generally as the molybdate, Mo(VI) species. A selection of these methods was chosen for further investigation and assessed on their ease of use, sensitivity, selectivity, colour difference between low Mo(VI) concentrations after complexing and the reagent toxicity.

The first method to be investigated used the previously investigated dye Xylenol orange.\(^1\)

A 0.05% w/v solution of this dye was used with a pH 1.5 hydrochloric acid/sodium acetate buffer and volumes of 1000\(\mu\)g ml\(^{-1}\) molybdate were added such that the final concentration in a 10ml volume ranged from 0.1 to 35\(\mu\)g ml\(^{-1}\). The solutions were left for 30 minutes before they were scanned for absorbance against a reagent blank at 560nm.

The complex colours varied from yellow in the blank and 0.1\(\mu\)g ml\(^{-1}\) tests to an orange colour in those tests containing greater than 15\(\mu\)g ml\(^{-1}\).

It was not possible to distinguish by eye between the colours formed by similar molybdate concentrations. The 0.5\(\mu\)g ml\(^{-1}\) test could just be distinguished from the 10\(\mu\)g ml\(^{-1}\) test.

A response curve, for concentrations 0 to 20\(\mu\)g ml\(^{-1}\) was plotted. see Fig 7. Once again a linear area is observed between 0 and 2.5\(\mu\)g ml\(^{-1}\), with a gradual loss in sensitivity at higher concentrations. The literature\(^1\) states that maximum colour intensity is observed at 65°C. This would be impractical for field use.

Catechol, provided another relatively straightforward method of determination. The literature\(^2\) was followed. 1.5 grams of sodium metabisulphite and 1.0 gram of catechol were dissolved in 50ml of 0.4% sodium hydroxide and the solution made up to 100ml with deionised water.

8ml of the prepared reagent, the pH of which was 6.48, was added to two mls of test solution containing molybdate concentrations 0.5 to 40\(\mu\)g ml\(^{-1}\). Colour was registered immediately. The solutions were then scanned at 400nm against a reagent blank. A response curve was drawn, with a sensitivity of 0.069 and intercept of 0.011.
There was reasonable colour difference between the sets of tests. This colour ranged from pale yellow with the 0.5 and 1.0μg ml⁻¹ concentrations through to orange in the 10 and 15μg ml⁻¹ tests and red/orange in the 40μg ml⁻¹ test.

The Mo(VI) catechol complex appeared to be stable for over 24 hours. However the catechol reagent needed to be made fresh every day due to the oxidation of sodium metabisulphite.

A method using pyrogallol was adapted from the literature. With a view to producing a dry reagent test, phenyl acetic acid/sodium hydroxide buffer of pH 4.4 was used in placed of the standard acetic acid/ acetate buffer. Concentrations of Mo(VI), ranging from 0.1 to 10μg ml⁻¹ were made up in 5ml of buffer, and 10μl of a 0.1% w/v solution of pyrogallol in 0.05M phenylacetic acid was added.

The tests were then scanned for absorbance after 60 minutes, at 545nm against a reagent blank. A response curve was drawn. see fig 7a, once again greater sensitivity is observed at lower concentrations.

It was possible to visually distinguish between those solutions containing less than 1μg ml⁻¹, as the colour ranged from a blank of colour pink/orange to pale purple with the 1μg ml⁻¹ test. Mo(VI), however with Mo(VI) concentrations greater than this all solutions were purple and could not be distinguished by eye.

Using a spectrophotometer the complex appeared to be stable for 2 hours.

Another reagent to be investigated was the catechol derivative tiron (disodium-1,2-dihydroxybenzene-3,5-disulphonate).

A pH 7.0 buffer was made up using potassium dihydrogen orthophosphate and dipotassium orthophosphate, both in 0.1M concentrations. A 10% w/v solution of tiron was made up in this buffer. Concentrations of Mo(VI) were made up in 5ml volumes of deionised water. 1ml of buffer and 1ml of the tiron solution were added and the volume made up to 10ml such that the final Mo(VI) concentration ranged from 0.1 to 20μg ml⁻¹.

The solutions were scanned for absorbance at 390nm against a reagent blank after 10 minutes. A response curve was drawn fig 7b, again a linear area showing a high degree of sensitivity can be seen at low concentrations, whereas at concentrations greater than 5μg ml⁻¹ sensitivity is almost lost.
All the concentrations were easy to visually distinguish, ranging from darkening shades of yellow with increasing concentration, to orange in the 10μg ml⁻¹ test.

Other methods that were investigated used the reagents gallic acid,⁵ and diphenylcarbazone⁶, however although reasonable results were obtained both reagents required an ethanolic solvent and were practically insoluble in aqueous solutions, therefore they were not pursued further.

7.3. Investigation of the tiron test.

As the tiron method of molybdate determination appeared straightforward, had high sensitivity at low concentrations and good visible colour distinction between molybdate concentrations, it was selected as that method which would be most suitable for further investigation.

7.3a. Interferences.

According to the literature⁴ certain cations interfere with the test by producing a coloured complex. These ions were Fe(III), Fe(II), OsO₄²⁻, Cu²⁺, UO₂²⁺, VO²⁻ and Ti⁴⁺. However at the pH used for Mo(VI) determination copper ions do not interfere. It is unlikely that any of the ions apart from iron would be found in significant amounts in environmental samples and therefore these were not investigated during the course of this work.

When the experiment was repeated with iron(III) concentrations of 1 and 10μg ml⁻¹. A pale pink solution and a blood red solution were observed, the iron(III) tiron complex showed a peak maximum at 470nm when scanned for absorbance against a reagent blank.

When a solution containing 1μg ml⁻¹ of both iron(III) and Mo(VI) was used in the tiron reaction a yellow solution was produced the absorbance of which, when measured at 390nm was equivalent to that obtained previously with a 2.5μg ml⁻¹ Mo(VI) concentration. An absorbance increase of approximately 5%.
A 10μg ml\(^{-1}\) Mo(VI) concentration failed to mask any of the colour produced by the 10μg ml\(^{-1}\) iron(III) concentration.

A 10μg ml\(^{-1}\) concentration of iron(II) also gave a red complex with tiron, the maximum absorbance of which was at 484nm.

To remove the iron interferences EDTA was used. The reaction was repeated with the exception of a 3ml volume of 1M EDTA being used in place of a 3ml volume of deionised water. The 10μg ml\(^{-1}\) iron(III) and iron(II) concentrations gave no colour change and no absorbance at 470 and 484nm. When this concentration of EDTA when added to a 1μg ml\(^{-1}\) Mo(VI) concentration the absorbance obtained at 390nm was only 34% of the value obtained for this concentration when no EDTA was used.

Experiments were carried out to reduce the amount of EDTA required to remove the 10μg ml\(^{-1}\) iron interferences. This limiting concentration of EDTA was found to be 74mg, i.e. 200μl of a 1M solution. Using this concentration of EDTA the absorbance of a 1μg ml\(^{-1}\) Mo(VI) concentration was similar to that obtained without EDTA addition.
Fig 7, Response of xylenol orange to Mo(VI), the sensitivity of the initial portion of the curve 0 to 2.5 µg ml\(^{-1}\) is 0.01µg\(^{-1}\) ml, from the linear regression \(y = 0.0104x + 0.00387\). Note the low absorbance.

Refer to section 7.2.

There was insufficient data to include error bars.
Fig 7a, Response of pyrogallol to Mo(VI)

The region below 1 μg ml⁻¹ has a sensitivity of 0.041 μg⁻¹ ml, from the linear regression 
y = 0.0411x - 0.00164.

Refer to section 7.2.
Fig 7b, Response of tiron to Mo(VI)

The sensitivity between 0 and 2.5 μg ml⁻¹ is 0.227 μg⁻¹ ml, from the linear regression

\[
y = 0.2268x + 0.1847.\]

Standard error bars of +/-5% are shown, appropriate to the data obtained.

Refer to section 7.2.
7.4. Sensitisation of the Tiron method by Polyvinyl pyrrolidone (PVP) K30.

It was observed in the alizarin red S aluminium reaction (section 6.5) that addition of a solution of PVP resulted in an increase in the intensity of the complex colour, combined with a bathochromic shift in absorbance. Experiments were carried out on the tiron Mo(VI) complex to investigate any similar effects.

A 5% w/v PVP K30 solution was made up in deionised water. To a 5ml volume of 2.5μg ml⁻¹ Mo(VI), was added 1ml of pH 7.0 buffer, 1ml of the tiron solution and varying volumes of 5% PVP solution, the final volume was made up to 10ml with deionised water. The absorbance at 390nm was determined after 10 minutes and the 2.5μg ml⁻¹ Mo(VI), PVP containing solutions were compared with the previous 2.5μg ml⁻¹ test containing no PVP. The results are shown in fig 7c.

From these results it appeared that the PVP increases the absorbance of the Mo(VI) tiron complex. The PVP test was also more strongly yellow coloured. This colour intensity was visibly similar for all PVP concentrations.

A PVP concentration of 0.75% w/v, (1.5ml of a 5% w/v solution in a 10ml volume) was selected for further investigation. A tiron calibration was carried out as before with this concentration of PVP added. The intensity of the complex colour, ranging from pale yellow to orange, was visibly greater than previously obtained. A response curve was plotted fig 7d, using concentrations 0 to 5μg ml⁻¹. From this plot, the sensitivity of this test is indicated.

7.5. A soluble polymer matrix test for Molybdenum.

Polystyrene microcuvettes, volume 2.5ml, were used. A paste was made up using PVP K30 powder, 3.80μl of pH 7.0 buffer, 1ml tiron solution and 200μl of EDTA 1M solution. The paste was stirred to a uniform consistency and then quantities of this paste of equal volume, judged visibly, and approximate mass 0.3g, were transferred to the microcuvettes.

These SPMTs were then stored for 24 hours in the fridge. To sets of 8 SPMTs was added 1ml volumes of Mo(VI) solutions of concentrations ranging from 0.5 to 5.0μg ml⁻¹.
The solution was stirred into the SPMT using a small glass rod. The absorbance of the solutions was determined at 390nm after 60 minutes from solution addition. Table 7 below indicates the absorbance values and complex colours obtained.

Table 7, mean absorbance of molybdate SPMTs.

<table>
<thead>
<tr>
<th>Mo (VI) conc. µg ml⁻¹</th>
<th>colour</th>
<th>mean abs. 390nm after 60 mins</th>
<th>std.dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>colourless</td>
<td>0.002</td>
<td>0.004</td>
</tr>
<tr>
<td>0.5</td>
<td>pale yellow</td>
<td>0.034</td>
<td>0.006</td>
</tr>
<tr>
<td>1.0</td>
<td>yellow</td>
<td>0.096</td>
<td>0.011</td>
</tr>
<tr>
<td>2.5</td>
<td>deep yellow</td>
<td>0.286</td>
<td>0.019</td>
</tr>
<tr>
<td>5.0</td>
<td>orange/yellow</td>
<td>0.551</td>
<td>0.026</td>
</tr>
</tbody>
</table>

A response curve was plotted, **fig 7e.** The sensitivity of the test, from this plot, is indicated.

### 7.6. Extraction of Molybdenum from soil samples.

As previously discussed, molybdenum is an essential trace element in soils. The tiron test was investigated for its ability to determine molybdenum in soil extracts.

A soil sample was collected and dried in an oven at 40°C. The soil was then crushed into fine particles with a pestle and mortar, and was weighed out as 5g masses. Aliquots of a 1M solution of ammonium acetate were added to the 5g soil samples such that the final weight to volume ratios were 1:5 and 1:10. These samples were then shaken for 5 minutes on a flask shaker.

The solutions were then filtered and the yellow filtrates stored overnight in the fridge. Solutions of the filtrates were then spiked with 0.1, 0.5, 1.0, 2.5 and 5.0µg ml⁻¹ concentrations of Mo(VI).

A large batch of SPMTs were made up as previously described and were stored for 24 hours in the fridge. To these SPMTs was added 1ml of each of the above samples, which were stirred into the paste. The tests were done in duplicate. The SPMTs registered colour almost immediately, and were scanned for absorbance at 390nm.
against a reagent blank. The absorbance scan of a 2.5µg ml⁻¹ Mo(VI) spiked 1:5 w/v soil sample filtrate is shown as fig 7f.

The mean absorbance and colours of the 1:5 and 1:10 samples are shown in the tables 7a,b below.

Table 7a, showing the colour and absorbance at 390nm of spiked 1:10 soil/solvent samples

<table>
<thead>
<tr>
<th>Mo(VI) conc (\mu g , ml^{-1})</th>
<th>colour</th>
<th>mean absorbance at 390nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>grey/colourless</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>pale yellow</td>
<td>0.00</td>
</tr>
<tr>
<td>0.5</td>
<td>pale yellow</td>
<td>0.045</td>
</tr>
<tr>
<td>1.0</td>
<td>yellow</td>
<td>0.196</td>
</tr>
<tr>
<td>2.5</td>
<td>deep yellow</td>
<td>0.704</td>
</tr>
<tr>
<td>5.0</td>
<td>orange</td>
<td>2.065</td>
</tr>
</tbody>
</table>

Table 7b, showing the colour and absorbance at 390nm of spiked 1:5 soil/solvent samples.

<table>
<thead>
<tr>
<th>Mo (VI) conc (\mu g , ml^{-1})</th>
<th>colour</th>
<th>mean absorbance at 390nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>colourless</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>pale yellow</td>
<td>0.155</td>
</tr>
<tr>
<td>0.5</td>
<td>pale yellow</td>
<td>0.171</td>
</tr>
<tr>
<td>1.0</td>
<td>yellow</td>
<td>0.413</td>
</tr>
<tr>
<td>2.5</td>
<td>deep yellow</td>
<td>0.821</td>
</tr>
<tr>
<td>5.0</td>
<td>orange</td>
<td>1.484</td>
</tr>
</tbody>
</table>

Response curves were drawn, using these mean absorbances, fig 7g. From these crude plots it can be interpreted that using a 1:10 soil:solvent allows for greater sensitivity in these tests.
7.7. Conclusions.

Attempts have been made to develop a soluble polymer matrix test for the determination of molybdenum as molybdate using the reagent tiron. Molybdenum concentrations as low as 0.05 to 0.1 \( \mu g \) ml\(^{-1}\) can be seen by a simple colour change procedure. There is good differentiation between the colour of the complex produced by different concentrations (seen by the same panel of 5 independent observers) and it is possible to visibly discriminate between low molybdenum concentrations. The soluble polymer matrix tests can also be determined spectrophotometrically by measuring the absorbance of the test solution at 390 nm against a reagent blank. Interferences from iron(III) and iron(II) are removed using EDTA. Although interferences have not been subject to a thorough analytical study. It is hoped that the SPMT may be used to determine molybdenum in soil extracts, following the preliminary investigations. The tiron molybdate complex forming reaction has been sensitised by polyvinyl pyrrolidone.

7.8. References.

Effect of addition of varying concentrations of PVP on the absorbance at 390nm of a 2.5µg ml⁻¹ concentration of Mo(VI) following complexation with tiron.

Refer to section 7.4. Sensitisation of the tiron method by PVP.
Fig 7d, PVP sensitised tiron response to Mo(VI)

The curve sensitivity is $0.468 \mu g^{-1} ml$, from the linear regression $y = 0.468x - 0.01277$.

Refer to section 7.4.
Fig 7e, Response of SPMTs to Mo(VI)

The sensitivity of the curve is $0.113 \mu g^{-1} ml$, from the linear regression $y = 0.113x - 0.00766$.

Error bars shown, calculated from one standard deviation.

Refer to section 7.5.
The absorbance scan of a 2.5μg ml⁻¹ Mo(VI) spiked 1:5 soil sample filtrate

refer to section 7.6. Extraction of molybdenum from soil samples
SPMT response to Mo(VI) contained in soil extracts

![Graph showing response of SPMTs to Mo(VI) spiked soil extracts]

Fig 7g, Response of SPMTs to Mo(VI) spiked soil extracts

The sensitivity of the curve produced using the 1:10 extract, 0.41μg/ml, from the linear regression $y = 0.413 - 0.257x$, is greater than that from the 1:5 extract, 0.29μg/ml, from the linear regression $y = 0.287x + 0.0847$.

Points shown are the mean of two absorbance readings, all of which correlated well, and hence using this number of data values, error bars would not give an accurate indication of the errors associated with a greater number of test results.

Refer to section 7.6.
8.0. Development of a Field test for Silver.

8.1. Reagents and equipment used.

All reagents were supplied by Fisons, Loughborough and were of Analar grade unless otherwise stated.

Acetic acid
Acetone
Aluminium nitrate
Ammonium acetate
Buffer tablets pH 7.0 phosphate
Bromopyrogallol Red (BPR) (BDH Ltd)
Calcium chloride
Copper nitrate
Ferric ammonium sulphate
Ferrous ammonium sulphate
Lead nitrate
Magnesium nitrate
1,10 Phenanthroline (1,10 phen) (SAF Ltd)
P-dimethylaminobenzalrhodamine
Potassium dichromate
Potassium hydrogen phthalate
Silver nitrate
Sodium chloride
Zinc nitrate

Philips UNICAM 8700 UV spectrophotometer
8.2. Determination of a suitable method for silver detection.

Compared to the other species investigated there are few methods in the literature for the spectrophotometric and colorimetric determination of silver. However three methods were chosen from the literature with a view to producing a soluble polymer matrix test.

The first method selected involved the use of potassium dichromate,$^1$ the principle being a silver solution would form a red precipitate of silver dichromate. This method was however unsuccessful in determining concentrations of silver at the $5\mu g \text{ ml}^{-1}$ level. 0.1g of p-dimethylaminobenzalrhodamine,$^2$ a red solid, was dissolved in an acetone:water, 1:4 mix. The resulting solution was filtered to leave an orange solution. Concentrations of silver, 0.05 to $5\mu g \text{ ml}^{-1}$ in 5ml volumes of water were added to 1ml volumes of the dye solution. No colour change was seen in those solutions of concentration less than $0.25\mu g \text{ ml}^{-1}$, the other concentrations developed a reddish tint. When these solutions were scanned for absorbance between 400 and 700nm against a reagent blank, a small peak was seen at approximately 545nm, this peak shifted to 560nm with solutions of lower concentration. This method was not pursued further. Greater success was had with the method of Dagnall and Deguti.$^3$ One drop each of 0.1M EDTA, 0.001M 1,10 phenanthroline, 20% ammonium acetate and 0.1mM bromopyrogallol red are added to the test solution, a blue colour indicates the presence of silver. A calibration was attempted using 1ml of test solution containing silver, concentration range 0.05 to $10\mu g \text{ ml}^{-1}$. The other reagents were added in 10μl aliquots. On addition of the 1,10 phenanthroline, colour was registered immediately.

*Table 8,* over, indicates colours obtained with this concentration range of silver.
<table>
<thead>
<tr>
<th>Ag Conc $^a$ (μg ml$^{-1}$)</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>pink</td>
</tr>
<tr>
<td>0.05</td>
<td>pink</td>
</tr>
<tr>
<td>0.10</td>
<td>pink/purple</td>
</tr>
<tr>
<td>0.25</td>
<td>purple/pink</td>
</tr>
<tr>
<td>0.5</td>
<td>purple</td>
</tr>
<tr>
<td>1.0</td>
<td>blue/purple</td>
</tr>
<tr>
<td>2.0</td>
<td>aqua blue</td>
</tr>
<tr>
<td>5.0</td>
<td>pale aqua blue</td>
</tr>
</tbody>
</table>

All solutions were scanned for absorbance between 300 and 700nm against a reagent blank. With those solutions containing less than 2μg ml$^{-1}$ silver a peak at 637nm was observed with a shoulder peak at 608nm. At greater silver concentrations the peak at 637nm disappeared and the peak at 608nm was dominant. Fig 8 shows the spectra of the 0.5 and 5μg ml$^{-1}$ silver concentrations. Response curves were drawn for both wavelengths, fig 8a. The sensitivities of these curves is indicated.

### 8.3. Order of reagent addition in the BPR/1,10 phenanthroline silver determination.

The literature$^3$ states the order of addition of reagents is unimportant, using bromopyrogallol red concentrations of 0.1 and 0.5mM this was found to be true. However when concentrations greater than 0.5mM were used it was observed to be essential to add the 1,10 phenanthroline after the bromopyrogallol red, no colour was seen if the reagents were added the other way round, the large excess of BPR possibly complexing the silver ions and masking the colour formed from the [phen-Ag-phen]$^+$BPR$^{2-}$ ion association system.
8.4. Complex destruction by anion addition.

Whilst increasing the quantity of acetate buffer in the reaction it was found that no characteristic complex colour was forming. Increasing the volume of 20% w/v ammonium acetate from 200 to 500\mu l, resulted in no colour change being observed, and this was reflected in the absorbance spectra in the region 600 to 650nm. Substituting the ammonium acetate (approximate pH 7.5) for a pH 7.0 phosphate buffer, resulted in a similar situation. Addition of 0.05g of PVP to 1ml of 0.5\mu g ml\(^{-1}\) silver solution before reagent addition markedly reduced the absorbance at 637 and 608nm, no colour change was observed. Addition of 0.2g of PVP to the reaction completely inhibited complex formation and no absorbance was observed between 600 and 650nm, however addition of these masses of PVP to a 0.5mM solution of bromopyrogallol red only produced a bathochromic shift in absorbance from 557nm to 573nm.

8.5. Effect of interferences.

To investigate the effect of cation interference on the reaction, 5\mu g ml\(^{-1}\) concentrations of the zinc, copper, lead, aluminium, calcium, iron(II), iron(III), magnesium and sodium ions were added separately to a test solution containing 0.5\mu g ml\(^{-1}\) silver. The dye and buffer reagents were then added. All cations except calcium, iron(II) and iron(III) produced a blue complex, similar in colour to that obtained with a 0.5\mu g ml\(^{-1}\) concentration of silver only. The calcium, iron(II) and iron(III) ions produced violet, orange and green complexes respectively. However when all the complexes were scanned for absorbance between 400 and 750nm, only the iron(II) complex, with a peak at 509nm, was different from the 0.5\mu g ml\(^{-1}\) silver only test. The 0.5\mu g ml\(^{-1}\) silver concentration appeared to be effective in spectrophotometrically masking the absorbance from the complexes formed by calcium and iron(III).

Attempts were made to remove calcium and iron(II) interference using EDTA. According to the literature\(^5\) the optimum pHs for complexation of these cations are 7 and 5 respectively. A 15\mu l aliquot of 1M EDTA was sufficient to remove interference from a 50\mu g ml\(^{-1}\) calcium interference. However, 500\mu l of this concentration failed to completely eliminate the iron(II) interference, visibly or spectrophotometrically.
8.6. Conclusions.

A reaction from the literature for the colorimetric and spectrophotometric detection of silver has been investigated with a view to producing a soluble polymer matrix test. It was discovered that this reaction would be of limited use as a SPMT due to masses of PVP as low as 50mg destroy the complex. It was also found that the order of reagent addition is important when the bromopyrogallol red concentration exceeds 0.5mM. Excess acetate and anions in general were found to destroy the complex. This phenomena is thought to be due to the complex being a stoichiometric compound formed by an ion association between BPR$^-$ and [phen-Ag-phen]$^+$. Hence addition of other anions such as acetate or phosphate provides competition with the BPR$^-$ in setting up the ion association system.

The blue colour of the ion association system is different from that of the white Ag(phen)$_2$ ion and the red BPR$^-$ ion, due to charge transfer between the oxidisable silver(I) ion in the [phen-Ag-phen]$^+$ and the reducible quinoid group in the bromopyrogallol red ion.$^3$

8.7. References.

5. West, T.S., *Complexometry*, 3rd ed,
Fig 8. Absorbance spectra of 0.5\( \mu \)g ml\(^{-1}\) and 5.0\( \mu \)g ml\(^{-1}\) silver concentrations following reaction with BPR and 1,10 phen. Reference: reagent blank.

refer to section 8.2. Determination of a suitable method for silver detection.
Fig 8a. The response of BPR/1,10 phen to silver at two different wavelengths.

Refer to section 8.2
9.0. Other Reagent Delivery Systems.


Capillary fill devices\(^1\)\(^2\) simply consist of two glass slides, such as those used for microscopy, separated by a distance, usually 50μm, one on top of the other. The top plate is shorter than the base plate in order that solutions containing the target analyte may be placed on the open region of the base plate. The top surface of the base plate has reagents dried down in discreet bands, in the required order for a reaction to take place. When the solution containing the target analyte is placed on the base plate it is drawn between the air gap by capillary forces, and encounters the dry reagent bands in a specific order. The complete reaction, often indicated by the formation of a coloured complex, may easily be seen by the eye and compared with a pre-prepared colour standard. The glass capillary fill device may also be sealed at one end and easily inserted into a UV spectrometer, for more accurate analysis. Advantages of this device include, they are inexpensive to manufacture, disposable and non or little sample preparation is required.

9.2. Capillary fill devices for aluminium detection.

The reagents used to detect aluminium previously were now applied to capillary fill devices.

Three substances were tried as separators of the two microscope slides used to make up the device.

i. Ballotini beads.

These glass beads are approximately 50μm in diameter and when mixed with glue and used to seal the plates gave an excellent water proof wall of uniform separation, measured with a micrometer screw gauge. However the nature of the glue meant that these devices could not be reused, and they were also time consuming to manufacture.
ii. 'Teflon' PTFE tape.
'Teflon' PTFE tape was simple to use and gave excellent uniform separation and water proof sealing, however the plates required a weight on the top to prevent them from being easily pulled apart as the tape is non adhesive.

iii. 3M Double sided adhesive tape.
Double sided adhesive tape was by far the most effective plate separator, being inexpensive, very adhesive and uniform in thickness, the thickness of the separation could be easily altered by using more or fewer strips of the tape, and the devices could be used more than once.
Double sided adhesive tape was the medium used for separating slides for all experiments.

9.3. The Quinalizarin test.

The reagents used previously for the determination of aluminium, see sections 3, 4 and 5, were applied to the capillary fill device experiments.
A band of pH 5.6 potassium hydrogen phthalate/sodium hydroxide buffer was dried onto the top of the base plate using a micropipette and a hot air gun. A band of 0.1% w/v ethanolic quinalizarin was also placed down on the plate, The two slides were sealed as described using double sided sticky tape.
A neutral solution of 10ug ml⁻¹ aluminium in deionised water was placed on the bottom plate such that it was drawn into the device.

Results and observations.

The aluminium solution filled the gap, on reaching the brick red quinalizarin band the dye immediately turned violet, characteristic of quinalizarin in alkaline medium.
The buffer appears not to be able to hold the pH at 5.6.
After the majority of reagents had dissolved (quinalizain has a much higher solubility in ethanol than water and hence the mass of dye dried down from ethanolic solution will not fully dissolve again in aqueous solution). The colour of the solution in the gap was still violet.
The bands were then separated to the maximum distance possible and the experiment was repeated. No difference was observed.

Sodium lauryl sulphate was added to the buffer 0.1%w/v in the hope that as a surfactant it may assist in dissolving the phthalate quicker, this too proved unsuccessful.

The quinalizarin was also particularly difficult to dissolve in aqueous solution without excessive agitation, and the time taken for a reasonable amount to dissolve was beyond the time frame boundary laid down for a successful test.

9.4. The Xylenol Orange test.

Separate distinct bands of a 1%w/v xylenol orange solution (5%v/v ethanol) and phenyl acetic acid as the buffer were dried down onto the top of the base plate using a micropipette and a hot air gun, and the slides were sealed as before using double sided sticky tape.

Aluminium spiked solutions of deionised water of concentrations 1, 2 and 5μg ml⁻¹, were introduced into the gap by capillary forces.

Results and observations.

All the three solutions gave a yellow colour similar to that obtained with deionised water only. However after 30 minutes, a pale but distinct orange tint was seen in all three tests.

A 500μg ml⁻¹ solution of ascorbic acid was dried down between the buffer and dye bands. As this was the concentration required in the wet method to remove the interference from a 50μg ml⁻¹ iron(III) solution. The experiment was repeated using the same concentrations of aluminium in tap water. A similar result was obtained, the orange tint requiring approximately 30 minutes to form.

This period of time required for colour development was probably due to the time required for the phenyl acetic acid to dissolve without agitation as it has a relatively low solubility. The final pH of the test was approximately 3.1. Rather low for full colour development for the test (3.8 stated in the literature) however by omitting the
sodium hydroxide or acetate, problems associated with dissolution rates within the buffer and formation of inconvenient masking colours due to alkaline solution of the dye were removed.

9.5. The Alizarin red S test.

Separate, distinct bands of pH 4.6 phenyl acetic acid/sodium hydroxide buffer, and a 1% w/v alizarin red S solution made up in this buffer were dried down on the base plate of the capillary fill device. The dye dried a purple colour characteristic of an alkaline medium, due to the presence of the sodium hydroxide, which being hygroscopic, absorbed water from the atmosphere.

Results and observations.

Introduction of deionised water to this system dissolved the dry reagents, and after a period of approximately 20 minutes, (2 minutes with agitation) the purple colour became yellow.

Aluminium spiked solutions of deionised water, concentration range 1 to 5 µg ml\(^{-1}\) were introduced into the gap and compared with the blank. The 5 µg ml\(^{-1}\) test showed some orange colour, and a hint of orange could also be recognised in the 1 and 2 µg ml\(^{-1}\) tests.


To attempt to increase the visibility of the complex colour, a micro cavity was drilled into the base plate, in the hope that this might concentrate the dye colour into a much smaller area. This cavity was filled with 5 µl of the 1% alizarin solution and dried with a hot air gun. Using the same aluminium solutions, the complex colour formed did indeed stay around the mouth of the cavity. However the dye took 20 minutes to dissolve to a good extent. After this time period an orange ring around the cavity was clearly visible in the 1 µg ml\(^{-1}\) test, which gradually dissipated into the yellow dye colour of the main solution.
A 500μg ml⁻¹ concentration of sulphate was introduced into the buffer solution and this new solution was dried down onto the base plate. Aluminium spiked tap water solutions were added, the orange ring was still visible around the micro cavity, however concentrations of aluminium lower than 1μg ml⁻¹ produced a colour which was not easily distinguishable from the blank, when observed with an untrained eye.

9.7. Conclusions.

Capillary fill devices have been used to produce two tests capable of determining aluminium at levels around 1μg ml⁻¹ in tap water, using the dyes Alizarin red S and xylenol orange, the tests are straightforward and cheap to manufacture, and the apparatus may be dismantled after use and reused. Interference from calcium may be removed with sulphate. The test, however require a time period of 15 to 20 minutes for full colour development, less if agitation is used.

Capillary fill devices showed some signs of success, however their sensitivity, 1\( \mu \text{g m}^{-1} \), was approximately a factor of ten too low to be of use for monitoring real environmental levels, and the time taken for full colour formation was also too long. In an attempt to improve on the capillary fill device work, dry reagent tubes, a more simple approach, was attempted. Dry reagent tubes simply consisted of a standard style sample tube containing the dry reagents required for the aluminium test dried down in the appropriate amounts. A sample containing the target analyte is then introduced into the tube which is sealed and shaken until the reaction is complete, the colour of the tubes contents can then be compared with a preprepared set of colour standard, or inserted into a UV spectrometer for more accurate comparison. The visible sensitivity of the test colour change should in theory be increased as the eye is looking down the tube and hence along a longer pathlength. A white background should also improve this sensitivity.

9.9. Experimental.

A large number of small sample tubes were cut down to a depth of 1 cm, in order that reagents could be easily dried down onto the base of the tube with a hot air gun. A 5 \( \mu l \) aliquot of a 1\%w/v aqueous solution of alizarin red S was dried down onto the base of the tube as a yellow stain. A 50 \( \mu l \) aliquot of the pH 4.60 phenyl acetic acid/sodium hydroxide buffer, containing 500 \( \mu \text{g m}^{-1} \) sulphate, in order to remove calcium interference, was dried down on top of the dye. The stain was now purple due to the presence of hygroscopic sodium hydroxide. Aluminium spiked solutions of deionised water and tap water were then pipetted into the tube in 100 \( \mu \text{l} \) aliquots, and the tube shaken until the reagents dissolved.
9.10. Results and observations.

Using both tap and deionised waters there was a good difference between the colour of the tests, the blank in the tap water test was very slightly darker in yellow colour than the deionised water test. Table 9 below indicates the colour changes that were obtained.

<table>
<thead>
<tr>
<th>Aluminium conc (µg ml⁻¹)</th>
<th>colour</th>
<th>mean absorbance at 500nm</th>
<th>std.dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>yellow</td>
<td>0.001</td>
<td>0.00</td>
</tr>
<tr>
<td>0.1</td>
<td>yellow/orange</td>
<td>0.021</td>
<td>0.001</td>
</tr>
<tr>
<td>0.5</td>
<td>orange/yellow</td>
<td>0.141</td>
<td>0.011</td>
</tr>
<tr>
<td>1.0</td>
<td>orange</td>
<td>0.236</td>
<td>0.016</td>
</tr>
<tr>
<td>2.0</td>
<td>orange</td>
<td>0.291</td>
<td>0.021</td>
</tr>
<tr>
<td>5.0</td>
<td>deep orange</td>
<td>0.698</td>
<td>0.043</td>
</tr>
</tbody>
</table>

The 0.1µg ml⁻¹ test was distinguishable from the blank in all ten tests carried out.

The test was repeated on a larger scale, using aluminium spiked samples of deionised water. The samples were scanned for UV absorbance at 500nm. The results are also shown in table 9. From the results obtained a response curve was plotted. fig.9 Error bars of +/-12% for 95% confidence limits are shown.

9.11. Conclusions.

A method whereby all the reagents for a determination are dried onto the base surface of a sample tube, has been used to determine aluminium, by a visual colorimetric method, at levels as low as 100 parts per billion (0.1µg ml⁻¹) in real samples. The increased path length of observation did indeed increase the sensitivity from that seen with capillary fill devices. The tests are cheap and easily mass produced, and due to the air tight nature of the storage tube may be stored for several months before use.

Fig 9  The response of the dry reagent tubes containing alizarin red S, to aluminium spiked samples of deionised water. The absorbance of the complex was measured at 500nm.

Error bars are shown for the standard deviations obtained.

Refer to section 9.10
10.0 Lead in the Environment.

10.1 Uses and Pathways into the body.

Lead occurs in highly concentrated ores principally as lead sulphide from which it is easily smelted, the amount of lead used ranks fifth behind aluminium, copper, iron and zinc and it is produced in much larger quantities than any other toxic metal.

Lead has a melting point of $330^\circ$C and an atomic weight of 82, it exhibits the oxidation states 0, +2 and +4. Natural lead is a mixture of 4 isotopes $^{204}$Pb, $^{206}$Pb, $^{207}$Pb, $^{208}$Pb the latter being the most common.

Most people are familiar with lead as a pollutant however it has proved itself a tremendously useful chemical species for thousands of years.

Use of lead in the UK is dominated by the production of storage batteries for cars and electric vehicles accounting for over 35%, other uses include pigments and paints (13%), glass, ceramics and plastics (12%), roofing, ammunition, pipes etc (10%), antiknock additives for petrol (2%), alloys (5%) and miscellaneous uses including radiation shields and weights.

Nearly everyone is familiar with leaded petrol, tetramethyl and tetraethyl lead are used as antiknock additives in fuel to aid smooth ignition by matching the engine's compression ratio to the octane rating of the petrol, lead also helps to help keep an engine's valve seals lubricated avoiding sinkage. Emission of lead from petrol vehicles depends on several factors including traffic density, driving patterns and fuel efficiency. With the introduction of legislation reducing and eliminating lead from petrol, and the rise in catalytic converters which are damaged by leaded petrol, on all new cars, the extent of pollution from leaded petrol additives has fallen in recent years.

The lead emitted from combustion engines is generally concentrated in the surface soil up to 2 metres away from the roadside often in levels as high as $800\mu$g g$^{-1}$, and falls away down to levels around $500\mu$g g$^{-1}$ at distances of 100 metres.$^1$

This lead is usually in the form of inorganic oxides, carbonates and halides, however soils generally have large capacities for immobilising lead due to the chelating properties of organic matter and the soil pH and type hence this lead is not readily taken up by plants and transfer to edible plant tissue is limited.$^{2,3}$
The contamination of roadside crops is primarily due to surface deposition rather than soil uptake.\textsuperscript{4,5}

Aside from the combustion of leaded petrol, other sources of airborne lead include smelting, waste incineration and natural sources such as windblown dust and volcanoes. Approximately 5\% of all airborne lead is said to be from natural sources.\textsuperscript{6}

In the absence of a major source of lead pollution it may be assumed that the major source of lead for man comes from the diet.\textsuperscript{7}

As already mentioned lead in crops results from fallout onto leaves and by build up of lead in surface soils available for uptake, fallout onto surface waters may be taken up by fish and may also increase the concentration in the municipal water supply.

The limits of concentration for lead in food have fallen over recent years to a level of 1µg g\textsuperscript{-1} for fresh and tinned foods and 0.2µg g\textsuperscript{-1} for infant foods.

The proportion of lead absorbed across the gut wall following ingestion is taken as 10\%, however this figure is probably closer to 5\%.\textsuperscript{8}

Concentrations of lead in food are generally less than those in human blood and due to the variability of the matrix it is difficult to determine accurately the concentration in foods.\textsuperscript{9} and hence there is often a tendency to overestimate the quantities.\textsuperscript{10}

High levels of lead have been found in alcoholic drinks especially beers often more than 10µg dm\textsuperscript{-3}, probably due to storage and dispensing contamination this is concerning as lead in a liquid matrix is absorbed more readily,\textsuperscript{11} hence drinking water can also be a major source of lead intake.

Lead can enter the water cycle by fallout, industrial effluents, runoff water from highways and from rivers flowing through mineralised areas.\textsuperscript{12} The concentration of lead in public supply water very rarely exceeds 10µg dm\textsuperscript{-3}.\textsuperscript{13} Lead pipes are no longer used in modern construction, however lead may leach out from soldered copper pipes and PVC pipes where lead stearate has been used as a stabiliser.\textsuperscript{14}

The WHO guidelines for lead limits in drinking water is taken as 50µg dm\textsuperscript{-3}.\textsuperscript{15}
10.2. Health effects of Lead

The absorption and retention of lead in adults has been studied extensively,\textsuperscript{16} however as experiments on children are ethically prohibited, little data has been gathered on these groups.

The main pathways by which lead can enter the body are inhalation and ingestion. Not all lead inhaled reaches the lungs, due to their diameter (1-10μm) some particles are trapped by mucous in the respiratory tract and transferred to the oesophagus. Experimental studies have given a figure for deposition between 30 and 50% depending on particle size and ventilation.\textsuperscript{17-19}

Virtually all the lead that gets deposited in the lower respiratory tract is absorbed,\textsuperscript{20} and this has been proved by the absence of such lead in post mortem studies.\textsuperscript{21} It is thought that this deposition rate is upto 2.5 times higher in children.\textsuperscript{22}

As already mentioned a figure between 5 and 10% is given as the proportion of lead absorbed by the adult gut. As expected the factors affecting absorption include particle size, solubility, amount of food ingested with the lead, and water lead levels. As found with aluminium the presence of citrate increases lead absorption as does the presence of gut affecting species such as ethanol. However increased dietary minerals such as calcium, phosphate and iron limit absorption, calcium also reduces the excretion of lead.

Approximately 99% of blood lead is trapped within the erythrocytes (red blood cells) where it is bound to proteins.

The other 1% is transported in plasma fluid to the various organs of the body.\textsuperscript{23}

The half life of lead in blood is 25 to 30 days,\textsuperscript{24} lead not excreted by the renal system is transported to soft tissues where the half life is several months, however over 90% of body lead is stored as phosphates in bone\textsuperscript{25} where the half life is 20 years. It appears to be harmless in this form but may be released with time.\textsuperscript{26}

Lead serves no useful purpose as a trace element in any organism.
The effects of low levels and early toxicity of lead in man are characterised by anaemia where the lead affects globin synthesis and kills red blood cells. The threshold for this effect is vague but thought to be in the region of 1.1μg of lead per ml of blood.27 Children are more sensitive than adults to lead anaemia and symptoms of acute lead poisoning including sickness, abdominal pain, constipation, listlessness, stupor, anorexia, irritability, ataxia and clumsiness have been observed in children with blood lead levels of 0.5 to 0.6μg ml⁻¹.11

There have been countless papers published in recent years investigating the link between blood lead and hyper tension,28,29 as it is thought increased blood lead levels may cause an increase in blood pressure. A great deal of study has also been concentrated around symptomatic childhood lead poisoning, levels of blood lead above 0.4μg ml⁻¹ are proven to result in permanent neurological and cognitive impairment of children, lowered IQ and other mental deficiencies.30
References.

7. DHSS, Lead and Health, Working party report, HMSO 1980
9. Sherlock, J.C., and Evans, W.H., Chem. in Britain, 1985, 21, 1019
14. Royal Commission on Environmental Pollution, 9th Report cmnd8852, HMSO 1983
15. Packham, R.F., Water Treatment Exam., 1971, 20, 144
22. James, A.C., NRPB Annual Report 1975, 71 Harwell.NRPB
30. Heavy Metals in the Environment Conference 1991, 1, 139
31. Handbook of Chemistry and Physics, 76th ed CRC press
10.3 Lead in blood, Project Outline.

Enviromed plc have produced a stripping voltammetric sensor for the determination of lead in whole human blood. The sensor works well when a small drop (100μl) of diluted blood (1:10 in 1M HCl) is placed on the screen printed electrodes. However the sensor fails to function when a whole blood sample is placed directly onto the electrode. This is because the lead in whole blood is bound to proteins within erythrocytes. The dilution of blood in HCl causes lysis of the erythrocytes, followed by release of the protein bound lead. In addition the pH is one at which the stripping voltammetry proceeds well.

In discussions with Enviromed plc it became apparent that the dilution step might be omitted if a thin film of appropriate reagents could be located on the active surface of the sensor.

The nature of the required membrane was to be determined by Loughborough University.

10.4 Reagents and equipment used.

Napthalene 2 sulphonic acid, (supplier Fisher Ltd)  
Oxalic acid dihydrate, (supplier SAF Ltd)  
Polyvinyl pyrrolidone K30 Powder, (supplier SAF Ltd)  
Venous whole human blood. (Sample from J.M.Lee)  
Screen printed three electrode sensor strip, Carbon counter, mercury plated carbon working, and Ag/AgCl references electrodes. (supplier Enviromed plc)  
Conductance meter used WP Acm35 meter, supplier Linton, Cambridge.  
Camera film, Ilford RP4.

All chemicals used were of Analar grade.
10.5 Production of a Thin Film.

From previous work polyvinylpyrrolidone (PVP), with a molecular weight of 40,000 was to be used to cover the working area of the sensor.

In order to obtain the low pH needed to lyse the erythrocytes and release the lead, a solid acid was needed to be incorporated into the PVP membrane.

Two solid acids were chosen to be incorporated into the PVP film. These were oxalic acid dihydrate and napthalene 2 sulphonic acid, both having suitable low pKa values of 1.27 and 1.15 respectively.

The pH of various concentrations (% w/v) of these acids is shown in the table below.

<table>
<thead>
<tr>
<th>oxalic acid</th>
<th>napthalene 2 sulphonic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>concn %</td>
<td>pH</td>
</tr>
<tr>
<td>1</td>
<td>1.543</td>
</tr>
<tr>
<td>5</td>
<td>1.115</td>
</tr>
<tr>
<td>10</td>
<td>0.977</td>
</tr>
</tbody>
</table>

The practical limit of solubility of napthalene 2 sulphonic acid at room temperature was 4.5% w/v. Filtering of the turbid solution was required.

From these pH values, acid concentrations of 1% w/v were discarded as being to high, as a pH nearer to 1.0 was required for lead determination by this method.

The pH of acid solutions containing various weights of PVP, mg ml⁻¹ are shown in the table over.
100μl aliquots of various polymer acid solutions were dried on microscope slides in an oven at 38°C for 24 hour periods.

The dried films were examined by eye and by microscopy under magnifications x63, x125 and x250.

The underlying trend that appeared obvious from visual examination was noted to be:

Films containing more oxalic acid than PVP by weight for example 5mg PVP, 10mg Oxalic acid in 100μl, and films containing equal amounts of oxalic acid and PVP, all showed long, white needle like crystals and some dendritic crystals, covering the whole film area or the centre of it.

A photograph of a 2mg PVP, 5mg oxalic acid film surface under magnification x250, is shown below. fig 10
Films containing greater weights of PVP than oxalic acid, were all clear and glass like, the films were thicker in the centre than the periphery, and cracking could occasionally be seen towards the edges of the film.

The photograph shown below fig 10a, shows a 12mg PVP:5mg oxalic acid film under magnification x63.
The films produced using napthalene sulphonylic acid were surface marked with a large number of tiny cracks. The photo below, fig 10b shows a good example; A film produced from 2mg PVP and 4.5mg napthalene 2 sulphonylic acid under magnification x63.

10.6 Effect of Blood on the Polymer/acid system pH.

Solutions with a 2:1 ratio of polymer to oxalic acid appeared to produce the best films. Films containing 10mg PVP and 5mg oxalic acid were taken as an optimum for thickness of film and quantity of oxalic acid present.

A 5ml polymer acid solution was made up, such that 100μl of this solution would contain 10mg PVP and 5mg oxalic acid.
This solution was stirred whilst whole blood was added in 0.2ml aliquots upto 1ml then 0.5ml aliquots until 5ml had been added. The experiment was repeated using deionised water addition as a dilution factor control.

The pH of the starting solution was 1.008. 2ml of blood was added before the pH of the starting solution was increased by 10%. The results may be seen in the graphs figs 10d and 10e. From the equation of the blood graph, a sensitivity of 0.076 and an intercept of 0.967 were obtained.

As 2ml of blood (or water) may be added to a 5ml solution of PVP and oxalic acid before the pH changes beyond 0.1 units and the 5ml solution contains 5mg of oxalic acid it may be calculated using a 5:2 ratio that 12.5mg of oxalic acid are needed to keep the pH of 100µl of blood at a pH of approximately 1.1. However it may be assumed that at the film/liquid interface there will not be total diffusion and only 20% of this amount of oxalic acid will be required for the pH to remain around 1.0.

10.7 Estimation of film thickness.

Films produced on the electrode working area were of a much more uniform thickness than those produced on a microscope slide. The area of these films was measured as 1.8cm² (working area of the electrode 1.5cm²).

The mass of the film was taken as the mass of polymer and oxalic acid dried down. The density of the film was taken as the density of oxalic acid, 1.90g cm⁻³ (33% of the film), added to the density of the polymer, 0.84 g cm⁻³ (66% of the film). The density of PVP Mw 40,000, was obtained by measuring the volume change on adding 25ml of hexane to approximately 5g of PVP. From the volume of hexane displaced, the density of the polymer could be calculated.
Hexane was chosen as PVP is insoluble in this liquid.

Using the equation volume = mass/density = area x thickness,

The thickness of an average film could be calculated. see the table below.

<table>
<thead>
<tr>
<th>mass of film</th>
<th>thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td>oxalic acid</td>
<td>PVP</td>
</tr>
<tr>
<td>5mg</td>
<td>10mg</td>
</tr>
<tr>
<td>2.5mg</td>
<td>5mg</td>
</tr>
<tr>
<td>5mg</td>
<td>5mg</td>
</tr>
</tbody>
</table>

10.8 Speed of film dissolution.

Experiments were carried out to determine the speed with which the films dissolved. 100μl aliquots of polymer, oxalic acid solutions were dried onto the working area of the Enviromed sensor. These films contained either 10mg PVP and 5mg Oxalic acid or 5mg PVP and 2.5mg oxalic acid, hence the film thickness were either 69 or 34μm.

The mercury electrode was connected to the conductivity meter and the sensor placed on a lab jack. A single coil of platinum wire of fixed circumference was clamped above the platform and also connected to the meter. It was important that both conducting surfaces were of fixed area to maintain a similar conductance value for all samples on complete film dissolution.

When a 100μl aliquot of liquid, either blood or isotonic 0.9% w/v sodium chloride solution, was pipetted onto the film the lab jack was raised until the liquid surface touched the platinum coil. The conductance was measured using a stopwatch every 30 seconds for twenty minutes.

The results with both blood and 0.9% w/v sodium chloride are shown in figs 10d and 10e.
It can be seen that in both cases the maximum conductance i.e. complete dissolution, is reached after approximately 30 to 60 seconds in the 5mg:2.5mg samples and after approximately 3 minutes in the 10mg:5mg samples.

10.9 Addition of Chloride ions to the film

In an attempt to increase the oxalic acid/PVP ratio from 1:2 to 1:1, the temperature of the oven was increased from 40°C to 65°C. A 100μl solution containing 5mg PVP and 5mg oxalic acid was dried at the higher temperature to produce a clear glassy solid, although some transparent oxalic crystals could be seen marking the surface. This experiment was repeated using a 0.1M solution of sodium chloride as the solvent, hence 58.5mg of solid sodium chloride would be present in the film. The chloride addition enables the effective functioning of the Ag/AgCl reference electrode.

The film coating was dissolved as before using a 0.9%w/v sodium chloride, the conductance was monitored every 30 seconds using the stopwatch. The conductance of two sets of films were compared, one set containing 10mg PVP 5mg oxalic acid and another set containing 5mg of both PVP and oxalic acid. The results are displayed as fig 10f.

10.10 Other Conductance experiments.

Preliminary conductance measurements were made using a strip of glassy carbon, in place of the sensor strip. Solutions containing varying masses of PVP and oxalic acid were dried onto the strip, using the oven set at 40°C. The mass ratios of PVP to oxalic acid varied from 40/20 to 5/2.5. All the films produced in this manner were much thicker in the centre than the periphery and an estimate of average thickness based on density and mass calculations would be inaccurate.

Conductance measurements were carried out using the equipment described earlier, the glassy carbon strip being connected to the meter and the film raised on a lab jack until the liquid surface just touched the platinum coil.
To prevent the 100μl aliquot of liquid (water and 0.9% w/v sodium chloride were both used), a ring of silicone grease was placed around the edge of the film such that no liquid could reach the glassy carbon strip unless it dissolved the film.

Samples containing 60mg of material failed to register any conductance after 20 minutes.
Samples containing 15mg of material tended to take between 7 and 10 minutes to register maximum conductance.
Samples containing 7.5mg of material tended to take 2 to 5 minutes to register maximum conductance.
Samples containing 11mg of material took approximately 8 minutes to dissolve fully.

The results appeared to be variable and dissolution times much longer by this method. This can be explained by the fact that the exposed film area was much smaller due to the grease ring, and the area that was exposed was of a much greater thickness than the covered film, however these results provided much important preliminary information.

10.11 Crystal Growth.

When cooled at 40°C for 24 hours the oxalic acid, when in greater concentration than the PVP, recrystallised as long needle like and snowflake like crystals.

Nucleation is the process occurring in a supersaturated solution that results in the formation of small particles that are capable of growing to larger ones.

Crystal growth may result from homogeneous (spontaneous) nucleation where a cluster of ions or molecules form a nucleus in a supersaturated solution, or heterogeneous nucleation where the formation of the critical cluster is aided by a second phase. In analytical precipitation reactions, homogeneous nucleation is very rarely the predominant mechanism.
Heterogeneous nucleation of an ionic precipitate can be viewed as a sequence involving diffusion of ions to a surface and their adsorption and surface diffusion to form a cluster or island.\textsuperscript{32}

The number of nuclei formed depends on the extent of supersaturation and the effectiveness of the nuclei. If the nuclei are uniform there will be an equal number of nucleation sites, and the precipitate formed will be relatively coarse and uniform in particle size. If concentration is high enough, homogeneous as well as heterogeneous nucleation occurs, and large numbers of particles form.

Growth of nuclei to larger particles consists mainly of material diffusion to the surface followed by deposition. Mare\textsuperscript{33} found that most growth processes are second order, rather than first order as required by single ion diffusion mechanisms. The rate determining step may be loss of solvent from the solvated ions, near the end of growth diffusion may become rate controlling.\textsuperscript{34}

There are four principal types of crystal growth, Screw dislocation, two dimensional surface nucleation, dendritic growth and amorphous precipitation.

Screw dislocation is the most important type of growth for analytical precipitates giving large and well formed particles.\textsuperscript{35,36} Many substances on condensation form a supersaturated vapour and needle like crystals by screw dislocation, growth rate has been shown to depend on the extent of supersaturation.\textsuperscript{37}

Dendritic growth predominates at high supersaturation values, when crystal growth is limited by diffusion. This growth involves snowflake like crystals, there is diffusion of solvated ions to the growing crystal surface followed by release of solvent molecules and diffusion away from the growing surface.

Crystals fragment easily in dendritic growth\textsuperscript{38} and secondary nucleation results in the number of particles obtained far exceeding the number of nucleation sites as homogeneous nucleation does not occur fragmentation leads to smaller particle size.

170
The effect of temperature is complex, solubility usually increases so that the supersaturation ratio decreases with increasing temperatures, however the size of the critical agglomeration\textsuperscript{39} for nucleation decreases with increasing temperatures, hence an optimum temperature must exist for precipitate growth. It would be thought that at higher temperatures and hence lower supersaturation ratios long needle like crystals would develop by screw dislocation, and at higher levels of supersaturation dendritic growth would predominate and snowflake like crystals would form.

In our case, \textit{fig 10}, it can be seen that at an intermediate temperature, $40^\circ\text{C}$ both dendritic growth and screw dislocation have occurred.

Using Polyvinylpyrrolidone K30 molecular weight 40,000 and oxalic acid, films have been produced which cover the electrode area on the sensor strip. The optimum weight ratio for film production at 40°C is 5mg PVP and 2.5mg oxalic acid. This weight of material provides a film of a thickness which is rapidly dissolved (in under 1 minute), by a small aliquot of blood.

The mass of acid in the film is capable of holding the pH of blood at approximately 1.0, and provides an environment harsh enough to lyse the erythrocytes. The weight ratio of film materials may be increased from 2:1 to 1:1 by raising the drying temperature to 65°C, enabling a greater mass of oxalic acid to be contained within the film, to ensure the pH of the blood remains constant during lead determination. This drying temperature also allows chloride to be contained within the film to ensure effective functioning of the Ag/AgCl reference electrode. These films are also dissolved in under 1 minute.

The films are simple to produce and may be manufactured rapidly in large numbers. The films are smooth and glass like in appearance.

It is hoped that these sensor strips will be successful in determining blood lead by stripping voltammetry, and the results of this Enviromed trial are awaited with interest.
Fig 10c

Effect on polymer/acid system pH

Refer to section 10.6 effect of blood on the polymer/acid system
Fig 10d
rate of electrode film dissolution
addn of isotonic sodium chloride soln

% maximum conductance

time seconds

• 10/5 mg  • 5/2.5 mg  • 5/5 mg

refer to section 10.8 speed of film dissolution
rate of electrode film dissolution
addn of whole human blood

Fig 10e

% maximum conductance

time seconds

- 10/5 mg
- 5/2.5mg

refer to section 10.8 speed of film dissolution
Fig 10f

rate of Chloride film dissolution

% maximum conductance

0 100 200 300 400 500 600 700 800 900

time seconds

- 5/5 mg
- 10/5 mg

refer to section 10.9 addition of chloride to the film
11.0. Conclusions and Future prospects.

The production of a paper dip test for the determination of nitrite and sulphite in aqueous solution at levels of 0.125 and 0.5μg ml⁻¹ respectively, was investigated. It would be desirable to improve on this work by obtaining more accurate calibration information with the dm3000 spectrometer. It would also be desirable to carry out further research into the effect of interferences, reproducibility and the usefulness of the paper tests after prolonged storage. It would be essential for the further development of these papers to test them against existing methods of analysis.

Less success was obtained with the production of a paper dip test for the determination of aluminium, even though the wet colorimetric method was capable of detecting aluminium at a sensitivity level of 0.05μg ml⁻¹, without interference from significant concentrations of common cations. This was thought to be due to the poor mobility of aluminium ions within a paper matrix, and the difficulty in removing interferences on paper. It would therefore be unwise to pursue the development of a dip test for aluminium further.

A reagent delivery system using the soluble polymer polyvinyl pyrrolidone (PVP), molecular weight 40,000 was developed and applied to the determination of aluminium in natural water samples and molybdenum in aqueous solution, at sensitivity levels of 0.05μg ml⁻¹ and 0.1μg ml⁻¹ respectively. Both tests were free from interference and showed some degree of reproducibility, although more in depth analytical studies would be needed to clarify this data. Again it would be necessary to calibrate the tests using matrix analysed water samples, or an instrumental method of analysis.

It would also be desirable to investigate the detection of molybdenum in soil samples. The novel soluble polymer matrix system (SPMT) was capable of holding layers of reagents in discrete bands so that a reaction could be carried out in the order required by the desired procedure. This was accomplished by producing blown polymer layers separated by films of PVP. The SPMTs were capable of being dissolved in under 3 minutes and had a resulting solution transmittance greater than 90%.

This method of SPMT production however was not capable of mass production by liquid line procedures due to the organic blowing agent separating out of the PVP.
mixture on standing. It would be an aim of future work to develop the manufacture technique in order that mass production may become a possibility.

Reducing the polymer mass in the test layers to a minimum negated the need for a blowing agent as these thin layers were quick to dissolve, however, reducing the mass of PVP in the layer did not allow for sufficient containment of reagent. A method whereby the solution of PVP and the reagents was evaporated until the PVP layer was formed, allowed sufficient reagent to be contained in an otherwise insufficient mass of PVP. However this obviously did not allow for the production of layered tests.

It would be desirable for further research to build on these results to produce a sequenced reagent test using the PVP, which would be capable of mass production whilst still allowing large amounts of reagent to be held in the polymer layers.

The technique of evaporating a PVP solution containing reagents to produce a solid thin layer was applied to develop a stripping voltammetric sensor for the determination of lead in blood, by negating the need for sample pretreatment.

It is hoped that this layer will prove a success following the electrochemical trials by Enviromed plc.

It is also hoped that in the future this novel technique may be used in a similar manner to improve and develop other chemical sensors and field monitors for the determination of both environmental and clinical samples.