Automated Analysis of Ions in Solution

The following are summaries of five of the papers presented at a Joint Meeting of the Automatic Methods Group and the North East Region held on September 25th–27th, 1985, in the University of York.

Potentiometric Methods of *In Vivo* Analysis

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The monitoring of patients in hospital, both during and after treatment or surgery, is an essential part of health care that is rapidly growing in sophistication in order to reduce both complications and hospital bed time. Already the monitoring of ions, gases and other blood metabolites is widespread in intensive care and surgical units. Potentiometric *in vivo* transducers for patient monitoring are highly relevant in this context, and have made an important contribution to patient management in clinical practice and physiological research by providing the clinician and scientist with answers to their questions in the operating theatre, at the bedside and in the laboratory.

Clinicians generally require small, robust, problem free, low drift, long lifetime devices that are easily calibrated.

Table 1 details the normal ranges for blood electrolytes, as expressed in mmol l^{-1} ± 2 standard deviations, with their corresponding physiological activity ranges.

Table 1. Normal ranges for blood electrolytes

Ion	Range ±2 SD/ mmol l^{-1}	Theoretical change in potential at 37 °C/mV
Na^+	135–150	2.82
K^+	3.5–5.0	9.54
Ca^{2+}	1.0–1.2	2.44
Cl^-	95–110	3.92
HCO_3^-	21.3–20.5	5.84

A number of these blood constituents can be monitored *in vivo* by potentiometric transducers. These can be specific, and some also lend themselves to micro-miniaturisation.

Ion selective electrodes (ISEs) are widely used in industry and science, but their introduction to clinical medicine has been comparatively slow. This has been due to: (i) the unreliability of the early sensors; (ii) the failure to provide equipment fulfilling particular and specific clinical needs; and (iii) the natural conservatism of the medical profession.

More tangible scientific disadvantages of *in vivo* ISEs are that: (i) they are delicate and require careful handling; (ii) they only respond to free ionic species in solution and will not respond to ions in an insoluble or complexed form; (iii) the electrode response is affected by the presence of proteins or organic constituents as a coat or clot on the ISE membrane. This may cause a sluggish response to be observed, or may change the observed emf and the selectivity due to the presence of active sites on the coating molecules superimposing their characteristics on to the normal electrode behaviour; (iv) due to liquid junction effects, ISEs may be subject to large-scale drift; (v) blood electrolytes have to be monitored invasively by inserting a catheter electrode, which may be 1.0–1.5 mm in diameter, into a blood vessel. The most useful results are obtained in flowing blood because there is less chance of clot formation. Therefore electrodes have to be inserted arterially, *e.g.*, into the femoral, brachial or radial artery. This is a procedure fraught with problems such as thrombus formation, infection, or perforation of the blood vessel, and as such is only done on very sick patients or in research animals. Finally, (vi), ISEs are difficult to re-calibrate once *in situ*.

The advantages of ISEs are: (i) they make possible the direct evaluation of ionised and physiologically active fractions and are therefore well suited for assessing biological systems; (ii) in acute care, *e.g.*, in the operating theatre or the bedside, ISEs can give the clinician much faster results than a laboratory analysis, as the result is from a continuous direct reading on whole blood from (iii) a system that is small and easily portable.

Accuracy

Current patient-safe mV-meters have an accuracy ±0.1 mV. An inaccuracy of 0.1 mV in the Na^+ or K^+ readings *in vivo* would correspond to an error of 0.5 mmol l^{-1} and 0.02 mmol l^{-1}, respectively. This is well within the clinical tolerance error levels where values of 2 mmol l^{-1} for Na^+ and of 0.2 mmol l^{-1} for K^+ would be realistic. Of more real value to the clinician than absolute accuracy is reproducibility, and the ability to detect a trend in plasma electrolyte levels over a given time period.

Liquid Junction Potentials

Potential fluctuations due to the liquid junction potential, E_J, between the reference system and biological fluids provides the main headache for clinical scientists. Tolerances in accuracy of 10% for plasma electrolytes require reproducibilities better than 0.3 mV for Na^+, 1 mV for K^+ and 0.2 mV for Ca^{2+}. Therefore, a highly reproducible E_J is required. However, it is upon the magnitude of the residual junction potential that the accuracy of the ISE measurement will depend as it affects the slope of linear calibration. Ideally, this residual E_J should be a minimum, preferably zero, and stable.

Standards and Calibration

To solve problems due to interfering ions and therefore lack of selectivity, it is customary to incorporate the main interfering ions into the calibration solutions. Therefore, all activity coefficients in the calibrants should try and closely match those in the samples. This is achieved by making up standards in a matrix with a background of a constant ionic strength that approximates that of plasma ($I \approx 0.15$).

Calibration of devices in vivo

This can be effected by the removal of a blood sample close to the site of the catheter or *via* a lumen built into the catheter construction.[1] Unfortunately, this is not possible when using devices inserted into tissues.

Electrode drift

This may be: (i) parallel (slope constant); (ii) anion dependent (slope not constant); or (iii) random (that is, the absence of regular trends, the slope being non-uniform in both direction and magnitude). Electrode drift may be due to: (i) E_j effects as discussed previously; (ii) temperature gradients (between electrodes and across individual electrodes); and (iii) membrane component leaching in polymer - membrane based transducers, which is really a loss of selectivity. If the drift is too great, replacement of the electrodes will probably remedy the situation.

Electrical Interference

With high impedance electrodes (> 1 MΩ), electrical interference is a major problem and effective cable screening is essential. Telemetry has been used to transmit transducer signals over short distances, up to a few metres.[1] This also avoids the problem of patient isolation. Normally one would have to conform to the British Safety Standard, BS 5724, and this can be achieved by purchasing a patient safe pH - mV meter, the cost of which is around £1400. Alternatively, an isolation circuit for standard non-patient safe equipment may be bought for £600.

Clinical Examples

The first potentiometric sensor to be clinically adopted for *in vivo* monitoring was the glass pH electrode.[2] However, glass electrodes are fragile and they have high electrical impedences, which cause problems when trying to carry out reliable measurements in electrically noisy environments, such as operating theatres and intensive care units. However, they have been used successfully *in vivo* to measure changes in blood pH due to hyperventilation[2,3] and to monitor continuously the scalp tissue pH of the human foetus during labour.[4]

Major advances have been made in recent years through the use of neutral ion carriers, which allow relatively robust, low resistance pH selective polymer membranes to be produced for medical purposes. These include *para*-octyldecyloxy-*meta*-chlorphenylhydrazonemesoxalonitrile dissolved in a block co-polymer elastomer,[5,6] and tridodecylamine (TDDA) in a PVC matrix for pH, developed by Professor Simon *et al.* in Zurich.[7] Applications using the TDDA ionophore range from foetal pH assessment[8] to intracellular proton activity measurements.[9]

Continuous intravascular potassium ion monitoring in both human and animal subjects has been carried out using valinomycin-based PVC membrane catheter electrodes,[1] whereas a neutral ion carrier for sodium, ligand ETH 227 in PVC,[10] has been used in the construction of a double barrelled Na^+-selective microelectrode, 1 μm in diameter, an application of which is assessing intracellular activity.[11]

Metal - Metal Oxide pH Electrodes

In order to avoid problems with fragile glass membranes, metal - metal oxide electrodes, *e.g.*, Ir, Pd and Sb, have been used for *in vivo* pH determination. In general, these polycrystalline electrodes tend to be irreproducible, unstable and subject to interference from oxidising and reducing substances, or from metal ions in the test solution. Recently, by using the close packed surface of a single crystal, an antimony - antimony oxide pH electrode (3 mm in diameter) which avoids the aforementioned problems has been developed[12] for the continuous monitoring of pH in the oesophagus of patients with pathological gastro-oesophageal reflux.[13]

In vivo ISEs have many exciting possibilities for medical science. No doubt the discovery of new ISE membranes together with the adaptation of existing materials for clinically relevant ions will broaden this field.

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Extended Range Calibrations by Flow Injection Analysis

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By far the most commonly used quantitative parameter in flow injection analysis (FIA) is peak height. In FIA, the raw data are evaluated in a similar way to the corresponding conventional method, in which samples are presented directly, or via some pre-chemistry, to instruments producing steady-state readings. Flow injection systems provide a safe, convenient and rapid method of handling samples, but offer nothing new in the evaluation of the results. Thus, peak-height measurements in FIA suffer from the same limitations as conventional procedures; they set an upper limit to the sample concentration because either the instrument response is "off-scale" (or in a non-linear region of the calibration function) or there is insufficient reagent to form the appropriate amount of product.

Both of these restrictions are by-passed if the width of the peak is measured. The experimental factors that make the peak height precise ensure that the peak width is also precise. However, the relationship between peak width and the concentration of the sample is not straightforward and depends on whether or not a chemical reaction occurs in the flowing stream, the dispersion, the relative concentrations of sample and reagent, and on the peak shapes produced by the physical dispersion processes. If it is assumed that the dispersed sample peak is exponential and that the leading and trailing edges are dispersed to the same extent, an equation may be derived relating the width of the peak to a logarithmic function of concentration.[1] This equation is simplified if, in the instance of chemical reaction, the sample is in excess in the profile centre.[2]

Table 1. Peak width based determination of OH^- and Fe^{2+}

Ion	Volume injected/μl	Manifold length/cm	Reagent	Range covered/M	Correlation coefficient
OH^-	125	35	10^{-3} M HCl + 4 p.p.m. bromocresol purple	4×10^{-3} to 10^{-1}	0.999, 0.997
Fe^{2+}	370	85	10^{-4} M, 1, 10-phenanthroline	4×10^{-5} to 10^{-2}	0.998

This version of the equation requires the peak width to be measured at the equivalence points, which are difficult to locate unless the product profile is followed, in which case doublet peaks are observed, as shown in Fig. 1.

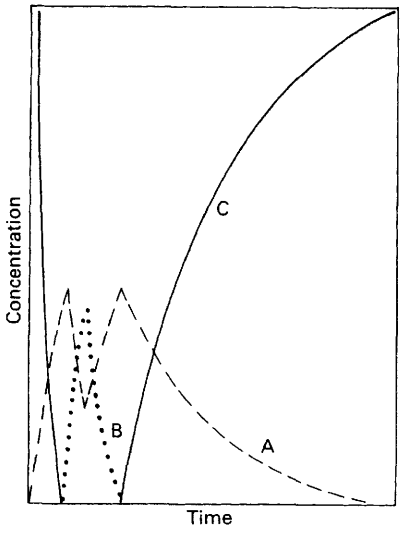

Fig. 1. Profiles produced when the sample is in excess in the profile centre. A, Product profile; B, sample profile; C, reagent profile

Although the basis of the linear relationship with the logarithmic function of concentration requires that the peak shape be produced by the passage of a plug of sample solution through a well stirred mixing chamber, the peak shapes produced by injecting relatively large volumes into relatively short length manifolds are quite good approximations to the exponential shape. The results for two single line manifolds with spectrophotometric detection are given in Table 1. For the determination of OH^-, two arbitrary peak widths above the base line were selected. The method may also be used with more complex manifolds such as that shown in Fig. 2 for the determination of NH_4^+. Measurements based on peak height showed a useful working range of up to 200 p.p.m. for a 40 μl sample volume. Using the peak width method, the working range was extended up to 16 000 p.p.m. Using a fully automated flow injection analyser (Tecator Model 5020), a throughput of 30 injections h^{-1} was possible. Precisions ranged between 1.8% RSD at 125 p.p.m. to 0.3% RSD at 4000 p.p.m.

To illustrate the application of the doublet peak method, copper was determined by injection of 500 μl into a 150 cm single line manifold with 10^{-4} M EDTA as carrier stream. A Pye Unicam PU4020 UV HPLC detector was used to monitor the copper EDTA complex. Solutions covering the range 1.6×10^{-6} to 0.16 M (0.1–10 000 p.p.m.) Cu were injected. A graph of the peak separation against the logarithm of the copper concentration had a correlation coefficient of 0.992.

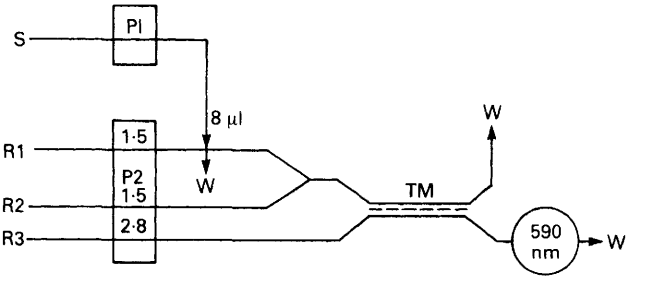

Fig. 2. Manifold for the determination of NH_4^+. The injected sample, s, in a water carrier stream (R1) is merged with a stream of 0.1 M hydroxide (R2) and the NH_3 generated diffuses across a Teflon membrane (TM) into an acceptor stream of bromothymol blue at pH 6.4 (R3). The absorbance at 590 nm is monitored. P1 and P2 are pumps (flow-rates shown in ml min^{-1}) and W is waste

In principle, measurements of peak width could be made to the nearest 0.01 s and with precisions of about 1% RSD, enabling small relative differences in concentration to be distinguished. If the uncertainty obtainable by any real system was unacceptable, the peak method could be used for rapid screening, allowing the dilution factors, necessary to bring the sample concentrations on to a more precise calibration range, to be readily calculated.

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Chromatography of Organic Anions of Clinical Interest in Physiological Fluids

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Organic acids are intermediates in many pathways of human metabolism, and body fluids contain complex mixtures of aliphatic and aromatic carboxylic acids and their conjugates.[1–3] Nutritional status, dietary composition, drugs and assorted therapeutic procedures may alter the organic acid profile. Their accumulation in physiological fluids in various patho-

logical conditions, such as diabetes (β-hydroxybutyric, acetoacetic), renal stones (oxalic), certain tumours (phenolic catacholamine metabolites), and inborn errors of phenylalanine and tyrosine metabolism (phenylketonuria, tyrosinosis, alkaptonuria), is well established.[1] In the past two decades, a group of inherited defects in aliphatic acid (propionic, methylmalonic, isovaleric, lactic, glutaric, etc.) metabolism, which frequently result in severe, life-threatening illness in the neonatal period, have been recognised.[4] In consequence, rapid methods for organic acid analysis must be included in the metabolic investigations applied to acutely sick children.[4,5]

Choice of Technique

Spectrophotometric analysis of organic acid products following enzymatic or chemical conversion is valuable for specific analytes, but comprehensive investigation requires chromatographic techniques. Certain important groups of compounds, such as amino acids and long chain fatty acids, are excluded by sample preparation procedures or the limitations of the separation techniques, and are assayed separately. Derangements in phenolic acid and ketoacid metabolism can be detected by semi-quantitative, thin-layer chromatographic procedures,[1] but general location reagents for aliphatic acids are insensitive and are affected by minor pH changes in the layer.[1,6]

The introduction of gas - liquid chromatography with flame-ionisation detection, and more particularly, combined with mass spectrometry, allowed the systematic investigation of organic acids in man.[2,3] Multi-step sample preparation, including fractionation, concentration and conversion to volatile (*e.g.*, trimethylsilyl) derivatives is required for straightforward GLC, and the capital cost of mass spectrometry equipment, coupled with the necessary operator expertise, has relegated this sophisticated technique to a few specialist centres.

Renewed optimism in the possibility of combining the speed and simplicity characteristics of TLC with the comprehensive detection and quantitative virtues of GLC has followed the description of rapid, high performance, liquid chromatographic techniques for organic acid analysis.[7] Separations based on partition, at low pH to suppress the ionisation of carboxyl groups, are more generally useful than anion exchange procedures,[7,8] although the latter is the method of choice for urinary oxalate - glyoxalate separations. Bonded silica reversed-phase columns have been favoured for aromatic acids, especially when combined with gradient elution and ion-pair reagents[7]; whereas ion moderated (ion-exclusion) partition on cation-exchange resins[8] has been preferred for aliphatic acid analysis of physiological fluids.[9–12] The simplicity of the isocratic elution (1 h cycle) and far ultraviolet (190–210 nm) detection system of the latter makes it attractive for the initial detection of clinically important organic acid disorders.[11,12]

Apparatus and Methods

A Spectra-Physics liquid chromatograph and Bio-Rad Aminex HPX 87H organic acid column (300 × 7.8 mm) protected by a Bio-Rad Aminex MPX-85H guard cartridge (40 × 4.6 mm), identical to that described by Daish and Leonard,[12] were used for this study. Dilute sulphuric acid (5 mmol l^{-1}) was used as mobile phase (0.6–1.0 ml min^{-1}) and a column heater (HPLC Technology) was added following the initial investigations.

The acids present in 100 µl of either plasma or urine diluted to contain 1 mmol l^{-1} of creatinine[10,11] were retained during their passage down a small (100 mg) column of Bond Elut SAX (aminopropyl, trimethyl chloride bonded to silica) and subsequently eluted with 500 mmol l^{-1} of sulphuric acid (200 µl) as described by Daish and Leonard.[12] Usually 100 µl of this extract was injected into the chromatograph.

Sample Preparation

Direct injection of urine (but not fluids containing proteins or lipids) on to polymeric cation-exchange columns has been considered suitable for screening for gross disturbances to organic acid metabolism.[9–11] However, fractionation of samples, using either SAX or solvent extraction,[3] indicated that many of the compounds detected at 210 nm following direct injection were not acidic. Additionally, the peak shape was frequently distorted, presumably due to non-acidic compounds affecting the initial binding of analytes to the proximal end of the column.

Anion-exchange methods of sample preparation[2] are more comprehensive and specific than solvent extraction procedures.[3] The single step SAX method[12] was the most suitable procedure for the isolation of organic acids prior to HPLC on polymeric columns. With the exception of uric acid (less than 10%) and orotic acid (40%), recovery of carboxylic acids was greater than 90%. Cations, neutral compounds and virtually all the proteins are removed, the eluate can be directly injected into the chromatograph without prior concentration (which may remove volatile compounds) and the sulphuric acid is frontally eluted in contrast to the organic anions used in other preparation methods.[2,6]

Attempts to simplify the mixture further by adding charcoal to remove aromatic acids had limited success when applied to urine extracts. If sufficient charcoal (200 mg ml^{-1}) was added to remove all aromatic compounds, 50% of dicarboxylic and some ketoacids were also removed, and reproducibility was poor. Alternative techniques, such as utilising hydrophobic interaction on reverse-phase materials, are at present under investigation.

Chromatographic Separation

Ion-exclusion and partition mechanisms are the primary influences on the separation of carboxylic acids on columns of cation-exchange resin at low pH,[10] but reversed-phase processes and hydrophobic interaction of non-polar groups with the aromatic backbone modify the elution order.[8,13] The capacity factors for most aliphatic acids[12] are low (less than 2) and tentative identification by retention time in the potentially crowded early region of the chromatogram depends on reproducible chromatography. Most investigators[9,11,12] have performed their chromatography at ambient temperature. Preliminary studies in this laboratory showed that retention times were approximately 10% lower at 30 °C than at 20 °C and that the relative position of some acids changed, illustrating the need for both column thermostating and the inclusion of run temperature with retention data for reference materials. A systematic study of the influence of both column temperature and eluent flow-rate on chromatographic resolution is currently being undertaken.

Detection

Detection of separated acids in the far UV range (210 nm) is a robust, non-destructive procedure for the initial detection of an abnormality.[11,12] The sample preparation methods ensure acid specificity but the structure determines the amount of energy absorbed by individual acids[9] and response factors must be determined for all compounds of quantitative interest. A second, in-line, different detector (UV, fluorimetric or electrochemical) may increase the specificity.[9,13] Additionally, contamination of samples at all stages, especially by detergents and plastic polymers, must be actively avoided.

Conclusion

The suitability of ion-moderated partition liquid chromatography, with appropriate sample preparation, for the detection of gross disturbances to organic acid metabolism has been confirmed. Comprehensive data on chromatographic behaviour and the sensitivity and selectivity of detection systems for the many acids known to occur in physiological fluids is required before the value of the technique for quantitative studies at near normal concentrations can be assessed.

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Determination of Dissolved Humic Substances in River Waters Using Flow Injection Analysis with Fluorimetric Detection

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Water authorities in England and Wales are responsible for various aspects of river management, including the investigation of "pollution." Sometimes pollution is only obvious because of a deterioration of a "function" served by a river, such as the support of fisheries. One investigation into declining fish stocks in otherwise clean rivers looks at the chemistry of rivers that are suspected of being affected by the run-off from "acid rain." Various chemical parameters are monitored, including dissolved humic substances.

Dissolved humic substances form the major component of dissolved organic carbon in "unpolluted" river waters. They consist of humic acids and fulvic acids and their chemistry has been widely studied.[2] Their quantitative determination is of interest to water chemists and methods for their determination have included UV absorbance measurements and fluorimetry.[3–5]

The method used in our laboratory uses flow injection analysis with a fluorimetric finish. It is based on the method of Brun and Millburn[5] for an automatic analyser. A mixed reagent is used containing: 1, a sodium borate buffer (pH 10.5), which serves to enhance the fluorescence and to prevent the precipitation of humic acids; 2, a sodium citrate - potassium sodium tartrate complexer which helps to eliminate interference from iron(III) and prevents the precipitation of metal hydroxides at the pH used.

The method has been used with a simple one-line flow injection system with manual injection (Fig. 1), and also with an automated system based on the Tecator FIAstar fitted with a type 1 manifold (Fig. 2). The detector in each instance is a Perkin-Elmer 3000 spectrofluorimeter fitted with a HPLC flow-cell. The fluorescence response (excitation wavelength 270 nm, emission wavelength 460 nm) is compared with that of standards prepared from a technical grade humic acid (Aldrich Chemical).

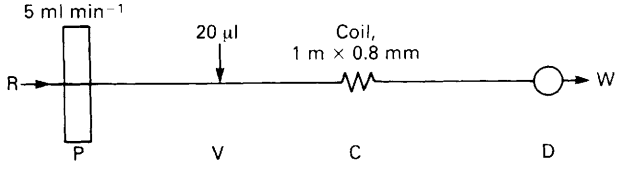

Fig. 1. Simple flow injection system. R, Mixed reagent; P, Isamtec Mini S820 pump; V, Rheodyne 5020 type valve. λ_{ex}, 270 nm; λ_{em}, 460 nm

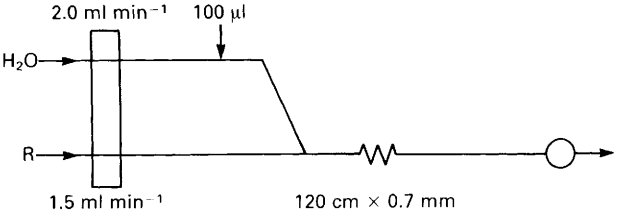

Fig. 2. Tecator manifold arrangement. R, Mixed reagent, λ_{ex}, 270 nm; λ_{em}, 460 nm

The automated method operates at around 105 samples h^{-1} and is calibrated up to a concentration of 20 mg l^{-1} of humic acid, which covers the range of concentrations of dissolved humic substances found in most river waters. The limit of detection is 0.3 mg l^{-1} and the relative standard deviation of 10 replicate measurements of a 10 mg l^{-1} standard was found to be 1.6%. Interference from iron(III) is checked by running a 10 mg l^{-1} standard containing 5 mg l^{-1} of iron(III) and should be 5% or less. Samples and standards are analysed in duplicate and drift correction standards are run at fixed intervals with the samples.

Flow injection analysis is a convenient method for measuring dissolved humic substances in river waters. Besides the interest in relation to pH in "acid rain" studies, dissolved humic substances are of interest because of their relationship to the production of trihalomethanes on the chlorination of water for potable use.[2] The method has also been used in this laboratory as part of an investigation into the effect of cotton industry wastes on rivers in industrial areas.

The permission of the NWWA, Rivers Division, to publish this work is acknowledged.

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Automated Suppressed Ion Chromatography as Applied to Acid Rain Research

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Ion chromatography has developed over the last decade to produce a reliable and simple technique that is especially useful for anion analysis. Chemically suppressed ion chromatography is particularly sensitive, and for sulphate determination provides a method that is superior to alternative techniques at the levels found in rain water. In addition, other anions can also be determined. Chloride levels are of particular interest in order to assess the marine influence and to complete the ionic balance sheet, whereas nitrate values are used in nutrition and pollution studies. A system was installed at Merlewood for the analysis of natural solutions from a range of ecological projects, and especially for use in acid rain studies. The development of an automated system capable of processing 8000 samples per annum is described, together with calibration and maintenance procedures.

Instrumentation

Hardware

A Dionex (Model 2010i) ion chromatograph fitted with an autosampler and sample load pump was installed for anion determination. A "four way" valve with a 50 ul sample loop replaced the standard "three way" valve in order to prevent pump damage in the event of an equipment malfunction. Guard and separator columns developed for their high sensitivity and capacity (AG4A and AS4A) were chosen, and an in-line filter was fitted to prevent blockages from particulates in the sample or eluent. Fig. 1 illustrates the hardware configuration and the components of the chromatograph that are computer controlled.

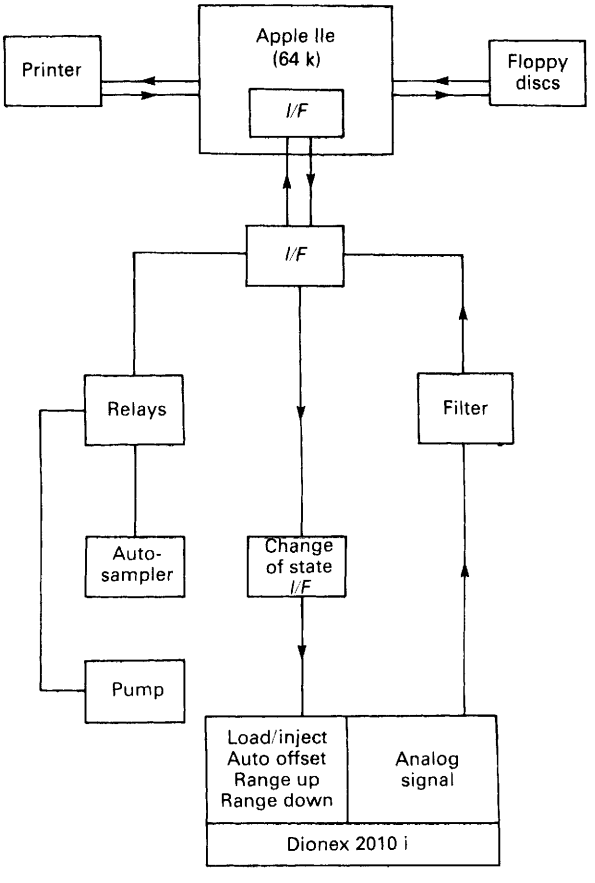

Fig. 1. Hardware configuration

A microcomputer package linked to the chromatograph gives control of the automation and data processing facilities. The system is based on an Apple IIe 64k microcomputer fitted with two floppy disc units and a dot matrix printer. A commercial interface (Adalab) provides analogue to digital conversion, timing controls and control lines. In addition, a Chromadapt interface, which connects to Adalab, facilitates external connection to relay control lines and to the analogue input from the chromatograph, and also provides signal buffering and amplification. Simple relays control the probe and sample advance on the autosampler, and also the 110 V supply to the sample load pump.

The number of programmable steps is limited, and, in addition, each function on the chromatograph requires two steps; a change of state interface was designed to send a pulse to simulate this two-step process. Hence, control was achieved for the autozero, injection, and range-change facilities. Analogue output from the Dionex was incompatible with the commercial software until a signal filter and improved integration algorithm had been added.

Software

"Chromatochart" is a comprehensive software package for the acquisition, analysis and reporting of chromatographic data, with facilities to control external devices. This control, through a 20 event program, is limited but is adequate with careful planning and the inclusion of the change of state pulse generator. Parameters for a routine integration method were evaluated and the peak height - area calculation was found to be reliable. Calculation using the standards comparison routine, which is based on a two point linear calibration, is unsuitable as these relationships deviate from linearity over the analytical range in operation. For both nitrate and sulphate, the relationship between peak area and concentration is non-linear and requires a 2nd order term in the regression analysis to minimise the variation about the fitted line, whereas the chloride graph is sigmoidal and requires a 3rd order term. Similar analytical graphs are obtained if peak height values are used.

The commercial software package made no allowance for instrumental calibration drift, and so a BASIC program has been developed. This is based on a single point calibration standard placed after every 10th sample and thus compensates for the drift, which can be up to 10% over a 24-h run. Part of this drift is associated with the sample matrix, but this has still to be fully evaluated.

Development

Before the installation of the chromatograph, it was appreciated that some of the solutions might contain substances that could reduce the ion exchange efficiency of the column. In particular, in the studies on the effects of acid rain on conifer species, rain collected beneath the dense tree canopy (throughfall) and solutions running down the tree stems (stemflow), was found to contain colourless soluble organic compounds that gradually fouled the column. Such a sample may influence the chromatogram obtained for the subsequent sample. The

"pseudo carry-over" was eliminated by passing these sample types through a clean-up cartridge (Sep-pak C18, Waters Associates). Further studies are envisaged to assess the impact of acid rain on the soil chemistry. Soil solutions were also suspect because evidence from other studies suggests that soil solutions from the litter and organic horizons contain humic and fulvic acid fractions, which also are liable to reduce column efficiency. However, in this instance, the guard column protects the separator column from long-term contamination and an initial clean-up is not required. Leachates from the lower mineral soil horizons may contain iron and aluminium colloidal complexes, but column efficiency does not appear to be adversely affected by these solutions.

Despite some gradual deterioration in guard and separator efficiency with time, it is possible to maintain analytical quality with careful monitoring and sample handling techniques, and effective column clean-up procedures. Typically, within a 24-h run, the precision was of the order of 0.5%, whereas day to day precision ranged from 0.5 to 1.3% (Table 1). To maintain good precision, the operating pressure and separating efficiency of the guard column were monitored daily, and remedial action taken immediately when required to minimise the contamination of the separator column. Several recommended clean-up procedures have been evaluated and 0.5 M $NaNO_3$ was found to be the most effective treatment. Hydrochloric acid (0.5 M) was also useful, but slightly less effective, whereas a mixture of 25% IMS - 1 M H_2SO_4 did not appear to remove any contaminants. It was also found to be essential to use high quality water for eluent preparation, and to remove particulate matter from both the eluent and the sample with an in-line filter.

Table 1. Estimates of precision

RSD, %	Cl^-	NO_3^-	SO_4^{2-}
Within-batch precision	0.53	0.64	0.44
Between-batch precision	0.50	0.98	1.30

Using the procedures outlined, we have operated with these improved 4A-type columns for eight months and processed approximately 6000 samples. Guard columns remained effective for about 2000 samples and the original separator column is still in use.