

THE USE OF ON-LINE COMPUTERS
IN ION SELECTIVE ELECTRODE POTENTIOMETRY

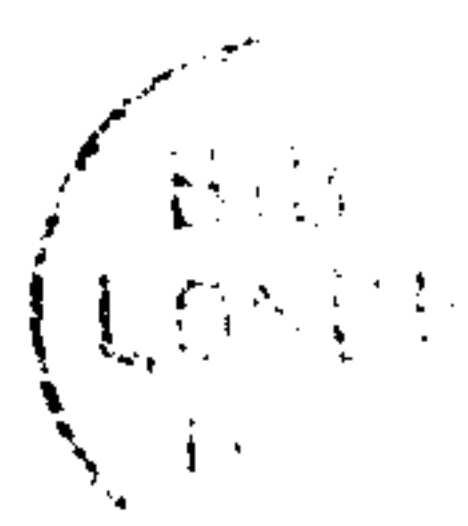
A Thesis Submitted for the Degree of
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ABSTRACT

The aim of the work presented in this thesis was to apply the control and data processing capabilities of an on-line minicomputer to analyses using ion selective electrodes in an automated continuous flow stream. The performance of ion selective electrodes in flow streams was examined and then analyses by bracketing standards, standard additions and single cup gradient titrations, using the on-line computer, were investigated. Particular attention was paid to the accuracy and precision of the techniques and the effects of interfering ions and complexing species upon the concentration measurements. Consideration was also given to the methods of calculating results from standard additions and to the calibration of single cup gradient titrations.

Then to illustrate the use of the above systems, various analyses of water, blood and urine samples were carried out.

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The work presented in this thesis was carried out between October 1975 and September 1978 and is original except where due reference is made.

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1.1 The Development of Ion Selective Electrodes

Ion selective electrodes, in the form of the glass pH electrode, have been in common use in the laboratory for many years, being introduced after Cremer (1) discovered in 1906 that the potential developed across a glass membrane separating two solutions depended upon the pH of the solutions. However it has been only in the last twenty years that electrodes selective to ions other than hydrogen with either solid or liquid membranes have become widely available. The development of these electrodes is briefly reviewed in Table 1.1. Now electrodes selective to many different ions have been prepared, Lakshminarayanaiah (2) listing twenty eight cations and twenty two anions for which at least one electrode has been described. In keeping with the great interest in ion selective electrodes in recent years many reviews have appeared in the literature, but especially useful have been those of Buck (3-7). Also several books have been published (2,8-15).

As well as conventional electrodes, electrodes for specialist purposes have been developed. These include microelectrodes which can be used for small samples or intra-cellular measurements (9,10) and ion selective field effect transistors (ISFETs) (16-20) in which the gate of a FET is replaced by the membrane, changing membrane potential regulating the current flow through the transistor.

The range of compounds which can be analysed with ion selective electrodes has been extended by placing an intermediate chemical system between the sample solution and the electrode. Various gas electrodes have been prepared by covering the electrode with a film of solution which is separated from the sample by a gas permeable membrane (21,22) or an air gap (23). The gas diffuses through the membrane or across the

Membrane Type	Comments
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Glass

By varying the composition of the glass membrane, electrodes sensitive to most of the alkali and alkaline earth metals have been prepared. Glass electrodes have been the subject of a comprehensive text (8).

Heterogeneous solid state -
a sparingly soluble salt or
metal chelate dispersed in
an inert binder material

The first working example, a calcium electrode consisting of calcium oxalate in a paraffin matrix, was described in 1957 (26). Other electrodes have been reviewed by Covington (27), but these designs have been largely superseded by homogeneous solid or liquid membranes.

Homogeneous solid state -
a crystal of either one or a
mixture of two sparingly
soluble salts

Silver chloride, bromide and iodide crystals were found to be responsive to the respective halide ion as early as 1937(28), but it took until the late 1960s for workable electrodes to be introduced. A highly specific fluoride electrode was prepared with a membrane of lanthanum fluoride (29), improved electrodes for the other halides were based upon mixed silver sulphide-silver halide crystals and copper, lead and cadmium electrodes were made with silver sulphide-metal sulphide membranes (30).

Liquid -
Charged ion exchanger

Certain organic ion exchangers were known to be highly selective for specific ions, but problems incorporating them into a suitable membrane were encountered until 1966 when Ross (31) described a calcium electrode prepared from a calcium didecylphosphate solution supported by cellulose dialysis tubing. Many other such membranes have been described, responding to various cations and anions (2).

(Continued overpage)

Membrane Type

Comments

Liquid -

'Neutral Carrier' ion exchanger

In 1964 it was discovered that certain neutral macrocyclic antibiotics caused selective ion permeation in biological membranes (32) and soon afterwards the first neutral carrier electrode, sensitive to potassium was described (33). Later designs based on the antibiotic valinomycin proved to be superior (34,35) and much work has since been carried out on both the investigation of natural macrocyclics and the preparation of synthetic neutral carriers, especially by Simon (36), again resulting in membranes responding to many other ions, particularly sodium and calcium.

Immobilised liquid

Both the above type of liquid membrane electrodes suffered from the disadvantages of being difficult to prepare and being mechanically weak. These factors have been overcome by immobilising the liquid exchanger and solvent in a compatible, inert matrix. The most successful of these immobilisation techniques has been that of Moody and Thomas (37), using a P.V.C. matrix to give a rugged, rubbery membrane with similar properties to the non-immobilised membrane, but with a longer life. Attempts to increase the lifetime of these electrodes further by grafting the exchanger onto the matrix have proved disappointing (38). However all the electrodes used in this work were of this type because of the wide range of species that could be analysed, and the simplicity of preparation and the durability of the membrane.

Table 1.1 The Development of Various Types of Ion Selective Electrodes

gap and dissolves in the film, affecting a chemical equilibrium, the position of which is monitored by the electrode. By surrounding the membrane with a layer of immobilised enzyme electrodes selective to many different organic species have been prepared (2,24-25). In the case of these enzyme electrodes the concentration of a product of the enzyme reaction is followed.

1.2 Membrane Potential

All ion selective electrodes respond roughly as predicted by the Nernst equation:-

$$E_{\text{mem}} = E_o + \frac{RT}{nF} \log_e \frac{a'}{a''} \quad 1.2-A$$

where E_{mem} is the potential across the membrane, E_o the standard membrane potential, R the gas constant, T the absolute temperature, n the charge of the ion, F Faraday's constant and a' and a'' the activities of the ion sensed on either side of the membrane. This was first shown to apply to a glass pH electrode in 1911 (39). The membrane potential cannot be directly determined, but is derived from the e.m.f. of an electrochemical cell comprising of the membrane separating the solutions and an internal and an external reference electrode. If reference electrode potentials, liquid junction potential and activity of the standard filling solution remain constant the cell potential (E_{cell}) is given by

$$E_{\text{cell}} = \text{constant} + \frac{RT}{nF} \log_e a_i = \text{constant} + S \log_{10} a_i \quad 1.2-B$$

where S is the slope of the electrode and a_i the activity of the ion of interest in the sample solution.

Early theories explaining the behaviour of glass pH electrodes assumed the membrane to be porous or semipermeable, e.g. the T.M.S. theory which divided the membrane potential into two surface potentials and a diffusion

potential across it (40-42). However the effect of interfering ions could not be satisfactorily explained by these theories and Nicolskii introduced the concept of potential formation by means of an ion-exchange reaction (43). His original equation has been extended to cover interfering ions with charges not equal to that of the primary ion:-

$$E_{\text{cell}} = \text{constant} + S \log_{10} \left[a_i + \sum_{j=1} K_{ij} (a_j)^{n_i/n_j} \right] \quad 1.2-C$$

where a_i is the activity of the primary ion of charge n_i , a_j the activity of any other ion in solution with charge n_j and K_{ij} is the selectivity coefficient of the membrane.

This equation was derived for solid state membranes where the position of the ion exchanging groups is fixed, but it has also been shown to hold for liquid membranes where the sites are mobile. The theory of solid and liquid membrane potentials has been reviewed by Eisenmann (44) and Lakshminarayanaiah (2) and Simon has derived an equation describing the e.m.f. of an electrochemical cell containing any type of ion selective electrode (45).

1.3 The Performance of Ion Selective Electrodes

The usefulness of an electrode to perform a given analysis depends on several of its properties, including its working range, selectivity and time response. The lower limit of detection has usually been ascribed to the solubility of the ion exchanger in the solution and in the case of solid state membranes relationships between the solubility product and other properties of the membrane and the detection limit have been derived (46,6) and give fair agreements with the experimental results. However the solubility of the components of liquid membranes is less well defined and it is possible that the interfering effects of hydrogen or hydroxide ions cause the levelling off of response. In practice the

lower limit of detection of liquid membrane electrodes is about 10^{-5} to 10^{-6} M while solid membranes may be sensitive to lower activities. The upper limit of an electrode's range is often regarded as being when uncertainties in the value of the activity coefficient occur due to the high ionic strength of the solution, although deterioration of the membrane surface and the entry of counter-ions into the membrane may also contribute.

Naturally any electrode is practically useless if it is incapable of distinguishing adequately between the primary ion and any interfering ions. The factors which determine the selectivity of a membrane have been reviewed with the theory of membrane potential (see 1.2) and many methods have been described for their experimental determination (47). However the value obtained usually varies with the method used, the age of the membrane and the activities of the primary and the interfering ions, as noted by Buck (48). However it is usually possible to ascertain from these values whether any other ion in a sample will interfere significantly with the determination of the ion of interest.

The response time of an electrode is another important characteristic since an electrode which takes an excessive time to come to a stable reading after a change in activity is obviously of little practical use. Buck (6) has shown that there are at least four possible time dependent processes that can occur in a membrane when a sample changes, but these are generally rapid compared with external factors such as the time required for the diffusion layer on the surface of the membrane to assume the concentration of the new solution and the migration of ions across any surface film of the membrane e.g. the hydrolised layer on a glass membrane. The response time of an electrode thus depends upon its type, the rate of stirring or flow of the sample solution, the activities of the primary ion before and after the change, the presence of other ions in solution,

the history and preconditioning of the membrane and the ambient temperature.

To confuse matters further there is no generally accepted definition of time response. The 1976 I.U.P.A.C. recommendations for the nomenclature of ion selective electrodes (49) include the definition of time response as 'the time to the first instant at which the potential of the cell becomes within 1mV of the steady state value', with such variables as the stirring rate, etc. defined. A rather serious shortcoming of this definition is that the response time becomes a function of the electrode slope; for a change in activity of an order of magnitude a monovalent electrode will be within 1.7% of its equilibrium potential but a divalent ion electrode only within 3.4%. Because of the small gradient of the response curve near the equilibrium potential these differences would lead to two electrodes with identical response characteristics being assigned substantially different response times. Definitions of response time which specify the time to a certain proportion of the potential 'step' overcome these problems. $T_{\frac{1}{2}}$, the time to half response has been used in theoretical work (50); while t_{95} , the time to 95% of response gives a better indication of the response in practical work (51).

Experimentally it has been found that solid membranes are capable of responding within a fraction of a second, while liquid membranes are somewhat slower, the response time being measured in seconds or even minutes. Response times may be greater if decreases in activity, rather than increases, are to be monitored or if interfering ions are present (51). However if the response of an electrode is too slow for a given purpose it may be possible to improve it by raising the temperature.

1.4 Analytical Techniques using Ion Selective Electrodes

1.4.1 Direct Potentiometry

The simplest method of analysis is to measure the cell potential of the ion selective electrode and the reference electrode when immersed in the sample. The activity of the sample may be calculated from either equation 1.2-B or 1.2-C if all the terms are known, but it is more normal to prepare a calibration plot over the range of activities of interest. If concentration rather than activity is required then both calibration and measurements must be carried out in an 'ionic buffer' of constant high ionic strength which ensures that the concentration-activity relationship is the same for all samples and standards, except in the case of matrix-ion interactions in the sample (e.g. complexation). If ions are present in the sample which interfere with the response of the electrode then these have to be 'masked' by some means or, if of a fairly constant concentration, accounted for in the calibration.

However all electrodes tend to drift and some show hysteresis (calibrating differently upon increasing and decreasing activities). This can lead to large errors, especially since the logarithmic nature of the Nernst equation means that small errors in the measurement of potential lead to substantial errors in the calculated activity. The accuracy of this method thus depends on the frequency of the calibration of the electrode and the closeness of the concentration of the sample to the calibrating solutions. Thus possibly the best method is to 'bracket' the sample with two closely matching standards, this technique being used in the work described in Chapter 4.

1.4.2 Standard Addition Techniques

Standard addition methods usually involve the addition of one or more aliquots of the species of interest to the unknown sample, ensuring that the increment does not significantly alter the activity coefficient or the liquid junction potential. Multiple additions have the advantage of minimising the effect of random experimental errors, but results can be more difficult to treat. One procedure is the graphical method developed by Gran (52,53). If additions of volume V_i of a standard of concentration C_i are made to a volume V_x of sample of concentration C_x the electrode potential E after the n th addition will be

$$E = E_o + S \log_{10} \frac{C_x V_x + \sum_{i=1}^{i=n} C_i V_i}{V_x + \sum_{i=1}^{i=n} V_i} \quad 1.4-A$$

which can be rearranged as

$$(V_x + V_s) 10^{E/S} = 10^{E_o/S} (C_x V_x - C_i V_s) \quad 1.4-B$$

where $V_s = \sum_{i=1}^{i=n} V_i$. A plot of $(V_x + V_s) 10^{E/S}$ against V_s will be linear with an intercept on the volume axis at V_e , where C_x is given by

$$C_x = - \frac{V_e C_i}{V_x} \quad 1.4-C$$

This method requires prior knowledge of an accurate value of the electrode slope. Without this information calculation of results becomes rather more complicated, requiring the use of a computer programme (54,55).

It has been claimed that the standard addition technique gives the total concentration of the species of interest, even in the presence of complexing agents (56), but it has been pointed out that this is only true if the fraction of the ion concentration existing in the

uncomplexed state remains constant (57). This, the effect of interfering ions and the use of standard additions in continuous flow analysis is considered further in Chapter 5.

1.4.3 Differential Potentiometry, Null-Point Potentiometry and 'Concentration Matching'

Differential potentiometric methods involve the replacement of the reference electrode by a second ion selective electrode, selective to an ion which remains at constant activity in the solution (58), the cell then being used for direct potentiometry, standard additions or as a titration monitor (58-60). This approach leads to the elimination of the liquid junction but errors may occur due to the 'reference' electrode drifting and from small changes in the activity of the ion it senses.

Another technique in which no reference electrode is used is null-point potentiometry, where two electrodes with identical response characteristics are placed in two half cells separated by a liquid junction (61,62). The sample solution is placed in one half and the activity of the solution in the other half varied until a zero potential difference indicates the activities to be equal. Highly accurate results have been obtained with this method, but it is difficult to set up, takes a relatively long time to run and error may arise from interfering ions in the sample.

To overcome these problems a 'concentration matching' approach has been suggested (63). A series of electrodes sensing both the primary ion and interfering ions were used along with a reference electrode. Electrode potentials were obtained for the sample and then a computer controlled flow system mixed standards to produce a solution which gave the same responses. This method was claimed to give fair accuracy and have great advantages over direct potentiometry when there were gross interferences.

1.4.4 Potentiometric Titrations

When compared with direct potentiometric methods potentiometric titrations generally offer greater accuracy and precision at the cost of increased time and inconvenience. They also allow the determination of some species for which no electrodes are available, if a titration product or the titrant itself can be monitored, and can be used in non-aqueous media where direct potentiometric data may be poorly defined.

The accuracy of the titration is limited by the standardisation and the volume measurement of the titrant, the completeness and known stoichiometry of the titration reaction and, perhaps most crucially, the determination of the location of the end-point. Compared with direct potentiometry titration results are relatively unaffected by drift, the presence of interfering ions and changes in ionic strength.

In most cases the end-point is taken as being at the position of the maximum rate of change of the electrode potential - volume of titrant added titration plot. However due to dilution effects this inflection point has been shown to precede the true equivalence point (64), but the errors involved are small and are unlikely to be detectable experimentally. Possibly more serious is the effect of ions which interfere with the electrode response, which can distort the shape of the titration curve, reducing the sharpness of the break and affecting the position of the inflection point (65). However to cause substantial errors the interference has to be so gross that direct potentiometric methods may be difficult or impossible and the effect may be lessened by calibrating the titration system with standards containing similar levels of interferent.

A convenient way of accurately locating the inflection point, especially if the titrant is being added at a constant rate, is to take the differential of the curve, when it is given by the position of a peak (maximum or minimum), or by taking the second differential, when it is given by the zero-crossing point.

These methods of end-point detection are discussed further in Chapter 6, along with the gradient titration, a type of potentiometric titration for use in continuous flow analysis.

1.5 Automated Analysis using Ion Selective Electrodes

Automation of chemical analyses, implying the full or partial elimination of human intervention, has gained considerable popularity in the last twenty years (66). Compared with manual methods there may be improvements in sample throughput, in cost with lower operator expenses and reproducibility of results with the elimination of human error. Most automated methods fall into one of two types - discrete or continuous. In discrete methods each sample is pretreated in separate containers, which are moved to each treatment unit and the measuring unit by mechanical means. Continuous methods rely upon the passage of the sample through a flow system, where it can be treated by the necessary reagent additions before being pumped through a flow-through detector. In comparison with discrete methods these are generally slower and may suffer from cross-contamination, but they are more reliable mechanically and are less expensive.

With ion selective electrodes as the detector discrete techniques have been mostly limited to 'batch' titrators. As long ago as 1947 an automatic titrator with a glass electrode detector was described (67). A motor driven syringe burette gave a stepwise delivery of titrant, the electrode potential being measured on a chart recorder. The addition of the titrant was controlled by the position of the chart recorder pen by means of a cam and microswitch system, so that addition was slow near the end-point, to give greater precision. Since then advances have been made in both the delivery and measuring systems. Automatic burettes, with solenoid operated taps, allow small or large quantities of titrant to be added and the use of control circuitry to recognize the end-point

by differential means has led to direct digital readout of the concentration.

Continuous titrimetry was first performed by mixing a constant flow of sample with a variable flow of titrant, using the signal from the electrode to change the rate of flow of the titrant until equivalence was reached (68).

However a much more widely used method of analysis using continuous flow has been provided by the Technicon 'Auto Analyzer', based on the work of Skeggs (69). A proportioning pump introduces reagents and samples in the appropriate volume ratios, the interaction of samples being minimised by the introduction of air bubbles, which also aid the complete mixing of the sample and reagents. The 'Auto Analyzer' was introduced with a colormetric detector, but ion selective electrodes adapted for flow through use have been extensively used since. One advantage of the 'Auto Analyzer' is its convenience, modules being available for sampling, separation of unwanted sample components and sample heating. Another is its flexibility, as well as choice of detectors, flow systems for various analytical techniques can be set up (see Chapters 4-6).

A recently introduced method of continuous analysis has been the Injection Flow system (70-73). A small known volume of sample is rapidly injected into a relatively fast flowing reagent stream, without air segmentation. A zone of sample is formed in the stream, which is mixed with the reagent by the turbulent flow in a small diameter tube and passed to the electrode. Here the height of the response peak gives the sample concentration, the system having been calibrated with standards. However to give accurate results several factors have to be optimised e.g. the line length, the tube diameter and the injection port geometry and others have to be reproduced within fine limits e.g. the sample volume, the speed of injection and the

electrode response. Using a rotary valve to automate the injection of the sample has greatly improved the sample introduction (74) and the technique is now capable of fair accuracy and precision.

As well as the analysis of individual samples ion selective electrodes have been used to automatically monitor the concentration of industrial process streams (75), the signal from the electrode either being displayed as a record of concentration with time or being used to trigger an alarm if certain concentrations are reached. Usually a small sample stream is bled off the main stream, treated as necessary and passed through the electrode cell, the electrode being recalibrated automatically after certain periods. However if the electrode does not suffer from interferences from the sample stream constituents, is insensitive to changes in pressure and flow rate and is not susceptible to drift it may be inserted directly into the sample stream, although it must be removed for recalibration. In these applications ion selective electrodes show several advantages compared with other methods of analysis - they need little attention, can be set up by unskilled operators, are small in size and are inexpensive.

The use of computers in association with automated methods of analysis has spread rapidly in recent years, particularly with the introduction of minicomputers and more recently microcomputers. At first most of the applications were limited to the off-line processing of data, but greater advantages have been accrued by using on-line data acquisition and experiment control. The use of computers in analytical chemistry and with ion selective electrodes are considered in the next two sections.

1.6 The Use of Computers in Analytical Chemistry

The first digital computers to be widely used by analytical chemists were operated exclusively in an 'off-line' mode, which is characterised by the experimental data being transmitted to the computer by the operator through some intermediate storage system such as punch cards or paper tape and by the processing timetable being determined by the computer according to the length and priority of the job. Typically large scale installations some distance from the laboratory were used and still are used because of their availability, the ease of programming in high level languages such as FORTRAN and the ability to carry out very complex data processing.

Unfortunately to utilise such an expensive piece of equipment efficiently any computer-experiment interaction was impossible, until the introduction into the laboratory in the early 1970s of relatively inexpensive minicomputers. Without the restraints imposed by other users an 'on-line' configuration was now possible, with an electronic interface taking data from the experiment and providing control of certain experimental parameters.

Advantages of the on-line approach fall into three main areas. The electronic interface is much more compatible with the computer in terms of speed than a human intermediate and the time elapsing between measurements being taken and results being presented is much lower. Secondly the control and data processing capabilities of the computer enable the complete automation of an analytical technique. Thirdly real-time interaction between the computer and the experiment is possible so that the computer can execute control modifications before an experiment has been completed and change the course of an experiment to provide better conditions.

One important aspect of using minicomputers for on-line work is that, as in the case of the work presented in this thesis, all

of the software must be written in assembly language. in this case the assemblers for higher level languages required more memory than was available, but also these languages have little or no provision for the input/output functions necessary for on-line operation and tend to be inefficient in their use of the available core space. Other disadvantages are that very complex data processing is not possible due to the limited memory and that experimental data may be completely lost in the case of computer failure.

Within the last decade great advances have been made in the development of integrated circuits, leading in 1971 to the appearance of the first microprocessor, a single chip capable of performing arithmetic and logical functions in a bit-parallel fashion under programme control. This device was originally designed to replace hard-wired logic circuits in instrumentation, a single microprocessor chip with the relevant programme stored in unalterable read only memory (ROM) being able to carry out the same functions as a complicated logic circuit consisting of many logic elements. Due to their low price and small size these have been installed in many scientific instruments and consumer goods, leading to a new type of computer operation, the 'in-line' mode where to the operator the computer is indistinguishable from the instrument, the operator may communicate with and direct the operation of the computer, but it is programmed at the factory.

At first the true power of the use of a microprocessor with associated random access memory (RAM) and input/output circuits as a user programmable microcomputer was only recognized by enthusiasts who built such microcomputers from the first commercially available microprocessors. Now complete microcomputers are available commercially and the cost of the necessary peripherals have in general become the limiting factor in the price.

Advances in the technology of microprocessors and their associated circuitry have resulted in the performance of microcomputers being equal, if not superior, to that of minicomputers in many aspects with respect to their use in on-line chemical instrumentation (76). One possible disadvantage of the microcomputer at present is the speed of execution of a programme, which is limited not so much by the cycle time of the microprocessor as by its word length. However this disadvantage is hard to quantify since some microprocessors have rather more complete and efficient instruction sets than some minicomputers.

The main advantages that microcomputers display in automated chemical analysis are their low cost, small size and that once software has been developed for a particular application it can be cheaply reproduced for the production of further units, even if the development of the software is more complicated than with a minicomputer.

1.7 The Use of Computers in Analysis with Ion Selective Electrodes

Calculations of results from the different techniques of analysis using ion selective electrodes are often quite complex because of the logarithmic nature of the Nernst equation. Therefore the use of off-line computer programmes to process data have become quite common over the last fifteen years. Dahms was the first to record the use of such a programme in 1967 (77), using it to evaluate data from an automated flow system for the determination of hydrogen, potassium, sodium and chloride ions in blood using a bracketing standard technique.

The manual processing of data from standard addition experiments was limited to single additions by direct calculation or for multiple additions by a graphical method (see 1.4.2). The accuracy of both of these methods was limited by the accuracy of the value of the electrode slope used in the calculation, but the use of a computer programme such as that described by Brand and Rechnitz (54) allowed the estimation of the best values for standard potential, slope and sample

concentration, the three unknown parameters, by a successive approximation technique.

Linearisation of titration curves as well as standard addition plots are possible using Gran's method (52), but again errors occur assuming a constant electrode slope over the range of concentrations covered. To overcome this Isbell et al. (78) wrote a comprehensive programme covering complexation and precipitation titrations with electroactive titrant or analyte, applying the rigorous nonlinear least squares method of Wentworth (79) to process the data.

The first on-line use of a minicomputer was proposed by Anfalt and Jagner in 1971 (80). They described a titrator with a measurement system consisting of a voltmeter interfaced to a Hewlett Packard HP 2114B computer, which could be used with any sensor delivering an input of up to 100V. However no practical uses of the apparatus with ion selective electrodes were described, only the photometric determination of alkaline earth metals in sea water (81). Later the equipment was extended to allow for the automatic generation of titrant by constant current electrolysis and was used to investigate the complex formation between tin(II), acetate and hydroxide ions using a tin amalgam electrode(82).

A similar apparatus was used by Keller et al. for acid-base titrations (83), using a minicomputer to control the coulometric generation of hydrogen or hydroxide ions as reagent and to monitor the signal from a glass pH electrode. This was found to be useful in conditions where the sluggish response of the electrode led to difficulties in manual methods and was also used to determine the dissociation constants of various weak acids.

Other uses of on-line minicomputers in the field of potentiometric titrations have included the successive determination of calcium and magnesium in drinking water by compleximetric titration to two

equivalence-points (84) and the titration of mixtures of a strong and a weak acid (85), where not only the concentrations of the acids but also the dissociation constant of the weak acid was found, even in some cases where visual inspection of the titration curve did not indicate the presence of two components.

Rather more flexible, although rather more involved than any of the above systems was that described by Frazer et al. (86). Both titrations and standard additions could be performed and a high degree of operator interaction in the experiment was possible, the computer performing the routine operations of control, data acquisition and mathematical calculation while the operator was left to observe whether the experiment was running satisfactorily. After initiation the computer automatically continued with a cycle, repeated up to 512 times, of addition of a set volume of titrant, waiting for a preselected time for the electrode to respond and then inputting and storing the data and displaying it on a oscilloscope, so that the operator could check that the right titration parameters had been chosen, that noise was not excessive and judge when the titration was complete. Then the data could be smoothed and a second derivative plot or a Gran function displayed along with the original data. A novel feature was the plotting of an 'error function' based on the difference between the intercepts obtained from Gran's plots of the data from two partially overlapping segments of the experimental curve. A minimum in the error function indicated the region of most ideal Nernstian response and thus the best area to evaluate results from.

To illustrate the principles used in this work titrations of chloride and bromide with silver nitrate and aluminium with fluoride were carried out, as well as the determination of fluoride by standard additions. Further work has shown the superiority of the error function technique over the standard Gran's plot and differential methods in

locating the equivalence point in silver-bromide titrations (87) and the apparatus has been used for the determination of phosphate in the presence of large amounts of fluoride by titration with lead perchlorate (88) and the determination of chlorine in water by standard addition (89).

Zipper, Fleet and Perone had described the use of an on-line minicomputer to monitor analyses by standard additions using ion selective electrodes in a flow stream (55) before the work of Frazer. In contrast to his method of making up to 500 additions with a time interval of only a few seconds between each, the electrodes were allowed to reach full response before the next addition so that only six additions were deemed necessary. Although analyses of $10^{-3}M$ fluoride solutions gave around only a 1% relative error, some criticism must be made of the flow cell since the time to 100% response for the fluoride electrode used was 12 minutes, so that a complete analysis took over 1 hour. The Gran's plot was rejected as the method of calculating the results because of its assumption of a constant electrode slope of a known value, and the rigorous least squares adjustment method of Wentworth (79) applied to the problem. Various data input subroutines were described, although the one used in the analyses involved the recognition of the plateau by the operator.

Another on-line system for standard additions was reported by Ariano and Gutknecht (90), this time using a potassium liquid membrane electrode in a beaker. After acquiring the cell potential for the unknown solution a predetermined volume of the standard solution was added from an autoburette and a second reading taken. Subsequent additions were not of a preset volume, but of a calculated volume to give a preset difference in potentials between additions, thus automatically adjusting the size of these additions as a function of the unknown concentration. This resulted in points being well distributed on the potential vs. log concentration standard addition plot

instead of becoming grouped together as more additions of equal volume were made. However results, calculated by the method of Brand and Rechnitz (54), for a 10^{-3} M potassium solution showed little or no improvement with the optimised method than with adding a constant volume, perhaps because of the influence of the first preset addition, perhaps because of the simple nature of the algorithm used to calculate the other additions or perhaps due to other factors such as the electrode response or the performance of the burette limiting the precision obtainable.

Perone and co-workers have used on-line minicomputers with ion selective electrodes in some novel ways. Under certain conditions the presence of iron(III) was found to give high readings with chloride electrodes (91). This interference manifested itself in the response curve of the electrode when the addition of a solution containing iron(III) and chloride ions was made to a chloride solution, the initial fast negative response to the chloride ions not being completed before a slower positive response to iron(III) occurred. To analyse chloride samples containing iron(III) the electrode was automatically calibrated, then a sample injected into a chloride solution and the response of the electrode stored in memory. The corrected value of the potential of the electrode without the effect of the perturbation by the iron(III) could then be obtained by back extrapolation of the positive slope to zero time.

Also a system was assembled to carry out the concentration matching technique described in section 1.4.3 (63). A potassium liquid membrane electrode and a sodium glass electrode were incorporated into a flow stream, a sample passed through, then various mixtures of potassium and sodium solutions until both sample potentials were replicated. A hierarchial minicomputer-microcomputer system was used, the microcomputer on instructions from the minicomputer performed input/output routines to the interface, the valves and pumps controlling the flow stream

and the electrode input multiplexer and if necessary returned information to the minicomputer, leaving the minicomputer in complete control of the experiment and to perform the data processing.

Other systems using microcomputers with ion selective electrodes have been described in the literature and have recently appeared commercially. Betteridge and co-workers described a microprocessor-controlled titrator (92), used for the sequential titration of adipic and boric acids as required for the control of the manufacture of nylon. The microprocessor controlled the addition of titrant to the beaker in 0.1ml increments and took in the electrode potential after a time interval varying from 0.35 to 5.5sec after addition. It was found that the precision and accuracy of the titrations increased as the time interval increased and the electrode response was more nearly complete. Detection of end-points was from smoothed first differential plots, a value for the equivalence volume being found to 0.01ml by linear interpolation of the two points surrounding the peak.

Another automatic titrator incorporating a microprocessor is the Radiometer DTS Digital Titration System described by Firstenburg and Malmvig (93). Only the first two additions of titrant were fixed, subsequent additions being of a calculated volume which varied inversely with the slope of the titration curve within a certain preset range, so that the rate of addition near an inflection point was relatively low. Between additions a reading was taken after the electrode potential had stabilised (i.e. was changing at a rate of less than 0.1mV sec^{-1}), unless this took more than 50sec when a reading was taken anyway. Equivalence was detected by taking the second derivative of the titration curve, and again precision was increased by finding the zero crossing point by linear interpolation of the points either side. After a selected number of inflection points had been passed or when the burette had emptied the titration was stopped.

The above systems show the potential of using microprocessors, in that automatic titrators have been produced that are relatively inexpensive, being of around the same price as 'set level' titrators but offering more flexibility and sophistication in end-point detection. However if the titration curve shows an abrupt change at, or is assymmetric around, the equivalence point some of the titration systems with on-line minicomputers would give more accurate results due to their greater data processing abilities.

Another application of the microprocessor is the Orion Research 901 Ionanalyzer, an instrument similar in appearance to a digital voltmeter. However instead of indicating electrode voltage a value of sample concentration is given from one of several operating modes. After precalibration a direct reading of concentration can be obtained, or if an accurate value of the electrode slope is entered, incremental methods based on a single addition or subtraction can be employed. While this machine takes in data from the electrodes and calculates results, much operator attention is needed for the entry of various parameters and for the changing of samples, addition of standards etc.

In the future it is certain that microprocessors will be incorporated into other automated systems using ion selective electrodes and that the level of sophistication will become higher.

2.1 The Electrodes and the Continuous Flow System

The ion selective electrodes used in this work were all of the immobilised liquid membrane type as supplied by E.D.T. Research Ltd., details of the membranes being given in Table 2.1. They were of the design described by Bound (60), with the ends threaded so they could be inserted into the flow cell shown in Figure 2.2.

All measurements were made relative to an EIL saturated KCl Calomel Electrode, model number 33-1370-710, fitted with a threaded adaptor to fit into the flow cell. This was found to give a satisfactory performance, although the use of other reference electrodes and the variation of the liquid junction potential between the sample solution and the internal filling solution have been discussed by Covington (27), Ives and Janz (94) and Midgley and Thomas (95).

The flow cell consisted of a number of perspex blocks on a rail, rubber 'O' rings preventing any leakage from the cell when the blocks had been tightened together by means of four nuts on a pair of threaded rods (see Figure 2.2). The number of blocks on a rail could be varied from two to six, to accomodate a corresponding number of electrodes. The blocks used were of two varieties, described in Chapter 3, found to give rise to different electrode response times.

The flow system was supplied by a standard Technicon Autoanalyzer III Proportioning Pump, sample solutions etc. being changed by a Technicon Autoanalyzer Sampler II. It was found that the use of a water wash between samples as recommended by Technicon caused problems when taking direct potentiometric readings because the electrode response was slow due to the change in concentration being large. Hence special timing cams were constructed and the sampler modified internally to eliminate the washing period from the sampling cycle. Also it was found advantageous when performing on-line gradient titrations to advance the sampler under

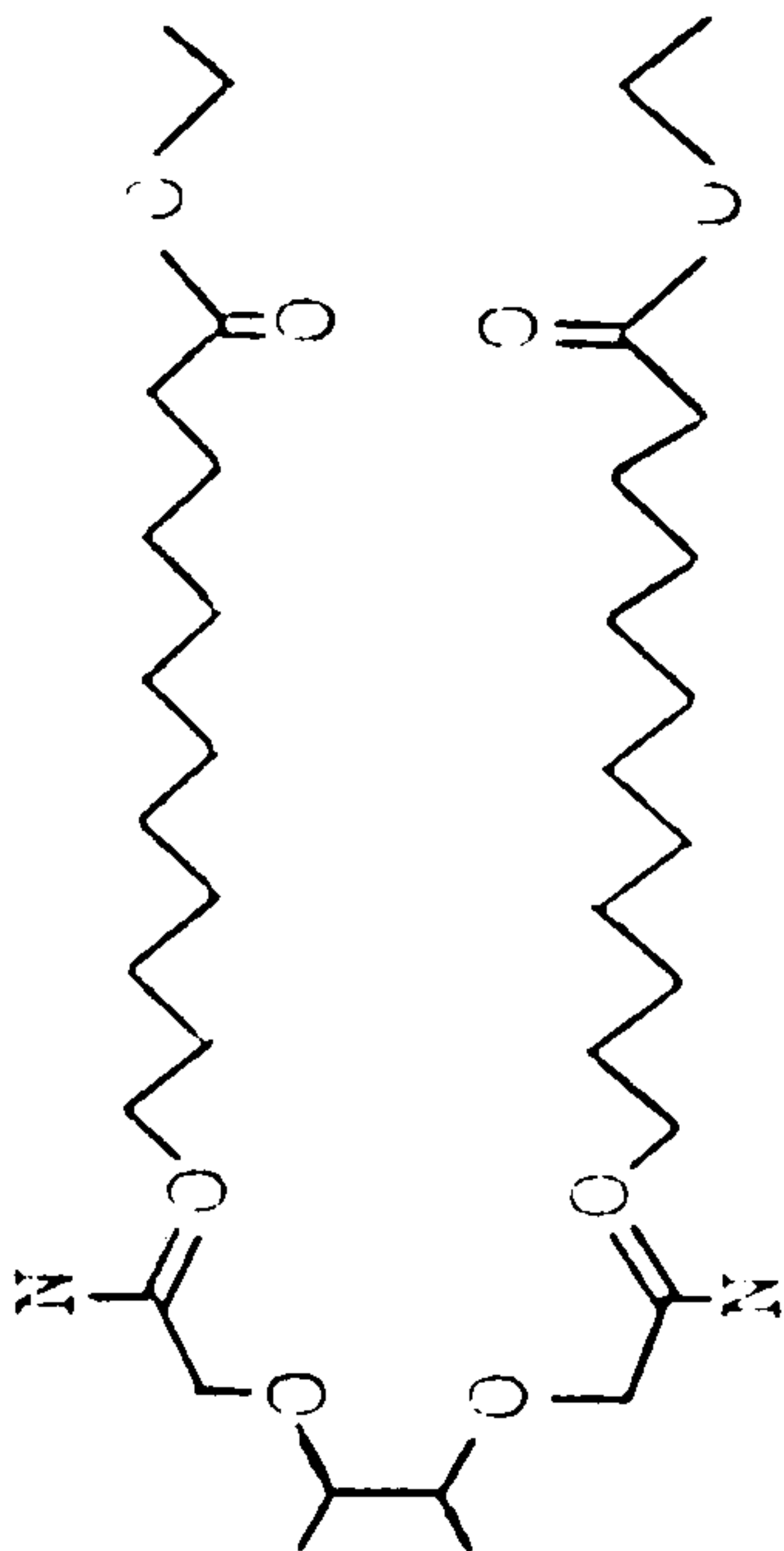
Electrode	Ion Exchanger	Ion Exchanger Type	Ref.
Ca^{2+}	1. Calcium di(n-octylphenyl)phosphate	Charged carrier	(96)
	2. 	Neutral carrier	(36)
K^{+}	Valinomycin	Neutral carrier	(34)
Water Hardness	Calcium didecylphosphate	Charged carrier	(60)
Ba^{2+}	Nonylphenoxypolyethylene ethanol	Neutral carrier	(97, 98)
NO_3^{-}	Tetradodecylammonium nitrate	Charged carrier	(99)

Table 2.1 Ion Selective Electrodes used

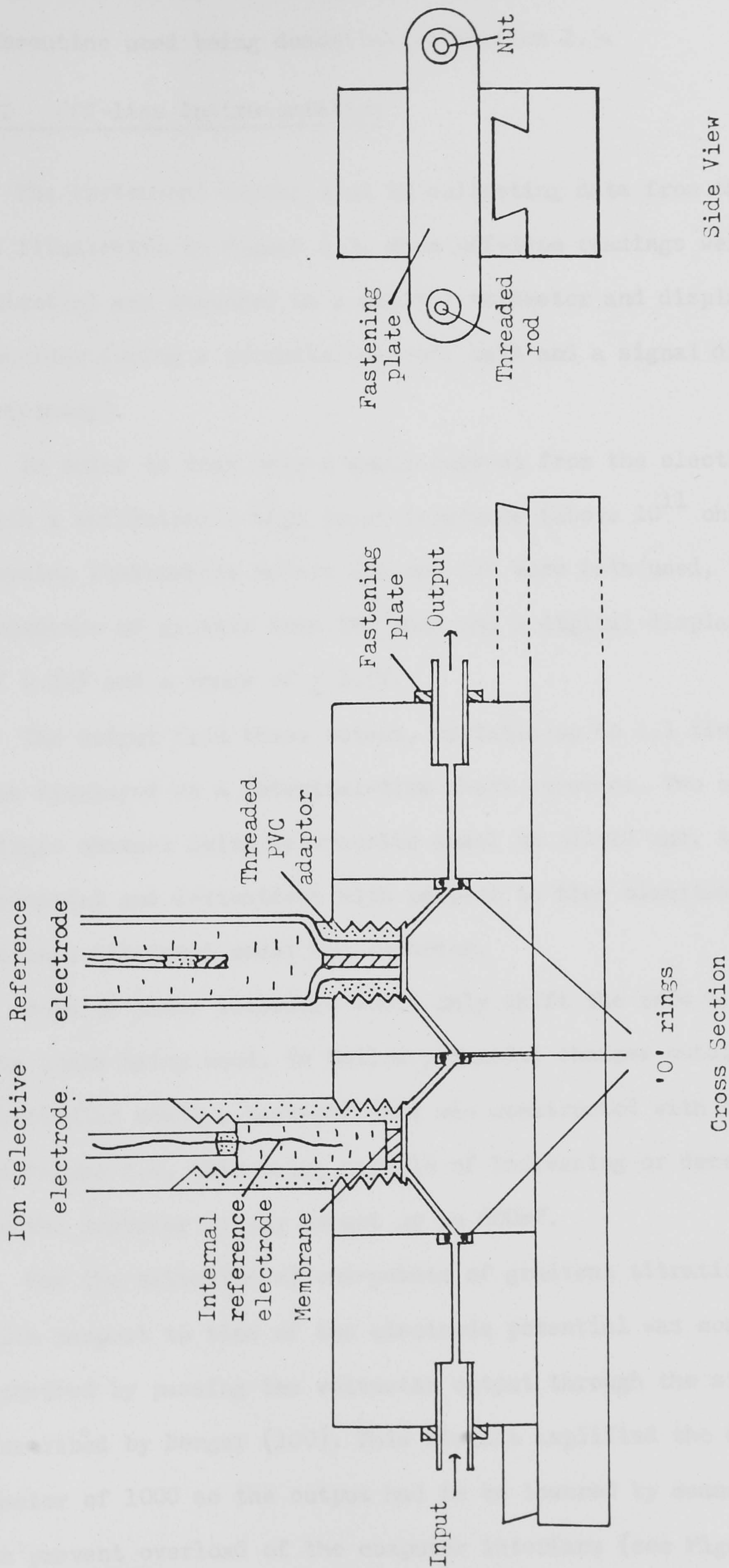


Figure 2.2 Electrode Flow Cell

computer control, the necessary modifications to the sampler and the subroutine used being described in Section 2.5.

2.2 Off-line Instrumentation

The instrument layout used in collecting data from the electrode system is illustrated in Figure 2.3. When off-line readings were being taken the potential was measured on a digital voltmeter and displayed on a chart recorder (using a potentialback-off unit and a signal differentiator as necessary).

In order to draw only a small current from the electrodes a voltmeter with a sufficiently high input impedance (above 10^{11} ohms) is required. Corning Instruments models 101 and 112 were both used, both having an input impedance of greater than 10^{12} ohms and a digital display with a resolution of 0.1mV and a range of $\pm 1.5V$.

The output from these meters, variable up to 1.1 times the input signal, was displayed on a potential-time chart recorder. Two models were used, a single channel Smith Servoscribe model RE 511-20 and, to display electrode potential and derivatives with respect to time simultaneously, a three channel Rikadenki model 401 recorder.

Both of these recorders would only shift the zero by around $\pm 100\%$ of the scale being used. To follow potential changes outside this range on a particular scale a back-off unit was constructed with the circuit shown in Figure 2.4, this being capable of increasing or decreasing the signal to the recorder by any amount up to 600mV.

For the detection of end-points of gradient titrations the differential with respect to time of the electrode potential was monitored, this being achieved by passing the voltmeter output through the signal differentiator described by Berger (100). This circuit amplified the differential by a factor of 1000 so the output had to be lowered by means of series resistors to prevent overload of the computer interface (see Figure 2.5). To smooth out electrode noise the input to the unit was damped.

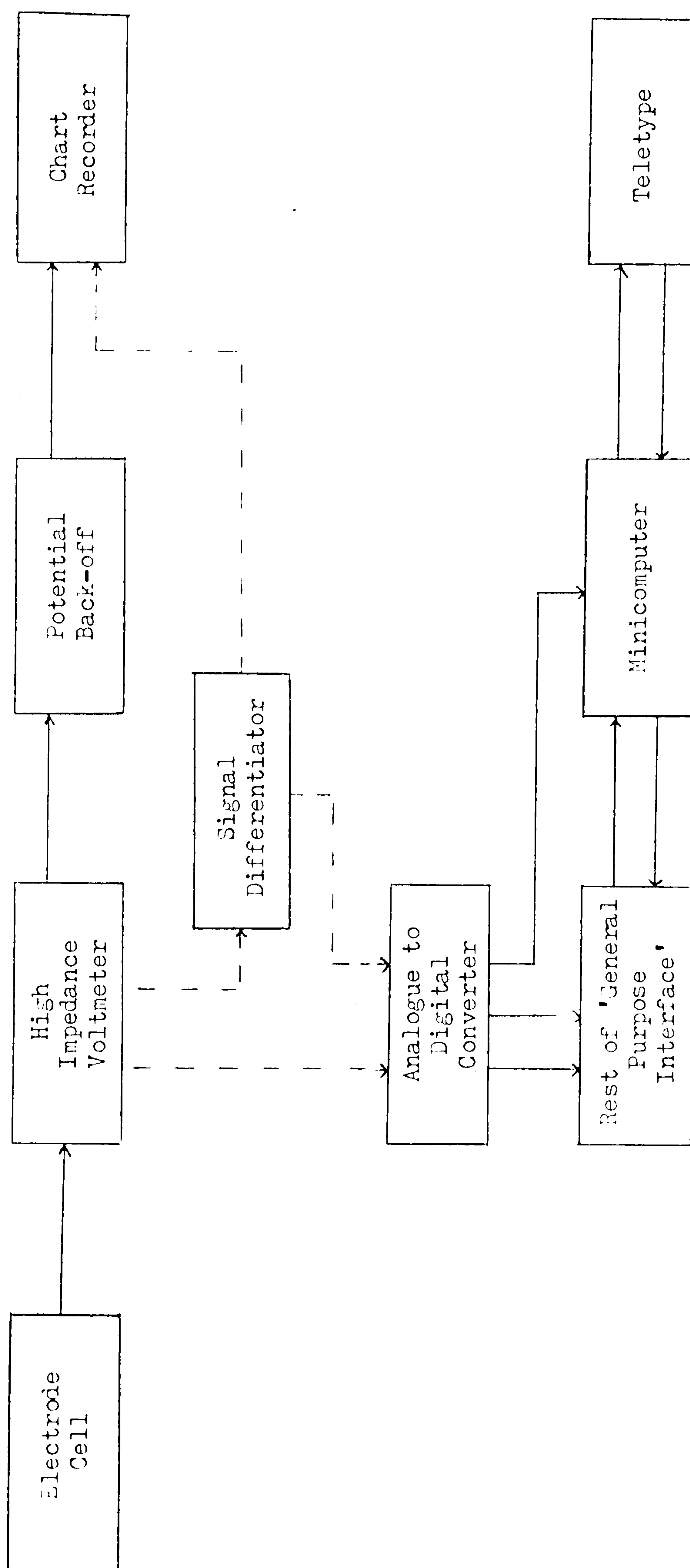


Figure 2.3 Instrumentation

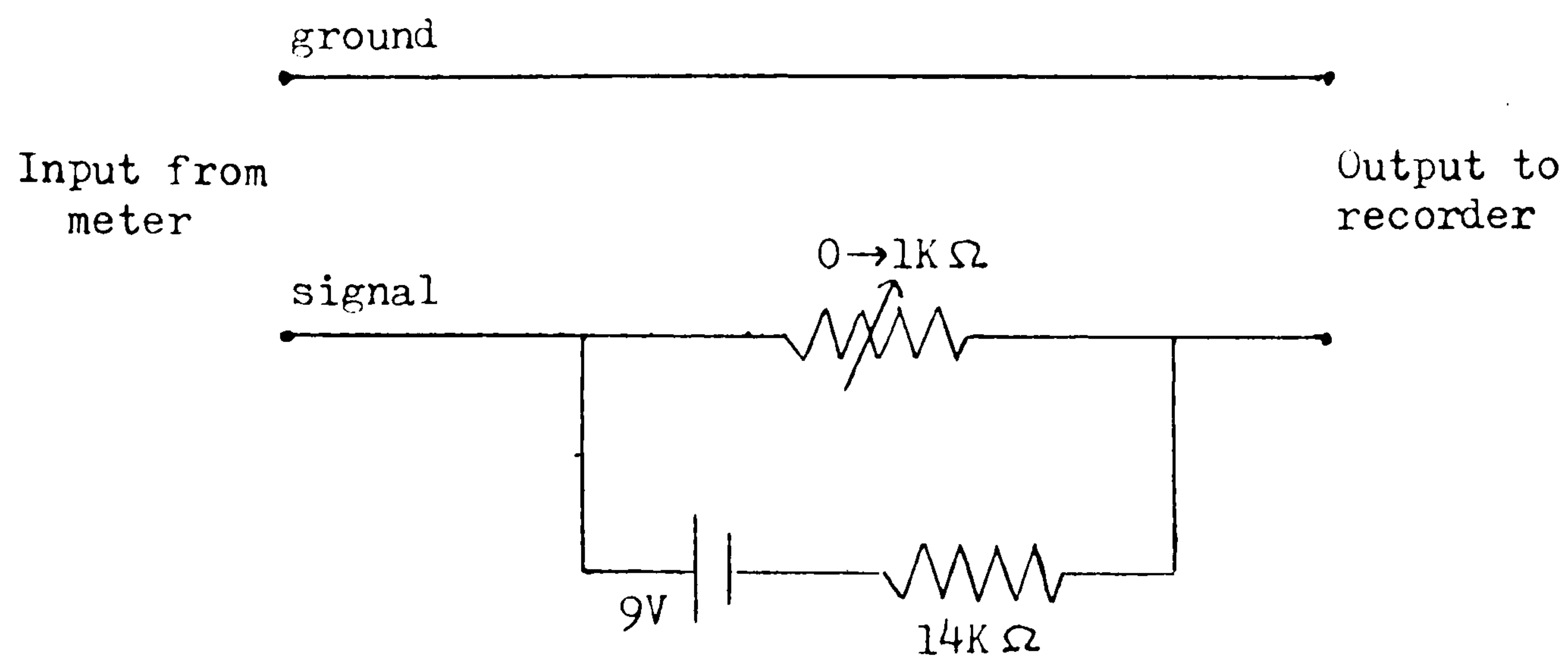


Figure 2.4 Potential back-off unit

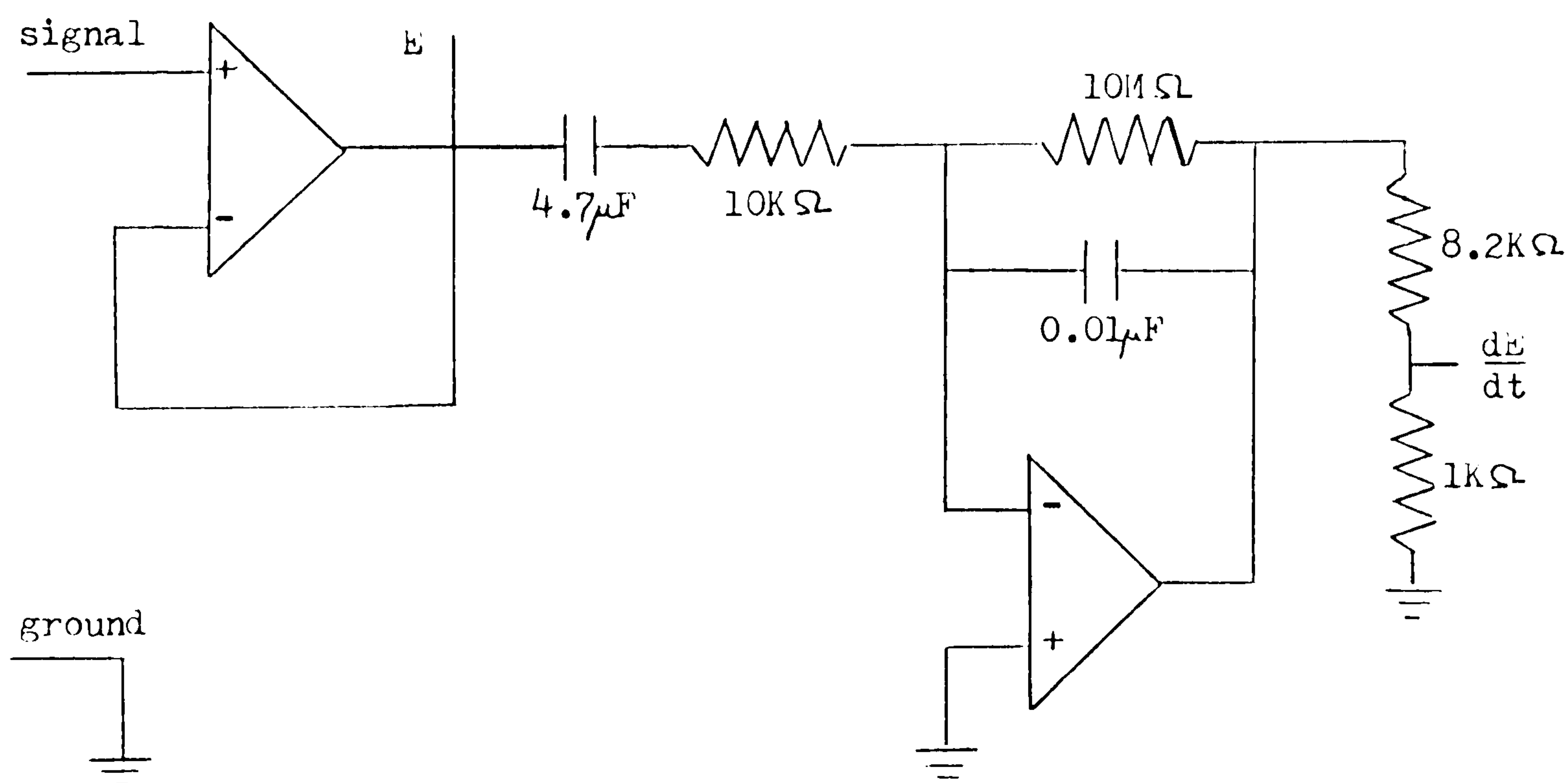


Figure 2.5 Signal differentiator

2.3 On-line Instrumentation

The signal from the voltmeter output or the differentiator was converted to a digital form and passed to the minicomputer by the analogue to digital convertor (ADC) (see Figure 2.3). The electrodes could have been connected directly to the ADC, but the voltmeter output was supplied so that the electrode potential could be conveniently monitored on the voltmeter and the chart recorder. The ADC was part of the general purpose interface (GPI) described by Farrell (101), but a short description of the equipment as used in this work and of the α -16 minicomputer used is given below.

2.3.1 Analogue to Digital Convertor

An Analog Devices ADC 1110K dual slope ADC was used, with a high impedance buffer connected to the signal input so ion selective electrodes could be directly connected. This model gave a 14 binary bit resolution (plus 1 sign bit and 1 input overload bit) over a $\pm 1V$ or a $\pm 10V$ range. The $\pm 1V$ range was normally used giving a resolution of $1/2^{14}V$ or about $0.0610mV$, this sometimes being called an ADC unit in the text.

A high to low transition triggered the conversion which followed the cycle shown in Figure 2.6. The voltage input was integrated for t_{in} sec (the input integration period, nominally $81.92msec$), then the integrator was discharged at a constant rate until it reached its initial state (the reference integration period, t_{ref}). The input voltage V_{in} was then given by

$$V_{in} = \frac{t_{ref} V_{fs}}{t_{in}} \quad 2.3.A$$

where V_{fs} is the full scale voltage, in this case $1V$. A pulse was then output to signal the end of conversion.

The maximum permissible input to the ADC on the $\pm 1V$ scale was $2V$, although any input over $1V$ would be signalled by an overload. A $2V$ input gave rise to a reference integration period of $163.84msec$ and a total conversion time of $245.76msec$, so a maximum of 4 conversions per second

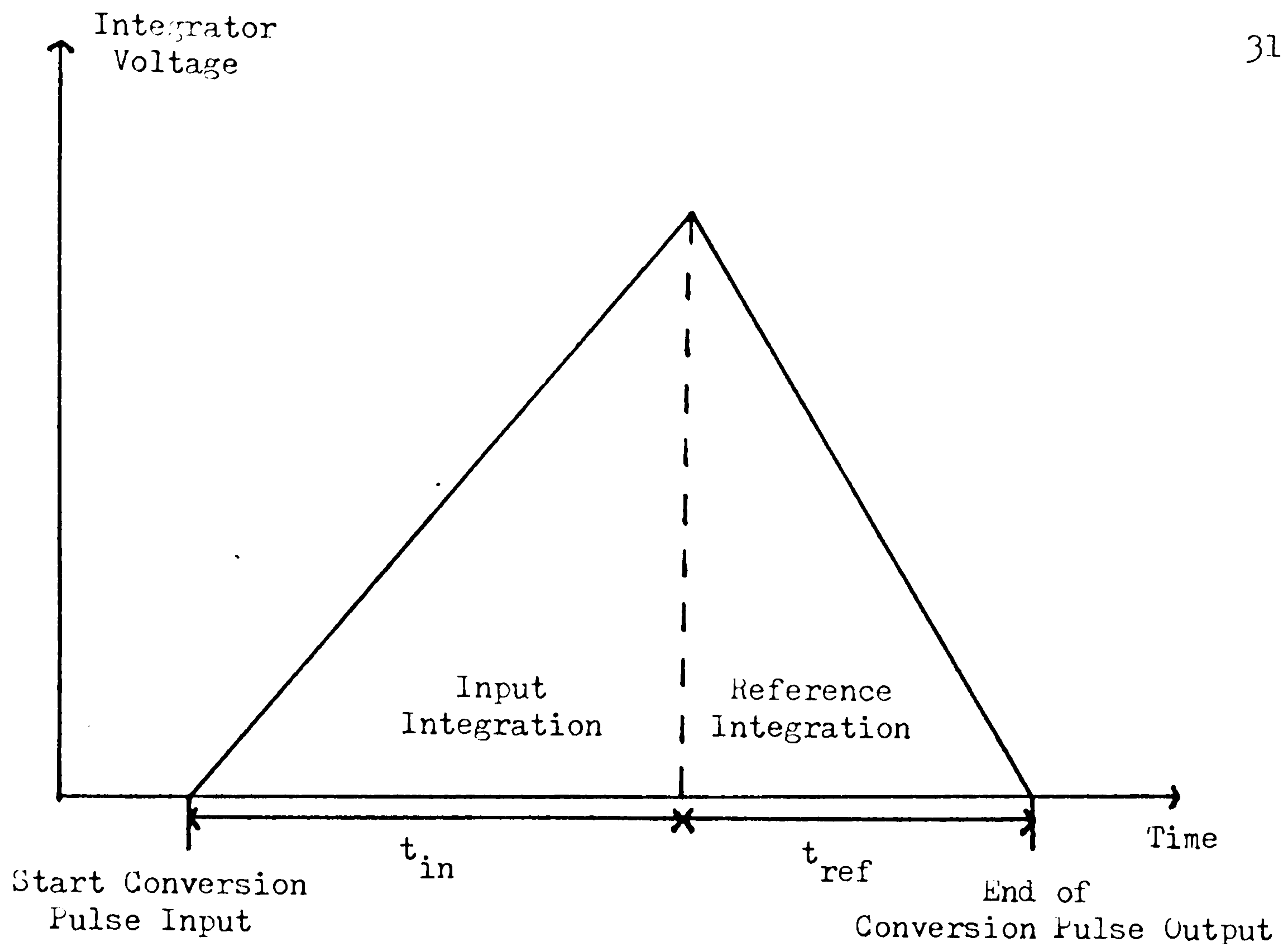


Figure 2.6. Conversion cycle of Dual Slope ADC

were possible, but the input voltage was only integrated for 33% of the time. Increasing the conversion rate to 8 per second increased this to 66% of the time, but reduced the input range to $\pm 500\text{mV}$. The data acquisition subroutines DATEX4 and DATEX8 described in Section 2.4 input one datum to the computer at these rates.

This conversion system was relatively slow, but had the advantage of integrating the input for up to around $1/6\text{sec}$ for each datum point, eliminating many of the effects of high frequency noise and reducing the amount of data processing necessary. Also the ADC covered the normal range of potentials obtained with ion selective electrodes and had a slightly better resolution than that usual with digital voltmeters.

2.3.2 General Purpose Interface

This provided for data to be passed from the experiment to the minicomputer and for the control of the equipment by the computer. It included the following:-

1. The ADC
2. A control bit, used to initiate conversions by the computer. This bit could be set high, then low, by software and this transition passed to the ADC to start a conversion.
3. A flag bit, which could be set only by a pulse input, but could be cleared by software. This was used to indicate the end of a conversion to the computer by sensing its status until it was set high by the end of conversion pulse from the ADC.
4. A clock with pulse output frequencies of 1 to 10^6 Hz in decade steps.
5. A multiplexer to input data to the computer from either the logic box or the ADC. This was set to channel A (ADC) by a low input and to channel B (logic box) by a high input. Data output from the computer was presented only to the logic box.
6. A logic box containing sixteen bits which could be set or reset by software and whose status could then be supplied to other equipment, also sixteen bits whose status was determined by input and could be transmitted to the computer if the multiplexer was set to channel B. Other facilities included various logic functions such as invertors, AND and OR gates which could be plugged in as required.

2.3.3 Minicomputer

A Computer Automation α -16 minicomputer was used, interfaced to an ASR-33 Teletype incorporating a paper tape punch and reader. The α -16 had an 8K memory and a fairly powerful instruction set of 145 basic instructions. A control panel enabled the display and alteration of certain registers and the contents of memory.

Supplied software which was used in this work included:-

1. The OMEGA assembler, which translated programmes written in assembler language by the operator into machine code, listing errors and allowing for their correction. All programmes in this work were written in OMEGA assembler language, but listings have not been included here for reasons of space, although flow charts have often been given.

2. The Teletype Utility Package, consisting of subroutines to perform common teletype input/output functions.

3. The LAMBDA loader, which loaded the object tapes produced by OMEGA into memory.

4. Arithmetic software, in particular the Floating Point Arithmetic and Elementary Functions Packages.

The teletype was used to type into OMEGA programmes in source code, to debug these, to produce programme listings and to print out experimental results. The tape punch was used to punch out onto paper tape the source and object codes and the reader to load tapes into memory.

2.4 Data Acquisition Subroutines

As shown in Section 2.3.1 the maximum rate of conversion with the full input range was 4 per second. This proved to be adequate for following the course of gradient titrations, but for direct potentiometric methods the greater signal averaging capabilities of converting at a rate of 8 per second, even with a lower input range, was called for. Hence two subroutines DATEX4 and DATEX8 were written to input data to the computer at these conversion rates, using the interface configuration shown in Figure 2.7.

DATEX4 subroutine took in one datum point from the ADC 0.25sec after being called and returned to the main programme with the datum stored in the A register in α -16 format (see Figure 2.8), overload being indicated by the X register being set to 1, otherwise this was set to

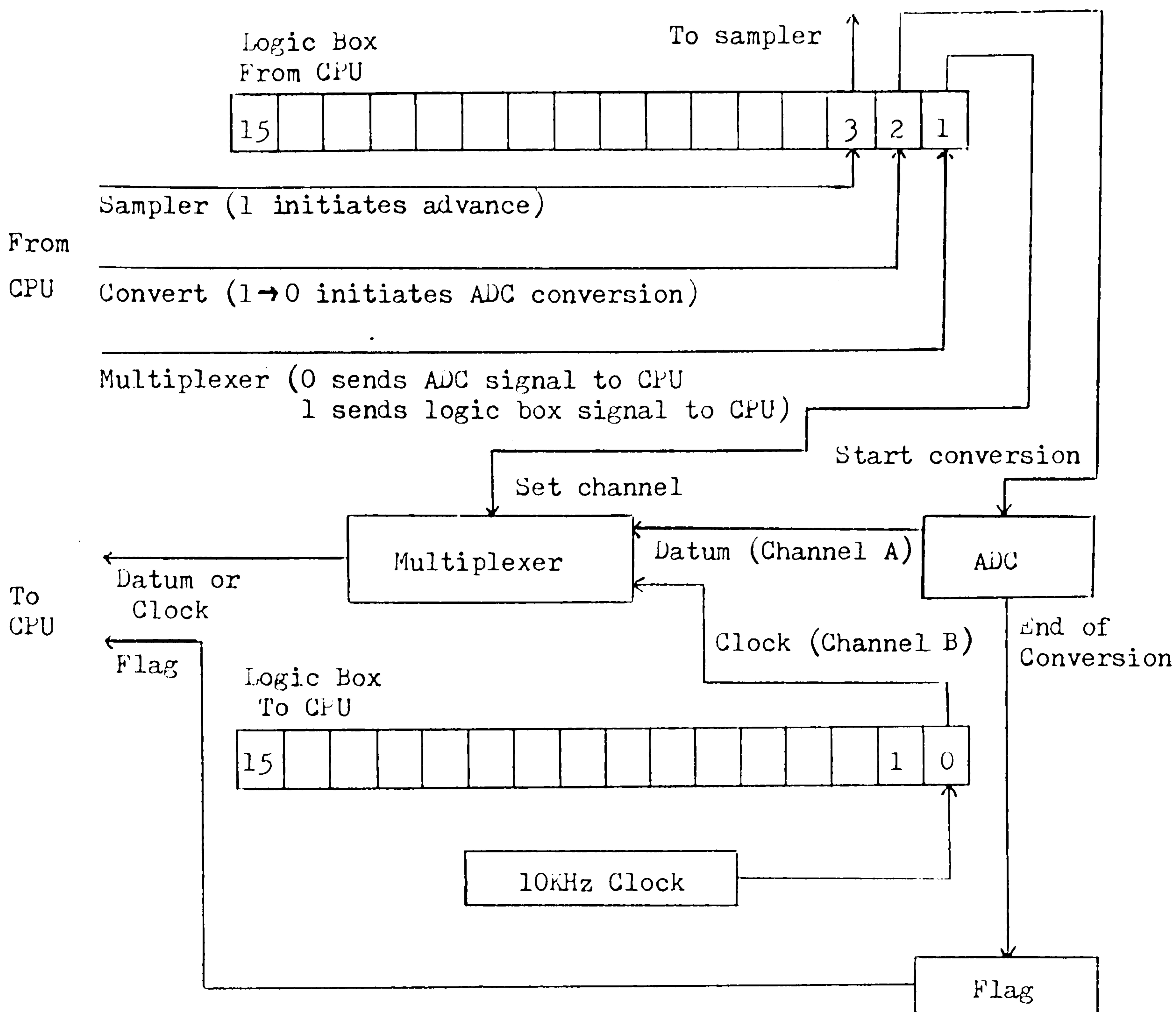
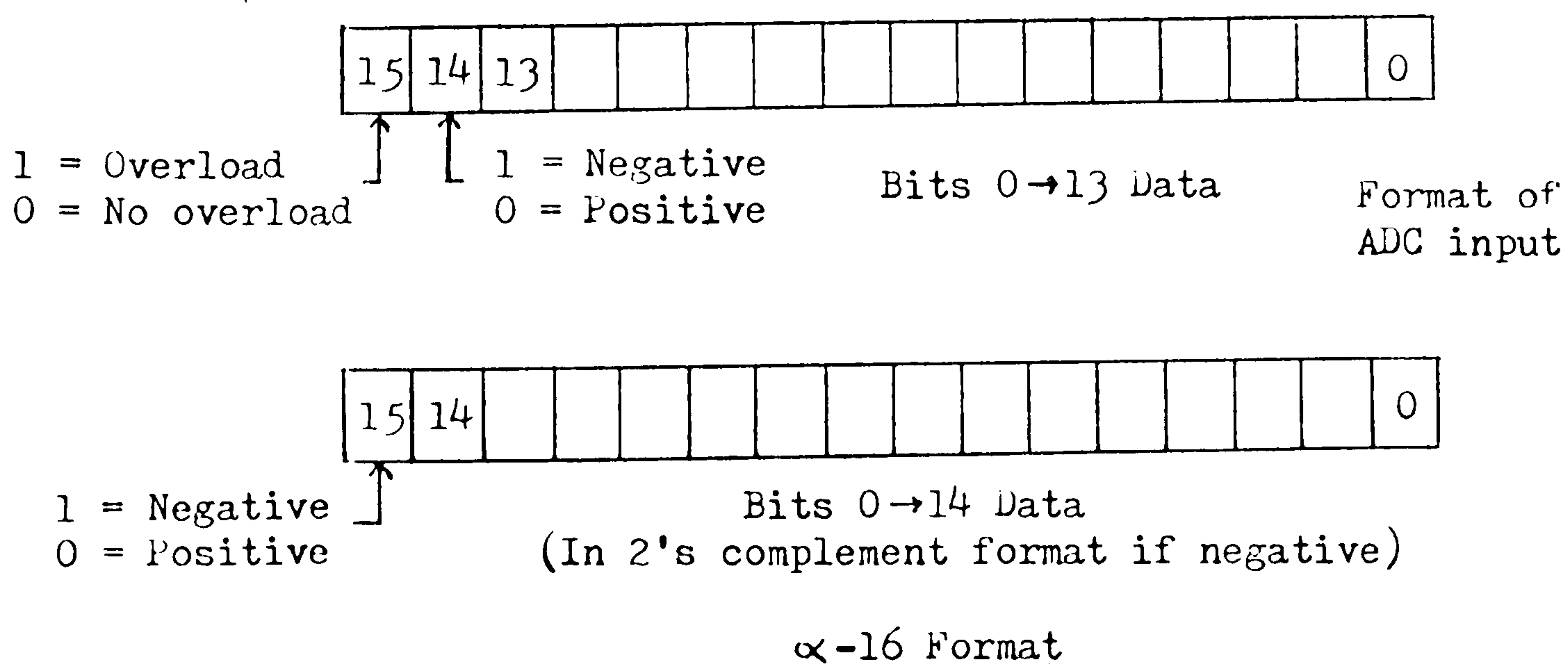


Figure 2.7 Interface Configuration for DATEX4 & DATEX8

Figure 2.8 Formats of input from ADC and of an α -16 word

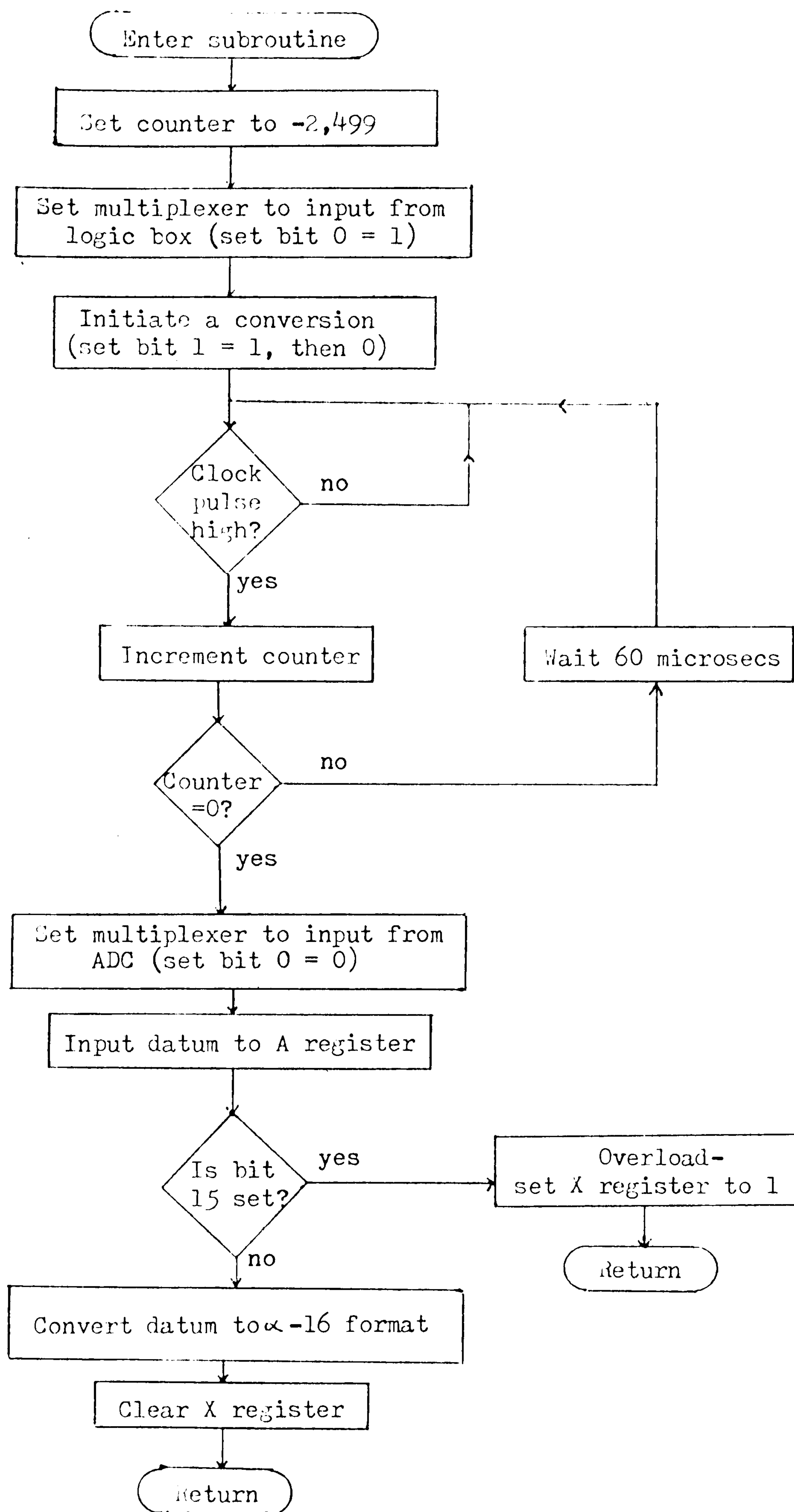


Figure 2.9 Flow Chart of DATEX4

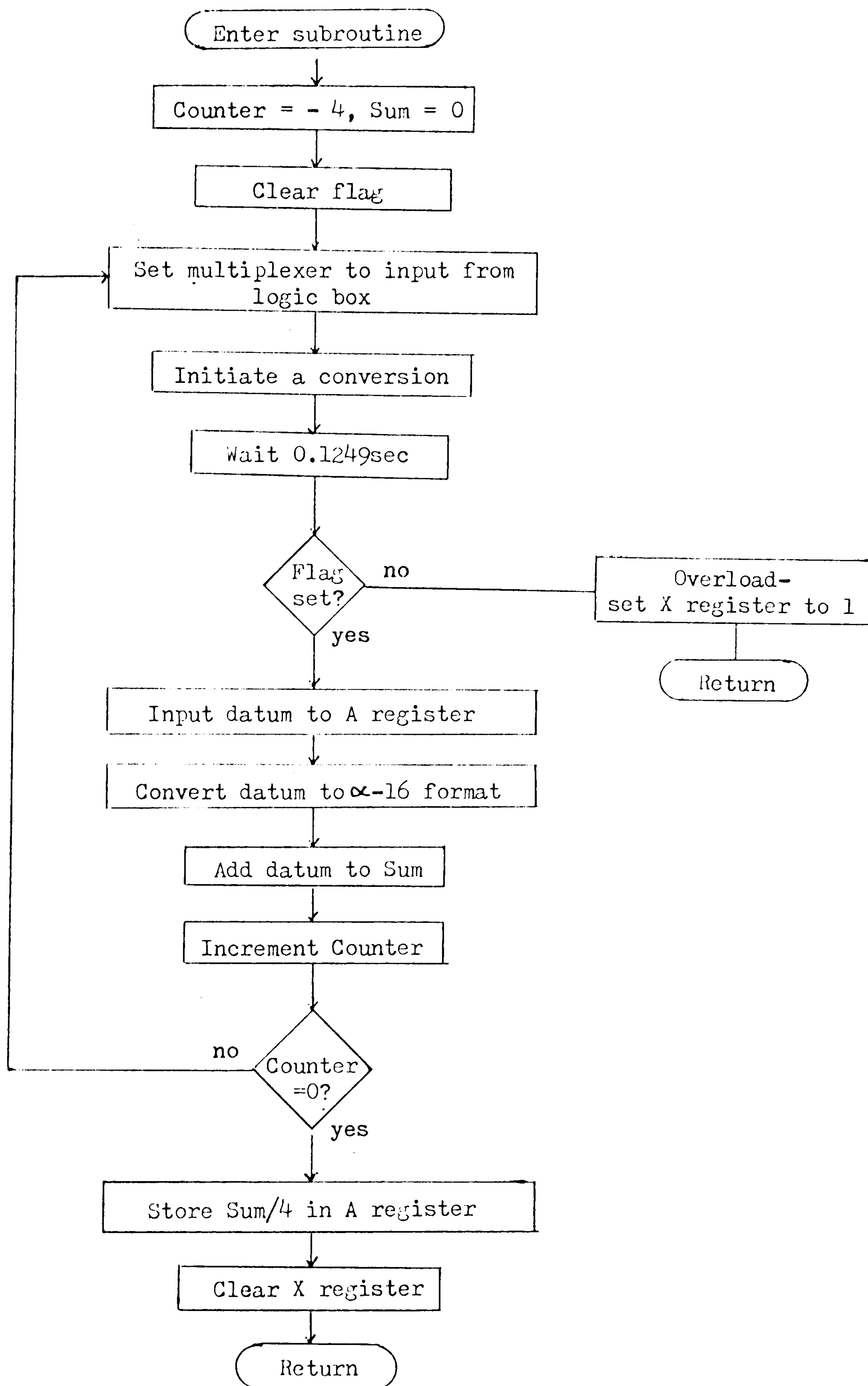


Figure 2.10 Flow Chart of DATEX8

zero. A flow chart of the subroutine is given in Figure 2.9. The execution of the subroutine, apart from the timing sequence, took around 100 microsecs bringing the total execution time to around 0.25sec.

DATEX8 took four data points from the ADC, one every 0.125sec, and stored the average in the A register. In contrast to DATEX4 the X register was set to 1, indicating overload, not if the overload bit was set but if the flag bit was not set after 0.125sec i.e. the conversion had not been completed, as shown in Figure 2.10. The average of four data points were taken so as to shorten any data buffers in the main programme and to shorten any signal averaging, since the four data points were of a maximum of 13 bits long and could be averaged by integer addition and shifting whereas any more would have required the use of the considerably slower floating point arithmetic.

2.5 Computer Control of Technicon Sampler

In order to advance the sampling probe when a gradient titration was complete, rather than to change samples after a set time, the Technicon Sampler II was modified so it could be advanced one position by computer software (Figure 2.11). The output from the 'from CPU' bit 2 was supplied to a Radiospares 349-428 TTL compatible reed relay, which switched another relay, a Radiospares 348-908, since the voltage to be switched inside the sampler (115V, 0.5A) was too high for the 349-428. When bit 2 was set high switch A closed, opening switch B, which stopped current flowing in the sampler, which initiated the sampling cycle. Setting bit 2 low opened switch A, closing switch B, which allowed current to flow, stopping movement of the probe.

SAMPLE subroutine was written to advance the sampler probe by one position. After initiating probe movement SAMPLE waited for 3.90sec, the time taken for one advance, then stopped the probe and returned to the main programme.

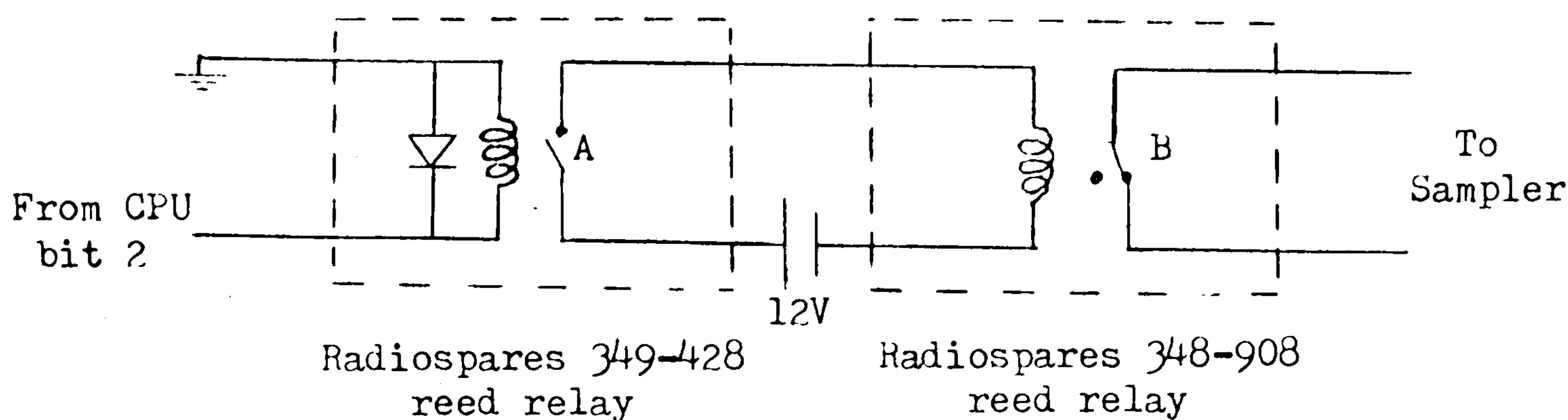


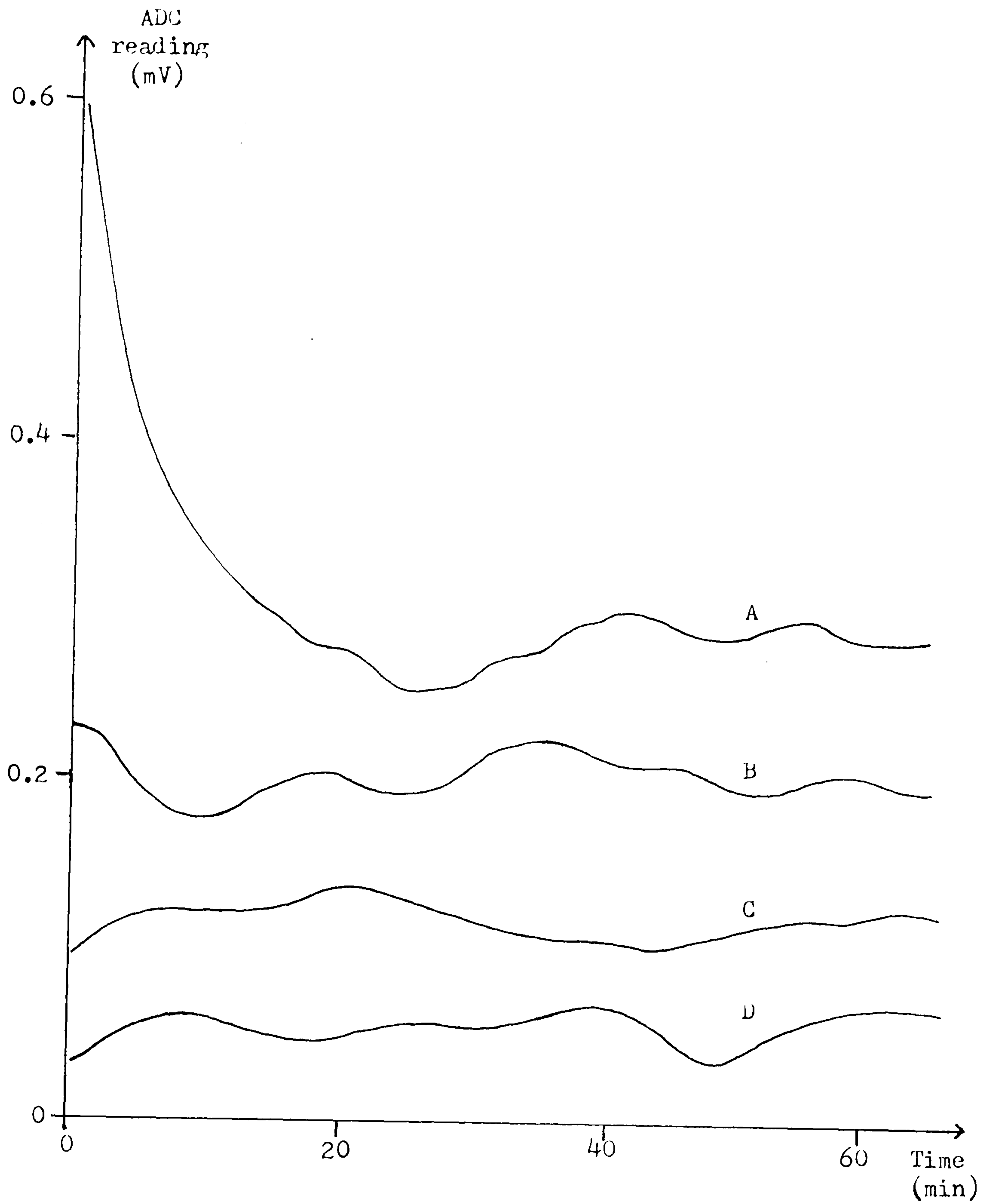
Figure 2.11 Switching mechanism for computer control of sampler

2.6 Appraisal of the Interface Design for Potentiometric Measurements

The linearity and the resolution of the ADC were checked by measuring the voltage from a MEI 1200 millivolt source simultaneously by the ADC and the Corning 112 meter. The resolution was found to be 0.0610 (± 0.0005)mV per bit over several different ranges and the response found to be linear within two or three bits over the ± 1 V range. The resolution obtained from the system in practice also depended on the gain of the meter output, but this was normally set to unity.

Two programmes, VARY and DRIFT, were written to determine the noise and long term drift of the system. VARY took in 'n' points using DATEX4 (n to be latched into memory using the control panel) and calculated the variance of the data points, storing the answer in floating point format in the memory. With the inputs to the ADC grounded the standard deviation of the points over 1 minute was consistently around 0.05mV, so the noise contributed from the measuring system was therefore satisfactorily low.

DRIFT indicated the drift of the system over 1 hour by averaging 120 successive inputs from DATEX8 60 times and storing the means as integers in a data buffer. As can be seen from Figure 2.12 apart from some initial drift when the interface was first turned on, the drift rates were low, generally within 2 ADC units an hour.



- A ADC inputs shorted: equipment just turned on
- B Voltmeter inputs shorted: voltmeter just turned on, ADC on 1hr
- C ADC inputs shorted: equipment on 1hr
- D Voltmeter inputs shorted: voltmeter and ADC on 1 hr

Figure 2.12 Drift of measuring system

CHAPTER THREE ION SELECTIVE ELECTRODES AS FLOW STREAM SENSORS

3.1 Introduction

While some of the properties of ion selective electrodes are the same whether they are used in an open beaker or are incorporated into a flow stream e.g. sensitivity and selectivity, other properties such as the time response and the noise level of the signal may vary with the nature of the system when used in a flow stream. Obviously it is desirable to have a low response time as possible to enable higher sampling rates in direct potentiometric methods and to enable end-points to be accurately positioned in potentiometric titrations. It is also obvious that noise and drift levels should be kept to a minimum to improve the accuracy and precision of analyses, especially by direct potentiometric methods. The magnitude and variation of these three factors within the flow stream described in Section 2.1 are dealt with in this Chapter.

3.2 Time Response

Although many examples of using ion selective electrodes in continuous flow systems have appeared in the literature little information has been published on time responses under these circumstances. Nevertheless a sampling rate of 60 per hour has often been quoted for Autoanalyzer systems, implying that the time response of the electrode to the full change in potential was well under a minute. Fleet and Von Storp found t_{95} for a cyanide electrode at a flow rate of 10ml/min to be 30sec for a step of $10^{-4}M$ to $10^{-3}M$ and 75sec for the reverse step, the times to equilibrium being almost double these values (102), this being many times slower than the response in a stirred solution in a beaker. Even longer response times were found in a later work (55) with a fluoride electrode monitoring a change from 2mM to 4mM taking around 10 minutes to reach full response at a flow rate of 2ml/min.

at a flow rate of 2ml/min.
at a flow rate of 2ml/min.
at a flow rate of 2ml/min.

However rather more work has been carried out at higher flow rates than those typically used in continuous flow methods (103,104). Rangarajan and Rechnitz (104) showed that at flow rates of 200 to 1,000 ml/min the time responses (t_{95}) of the silver sulphide-silver halide electrodes were in the range 0.5 to 1.5 sec and that t_{95} was a linear function of the inverse of flow rate. This behaviour was attributed to the ion to be sensed diffusing through a boundary layer on the electrode surface upon the change in concentration. However if these results are extrapolated linearly to flow rates used in continuous flow systems, values of t_{95} in the region of 60 to 120 sec are obtained.

These response times are clearly incompatible with the use of Autoanalyzer equipment for rapid and accurate analysis. One solution has been offered by Mertens et al. (105), incorporating a magnetic stirrer into the flow cell, reducing the time response considerably.

Again this suggested a diffusion model for the time response, the theory of which has been considered by Simon (106). In the case of ion exchange membranes where the process was considered to be the sole rate determining step

$$t_x = \frac{4\delta^2}{\pi^2 D} \log_e \frac{1 - a_0/a_1}{1 - (a_0/a_1)^{1-0.01x}} \quad 3.2-A$$

where t_x is the time to $x\%$ of the full response, δ the boundary layer thickness, D the diffusion coefficient of the ions and a_0 and a_1 are the initial and final activities.

For neutral carrier membranes it was shown that the properties of the membrane itself played a significant part in the time response and that

$$t_x = \frac{D'K^2\delta^2}{\pi D^2} \left[\frac{1 - a_0/a_1}{1 - (a_0/a_1)^{1-0.01x}} - 1 \right]^2 \quad 3.2-B$$

with D' the mean diffusion coefficient of the electrolyte in the membrane and K a partition parameter between the solution and the membrane, fully

explained in (106).

These equations successfully predicted different time responses with different values of the ratio a_0/a_1 , the longer response encountered when decreasing activities and also the effect of other ions in solution, which may affect the value of D . They also show that for rapid responses δ must be minimised since in both cases t_x was proportional to δ^2 .

To investigate the time responses of immobilised liquid membrane electrodes when inserted in the flow cell described in Section 2.1 t_{95} was measured for a potassium neutral carrier and a calcium charged carrier electrode for changes of concentration from 10^{-3} to $10^{-2}M$ by inspection of the response profile as shown on the chart recorder. This was repeated at various flow rates and the results are shown in Figure 3.1 with t_{95} plotted against the reciprocal of the flow rate.

The first flow cell used (Cell A) had relatively large input and output holes (around 1mm in diameter) which met at a common point on the membrane surface. The time responses were excessively high, even at the higher flow rates used, and this cell was not used for analyses. However the plots were similar to those obtained by Rangarajan and Rechnitz (104) at much higher flow rates, although they display slight curvature. Calcium response times were shorter than those of potassium, as may be expected if the effective boundary layer was equal for both, but the diffusion of ions across the neutral carrier membrane slowed the response of the potassium electrode. Calculation of δ using equation 3.2-A for the calcium electrode yielded values ranging from 0.2mm at 8ml/min to 0.5mm at 0.6ml/min. These figures are rather high, and probably incorporate the effect of the time taken for the mixing of the solution in the dead space of the cell.

To eliminate this problem cell B, which had input and outlet at opposite ends of the cell, was used. The diameter of the holes was much lower (around 0.3mm) so that the solution entered the cell at a much

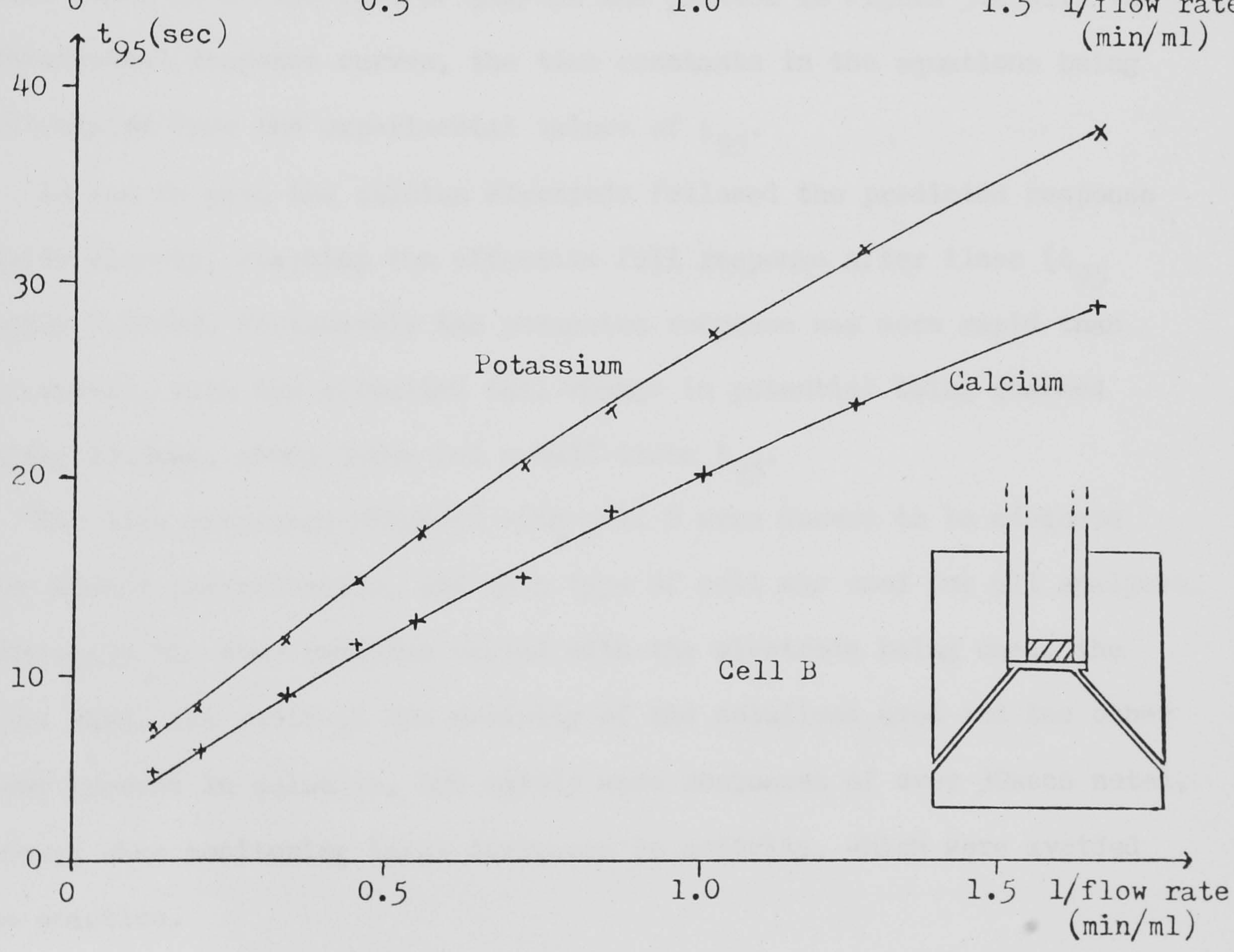
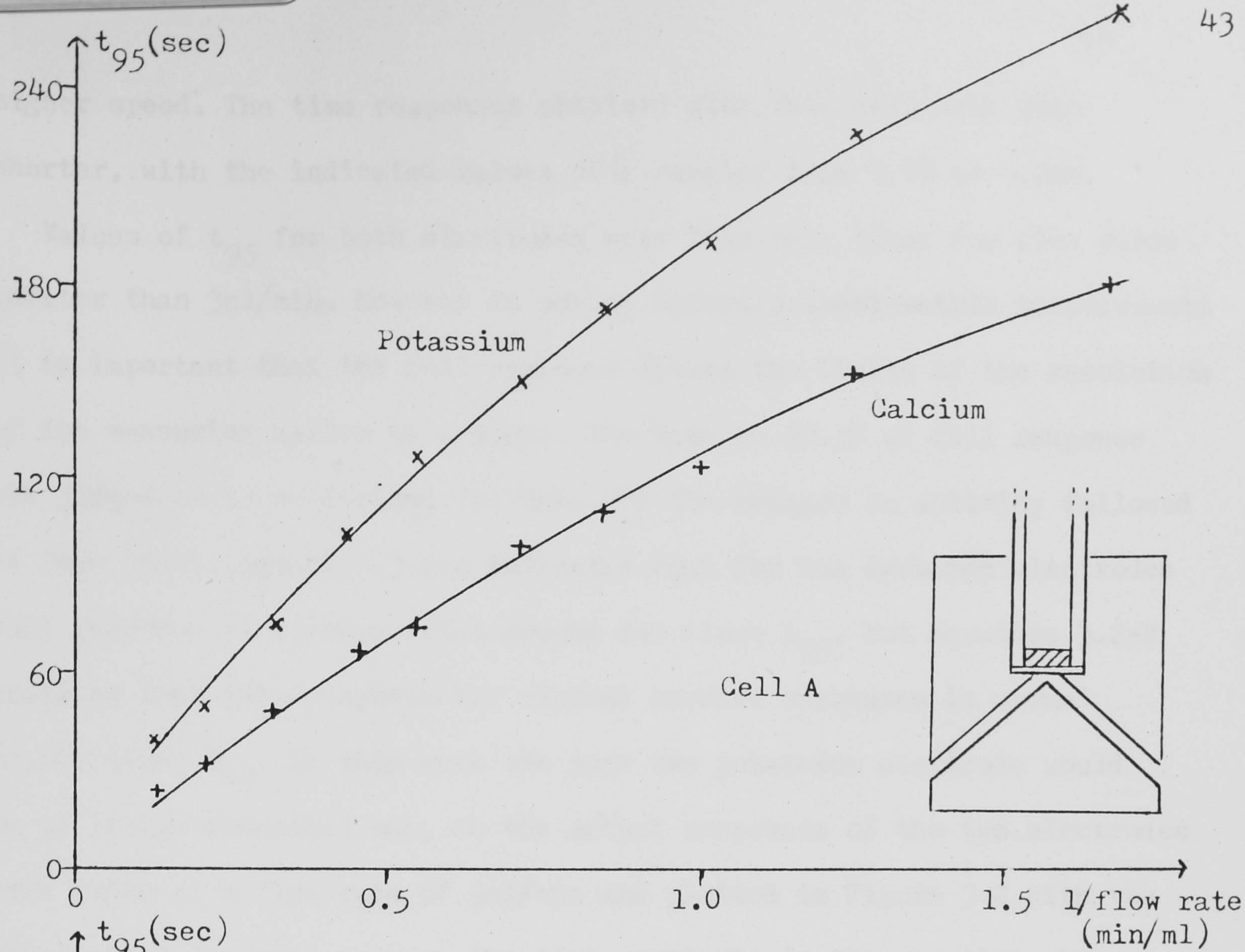


Figure 3.1 Values of t_{95} for potassium and calcium electrodes with varying flow rates using two different flow cells (Initial concentration = $10^{-3}M$, final concentration = $10^{-2}M$)

higher speed. The time responses obtained with this cell were much shorter, with the indicated values of δ ranging from 0.08 to 0.2mm.

Values of t_{95} for both electrodes were less than 10sec for flow rates greater than 3ml/min. However in making direct potentiometric measurements it is important that the full response within the limits of the resolution of the measuring system is reached. The time to 99.5% of full response was judged to be sufficient for this for the changes in activity followed in this study. Equation 3.2-A indicates that for ion exchange electrodes this response is reached after around two times t_{95} , but Equation 3.2-B predicts that this response for neutral carrier membranes is around 12,000 times t_{95} . If this were the case the potassium electrode would be of little practical use, so the actual responses of the two electrodes were taken at a flow rate of 5ml/min and plotted in Figure 3.2 with the theoretical response curves, the time constants in the equations being calculated from the experimental values of t_{95} .

As can be seen the calcium electrode followed the predicted response quite closely, reaching the effective full response after 11sec (t_{95} being 4.9sec). Fortunately the potassium response was more rapid than predicted, with the effective full change in potential being reached after 23.5sec, about three and a half times t_{95} .

The time responses obtained with cell B were deemed to be adequate for direct potentiometry, and this type of cell was used for all analyses. Obviously the time response varied with the electrode being used, the flow rate, the ratio of the activity of the solutions used and the other ions present in solution, but rarely were responses of over 30secs noted, except when monitoring large decreases in activity, which were avoided in practice.

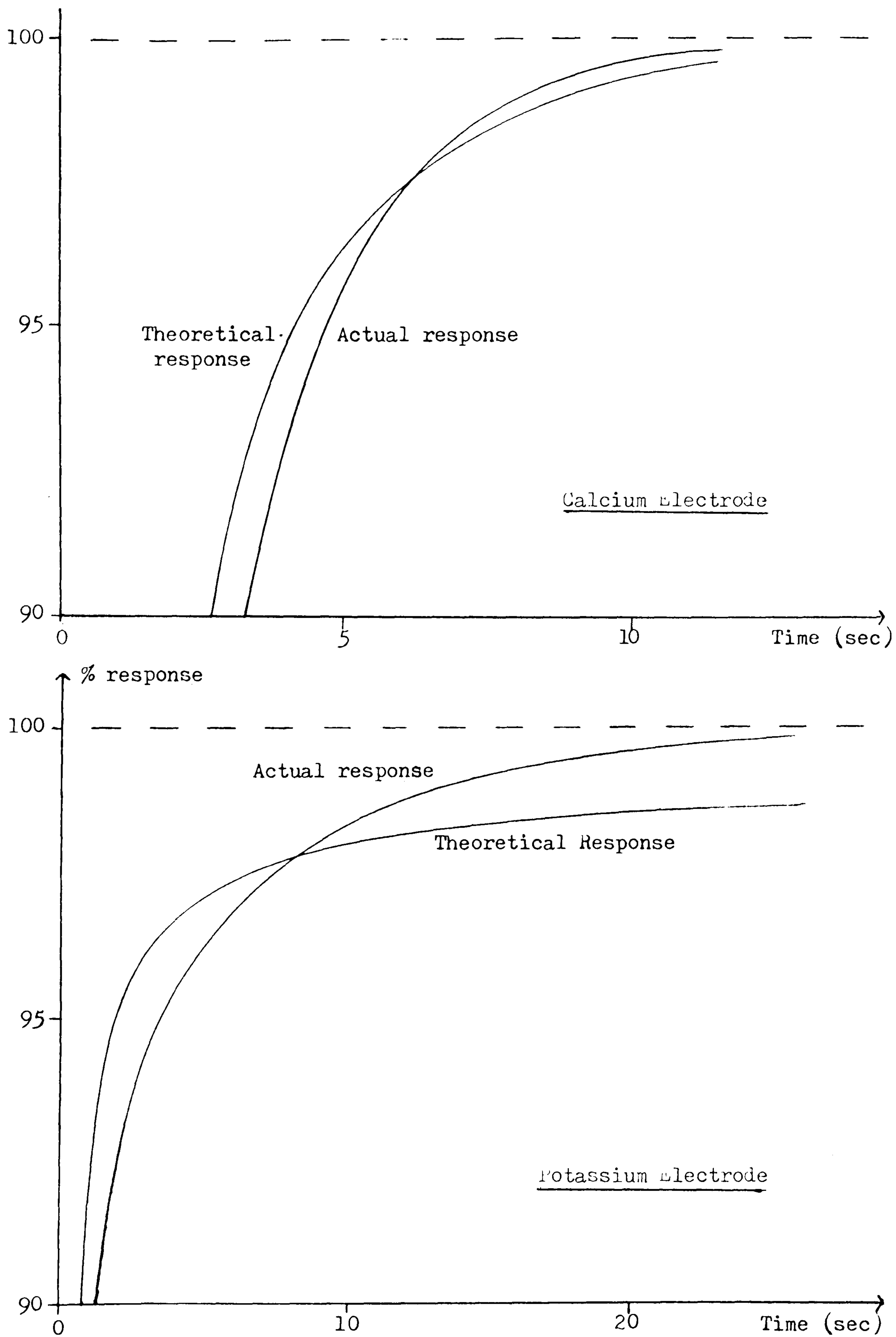


Figure 3.2 Theoretical and actual response of a calcium charged carrier and a potassium neutral carrier electrode

3.3 Noise

The noise associated with the electrodes fell mainly into two groups, high frequency noise (50Hz or higher) due mainly to electrical pick-up and low frequency noise (around 0.5Hz) due to the pulsing nature of the flow stream.

Ion selective electrodes are very susceptible to pick-up because of their high impedance, and it has also been suggested that some mains frequency noise may be transmitted along the pump lines if they are used in a flow stream (107). When measured on an oscilloscope this noise was found to be mostly 50Hz and up to 50mV pp in amplitude for a potassium electrode with a 10^{-3} M KCl solution flowing. This was decreased to 5mV pp by screening the electrodes in an earthed aluminium box and minimising the lengths of the leads to the voltmeter. Fortunately the ADC was of an integrating type and these levels of noise will be shown to have relatively little effect on the noise of the signal passed to the computer.

The low frequency pump noise has been shown to be caused by variations in streaming potential (108). The magnitude of this potential, set up across a liquid when it is passed through a tube, is proportional to the flow rate and will thus vary with time in the same way as the flow rate. For the peristaltic pump used this consisted of a pulse every couple of seconds, which resulted in the noise shown in Figure 3.3. This peak to peak value of 20mV was clearly too high, but was lessened considerably to about 1mV when the solution was passed through grounded platinum tubes before and after the flow cell. Also since the magnitude of the potential is inversely proportional to the conductivity of the solution, the noise was decreased to less than 0.2mV pp when an ionic strength buffer was applied.

To find the effect of these noise levels upon the measured signal

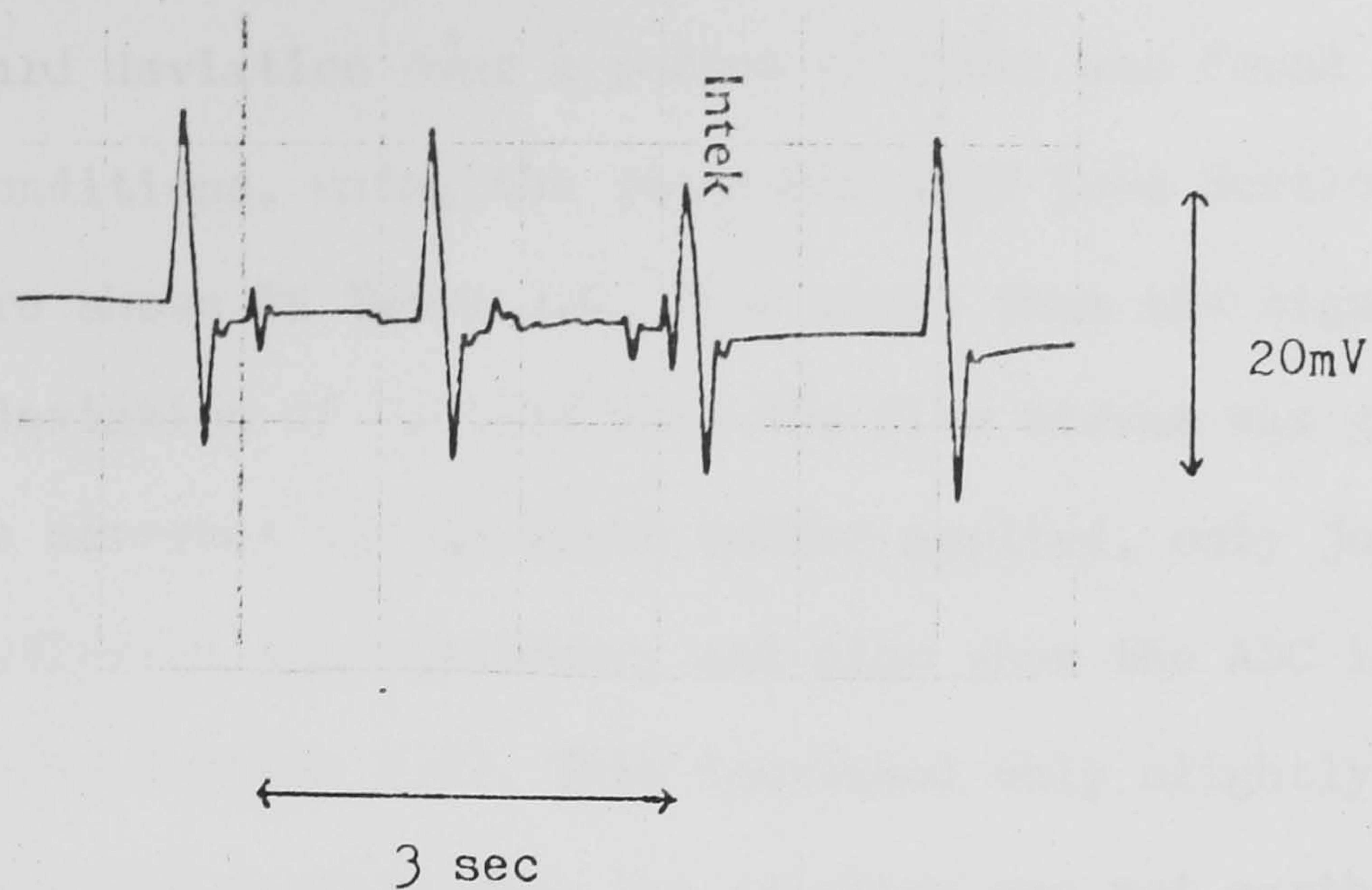


Figure 3.3 Noise of signal from a K^+ electrode with a $10^{-3}M$ KCl solution flowing at 4ml/min, no ionic buffer applied and flow stream not earthed.

Conditions	Standard Deviation (mV)
Normal	0.055
Inputs to ADC grounded	0.042
Zero flow rate	0.053
No ionic buffer applied (0.5M $MgSO_4$)	0.097
Flow stream not earthed	1.910
Electrodes unscreened	0.137

Table 3.4 Noise of signal from the ADC when supplied with the signal from the K^+ electrode normally screened, with flow stream grounded and ionic buffer applied; and its variation with change of conditions.

its standard deviation over a period of 60sec was found under these various conditions, using the programme VARY (see Section 2.6) and the results are shown in Table 3.4. This shows that the signal had a standard deviation of 0.055mV when the flow stream was grounded, the electrodes screened and an ionic buffer applied, only just greater than when the solution was stationary and also when the ADC inputs were grounded (see Section 2.6). This increased only slightly when no buffer was applied, but greatly when the solution was not earthed, showing that the effects of variations in streaming potential were greatly reduced by grounding the stream. The standard deviation of the signal with the electrodes screened was half of that unscreened, displaying the benefits of reducing pick-up.

Therefore despite the pick-up and streaming potentials, it proved possible to reduce noise levels to the point where they were limited not by the signal from the electrodes but by the measuring system itself.

3.4 Drift

Drift can be caused by many factors. The chief of these is change of temperature, since the electrode slope is proportional to the absolute temperature. This was minimised by letting all solutions equilibrate to the ambient temperature before use. Other possible causes are change in the value of E_0 with the aging of the membrane, change in the potential of either the electrode systems and variation in liquid junction potential.

Figure 3.5 shows the drift rates of a calcium charged carrier electrode in a 10^{-3}M CaCl_2 solution under various circumstances, measured using DRIFT (see Section 2.6). Under normal flow conditions and with a stationary solution drift rates were about 0.25mV/hr, considered to be satisfactory with the techniques used. However when the flow was first turned on the potential drifted upwards 2mV or more,

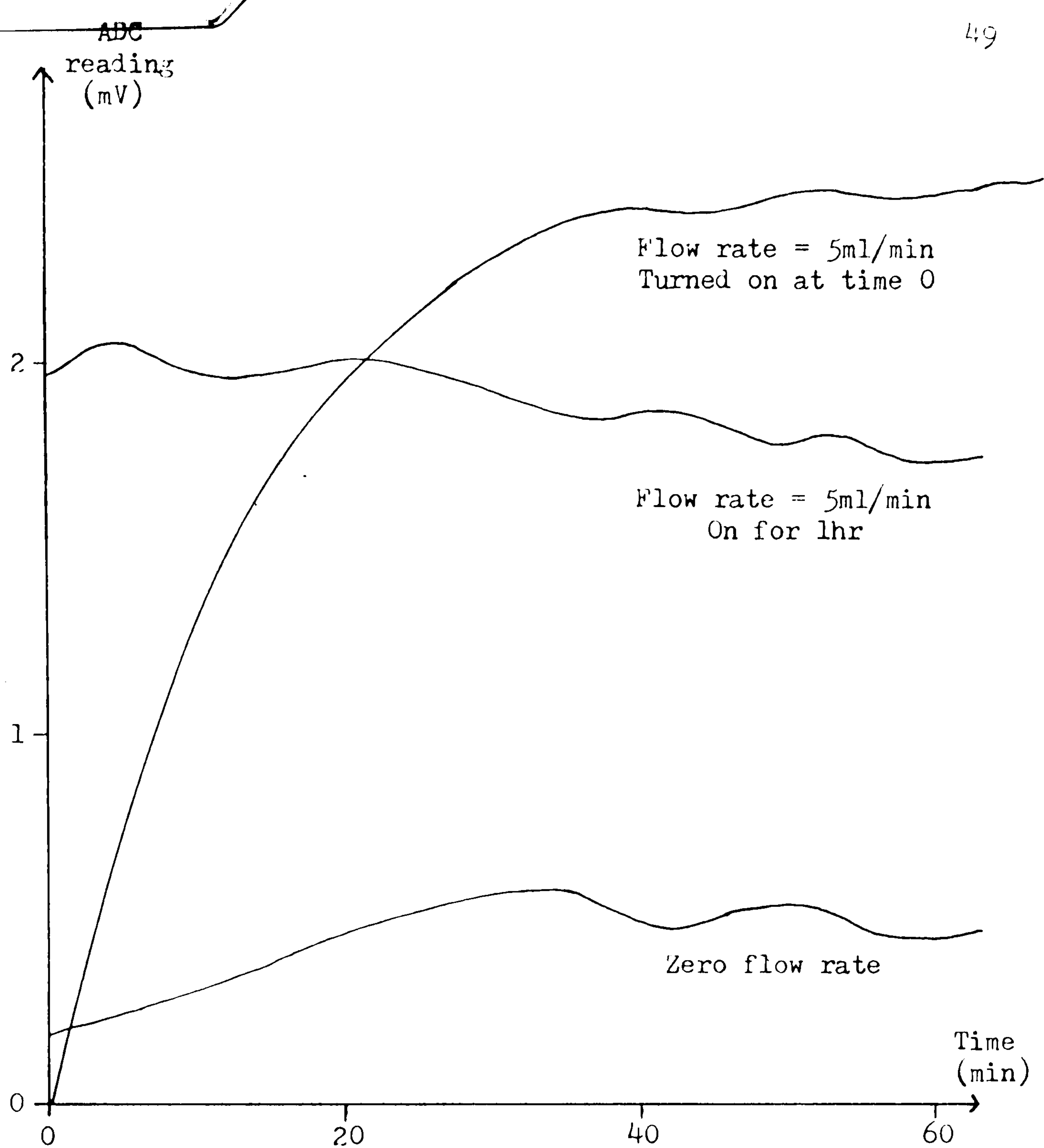


Figure 3.5 Drift of a calcium ion-exchanger electrode

possibly due to the heating effect of the pump on the solution or the stabilisation of the electrode-solution boundaries. However the ADC showed a similar 'warming-up' on switching on, so this initial drift was of little consequence since the system could not be used in this time.

4.1 Introduction

The use of standards interposed between samples is widespread in instrumental analysis when the signal of interest is liable to drift. When compared with direct potentiometric methods requiring precalibration the use of bracketing standards with ion selective electrodes not only reduces errors due to drift in electrode potential but also, if the standards match the sample concentration closely, errors due to variations in the slope of the electrode at different concentrations.

This technique can be used either to measure the activity of a sample, if standards of a known activity are used, or the concentration, if an ionic buffer is applied to both sample and standards. However if complexation of the species of interest occurs in the sample the concentration found will be that of the uncomplexed species. Also the presence of interfering ions may lead to grave errors unless the standards are made up in the same matrix as the sample.

Using the continuous flow system shown in Figure 4.1, if the electrode potentials given by the standards of concentration C_1 and C_2 are E_1 and E_2 and by the sample of concentration C_s is E_s , then -

$$E_1 = E_o + S \log (F_s / (F_s + F_b)) C_1 = E'_o + S \log C_1 \quad 4.1-A$$

$$E_2 = E_o + S \log (F_s / (F_s + F_b)) C_2 = E'_o + S \log C_2 \quad 4.1-B$$

where $E'_o = E_o + S \log (F_s / (F_s + F_b))$.

Solving equations 4.1-A and 4.1-B simultaneously gives

$$S = \frac{E_2 - E_1}{\log (C_2 / C_1)} \quad 4.1-C$$

$$E'_o = E_1 - S \log C_1 \quad 4.1-D$$

and the sample concentration is given by

$$C_s = \frac{E_s - E'_0}{S} \quad 4.1-E$$

To perform these calculations on the α -16 two subroutines were written, CALIB calculated values of E'_0 and S for values of E_1, E_2, C_1 and C_2 supplied to the routine using Equations 4.1-C and 4.1-D and NERNST calculated C_s from Equation 4.1-E for given values of E_s, E'_0 and S .

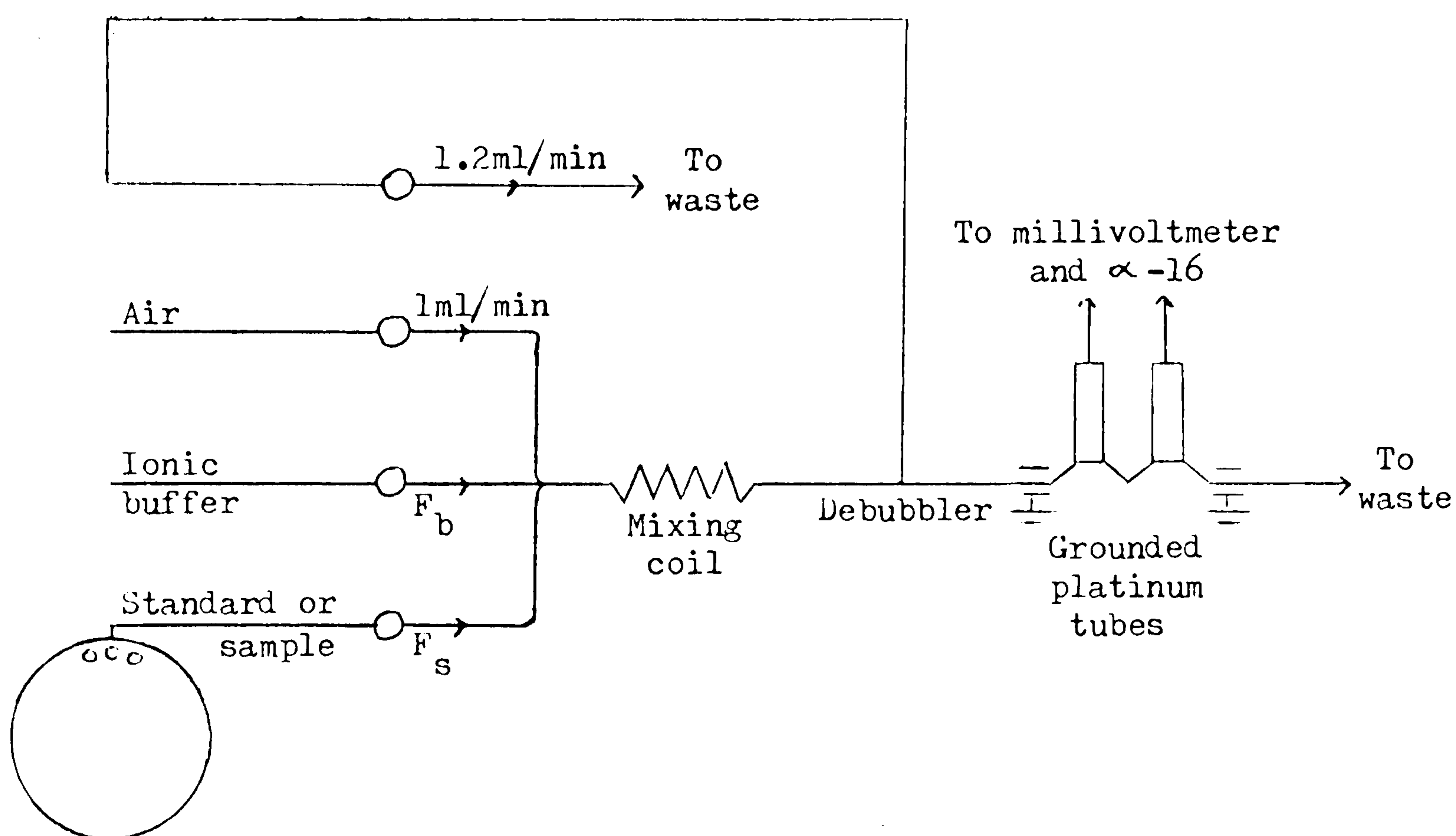


Figure 4.1 Flow stream for bracketing standards

4.2 On-line Bracketing Standard Systems

Two programmes, BRACKSTAN1 and BRACKSTAN2, were written to carry out bracketing standards on the apparatus shown in Figure 4.1. BRACKSTAN1 was for use when all the sample concentrations were known to lie between fairly narrow limits and the cycle of solutions standard 1, samples, standard 2, samples, standard 1, etc. was adequate. If the range of the

concentrations of the samples was higher BRACKSTAN2 was used. Part A of this took in the electrode potentials from two known solutions (CAL1 and CAL2), then the potentials from the sample solutions and calculated the approximate concentrations of the samples. Then the nearest values of the available standards, stored in memory, were printed on the teletype and passed to Part B. Part B took in the electrode potentials from a cycle of standard 1, sample, standard 2 and calculated sample concentrations assuming each of the standards to be that suggested by Part A.

To take in electrode potentials the same subroutines were used for BRACKSTAN1 and BRACKSTAN2 as well as for the standard additions technique (see Section 5.3). BREAK subroutine recognized the first solution, PTSIN took in the electrode data into the computer memory and these points were averaged by MEAN.

BREAK was called after the commencement of a programme, taking in data using DATEX8 until there was a jump in electrode potential of 1mV or more between two successive readings, that is the first solution was distinguished by the difference in concentration between it and the wash solution preceding it, then returned to the main programme so the data could be input. If no jump had been recorded within 1 minute of BREAK being called the subroutine returned with the A register set to 1 to indicate this. This procedure proved perfectly adequate although TITRATE used a rather more elegant method based on advancing the sampler (see Section 6.3).

PTSIN subroutine waited while a specified number (NW1) of data points were input by DATEX8, then took in the next NAV points and stored them in a preselected data buffer, then waited for another NW2 points to be input before returning. This enabled the computer to wait for the response time of the electrode and then take in data until the next solution arrived. The final wait was to avoid accidentally taking in data from the start of the next solution due to imperfect timing of the sampler cycle or

dispersion in the flow system. For both BREAK and PTSIN if the ADC was overloaded by the signal the subroutine immediately returned with the A register set to -1. For the electrodes and flow rates used in this work the time to 100% response was rarely more than 25sec, so generally a 1 minute cycle was used with $NW1 = 50$ (25sec), $NAV = 60$ (30sec) and $NW2 = 10$ (5sec).

Finally, to prevent too much of the memory being taken up by data buffers, MEAN subroutine was used to produce an average value of the data input by PTSIN. MEAN also detected whether or not the measured electrode response was steady, by establishing if any of the data points in the buffer varied from the average value by more than 1mV, this being the case the main programme being informed. There was a danger using MEAN that the timing of a programme may go out of synchronization with that of the sampler cycle, since MEAN used floating point arithmetic and took an appreciable time to run. In practice, however, this was never encountered.

BRACKSTAN1 (see Figure 4.2) took in the data using the above subroutines from NBATCH cycles of standard 1 (concentration CON1), a group of NSAMP samples and standard 2 (concentration CON2). Error messages were printed out if the input range of the ADC was exceeded at any time, if the first standard was not detected by BREAK or if the measured electrode response was unsteady, either due to a noisy or drifting electrode signal or to the sampler going out of synchronization. The results were then calculated and printed out on the teletype.

Compared with BRACKSTAN1, BRACKSTAN2 (see Figure 4.3) was slow and required more personal intervention in placing the selected standards into the sampler. The data for the first two standards was taken in from the electrodes, these standards normally being at either end of the range of interest. Values of E_0 and S over this range were then calculated and printed out as a check on the performance of the electrode. Since this printing took a few seconds the next solution to be passed to the

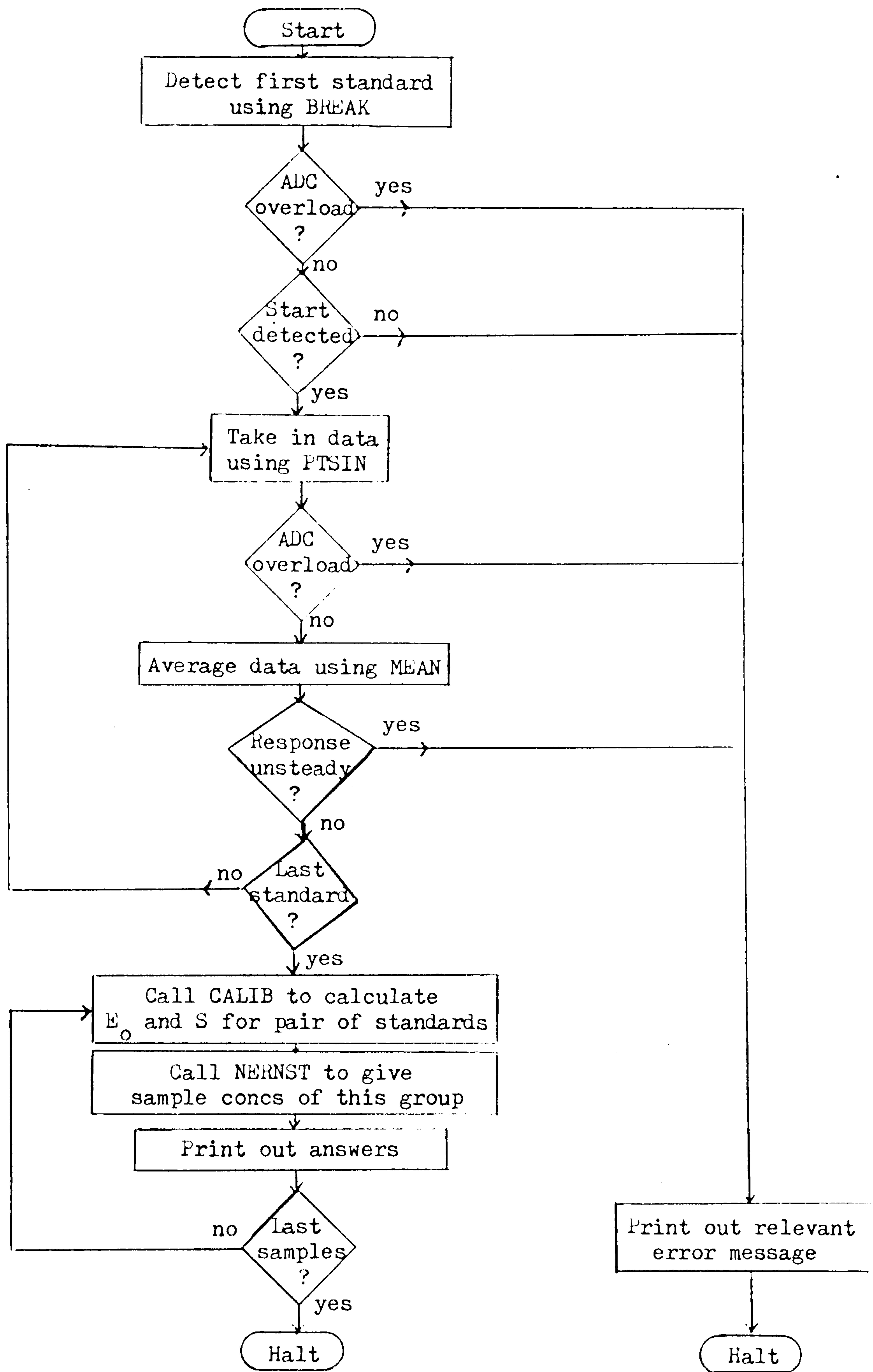


Figure 4.2 Flowchart of BRACKSTAN1

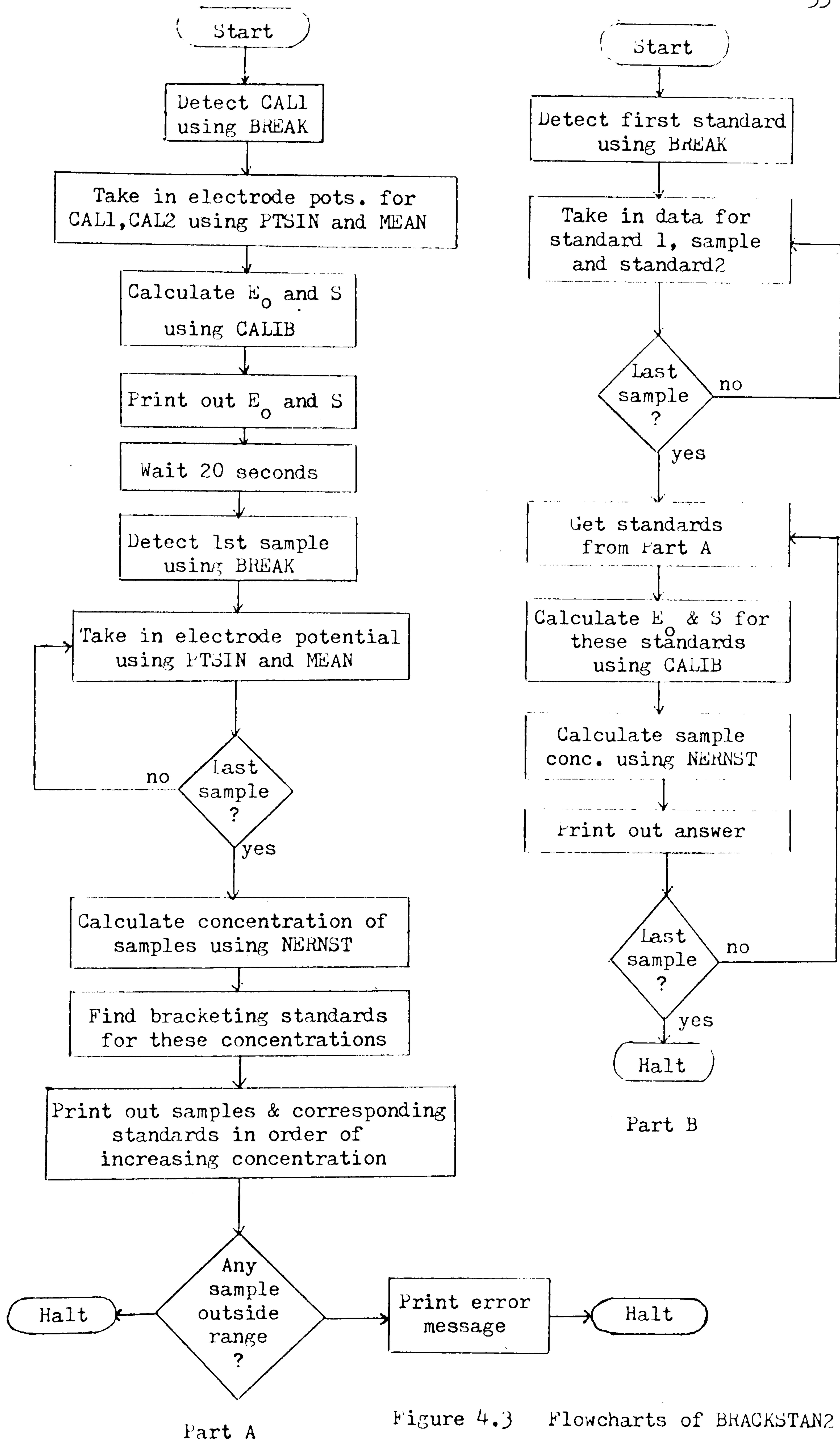


Figure 4.3 Flowcharts of BRACKSTAN2
Parts A & B (other error messages as
BRACKSTAN1)

electrodes was distilled water and the programme waited for 20sec for the electrode potential to stabilise before the detection of the first sample using BREAK. The samples were then passed through the electrode cell and the potentials for each taken in. Then the approximate concentration of each sample was calculated and the two standards which bracketed this concentration most closely were chosen from a list in memory. The sample concentrations and the suggested standards were then printed on the teletype in order of increasing concentration, so that the electrode response times in Part B would not be excessive due to drops in activity.

Then the standards and the samples were placed in the sampler in the suggested order so that Part B could be run. If a sample was found to be out of range in Part A it had to be omitted. Part B then took in the electrode potentials from the standards and samples, calculated the sample concentrations and printed these out.

As can be seen BRACKSTAN2 proved more inconvenient to run than BRACKSTAN1, but it was required to provide accurate results if the samples covered more than a narrow range of concentrations, as will be shown in the next section.

4.3 The Accuracy and Precision of Bracketing Standards

It has often been stated that the logarithmic nature of the Nernst equation leads to relatively small errors in the measured electrode potential causing large errors in the sample concentration e.g. that a 1mV error in potential leads to a 4% error in concentration for a univalent electrode, 8% for a divalent electrode etc. This, in fact, is an oversimplification since errors in the calibration have not been taken into account, but does show that large errors are very possible.

The potential accuracy of the method depends on the resolution of the measuring system, which in this case was 0.061mM, leading to a potential accuracy of 0.23% for a univalent electrode, 0.46% for a divalent

electrode etc. However in practice the accuracy obtainable with this method was limited by the behaviour of the electrode and by the presence of other species in the sample. As the rate of drift of electrode potential increased the accuracy decreased and for instance the number of samples which could be run between standards in BRACKSTAN1 depended directly upon the drift, but this proved a difficult effect to quantify. Similarly the change in electrode slope with concentration affected the accuracy, this being improved by the standards being as close to the sample concentration as possible. However this will be shown to lower the precision of the method, so a compromise had to be reached. Interfering ions present in the sample affected the accuracy greatly but these could be 'masked' or the standards made up in a similar matrix. If this was impossible a knowledge of the ions present at least indicated the likely accuracy obtainable. The position with complexing ions was similar, although if the concentration of the free ion was the parameter of interest this was not a problem.

The precision of the technique depended upon the precision of the measured electrode potentials, the concentration of the standards and the slope of the electrode, as shown below.

If $z = f(x, y)$ and x is independent of y then the variances of x , y and z , $V(x)$, $V(y)$ and $V(z)$ are related by -

$$V(z) = \left(\frac{\partial z}{\partial x} \right)^2 V(x) + \left(\frac{\partial z}{\partial y} \right)^2 V(y) \quad 4.3-A$$

Applying this equation to 4.1-C the variance of S is given by -

$$V(S) = \left[\frac{1}{\log(C_2/C_1)} \right]^2 (V_1 + V_2) \quad 4.3-B$$

where V_1 and V_2 are the variances of the signals from standards 1 and 2.

Solving equations 4.1-A and 4.1-B simultaneously gives -

$$E'_0 = \frac{E_1 \log C_2 - E_2 \log C_1}{\log(C_2/C_1)} \quad 4.3-C$$

Therefore the variance of E'_0 is given by -

$$V(E'_0) = \left[\frac{1}{\log(C_2/C_1)} \right]^2 ((\log C_2)^2 V_1 + (\log C_1)^2 V_2) \quad 4.3-D$$

Similarly using equation 4.1-E, if the variance of the signal from the sample is V_{samp} then the variance of the sample concentration is given by -

$$V(C_s) = \left[\frac{\log_e 10 C_s}{S} \right]^2 (V_{\text{samp}} + V(E'_0) + (\log C_s)^2 V(S)) \quad 4.3-E$$

Assuming that the variances of all the signals are equal, so that their standard deviations may be given by σ , the standard deviation of C_s ($\sigma(C_s)$) may be determined by substituting 4.3-B and 4.3-D into 4.3-E.

$$\sigma(C_s) = \sqrt{V(C_s)} = \frac{\log_e 10 C_s}{S} \sigma \sqrt{1 + \left[\frac{1}{\log(C_2/C_1)} \right]^2 ((\log C_1)^2 + (\log C_2)^2 + 2(\log C_s)^2)} \quad 4.3-F$$

Using this equation values of $\sigma(C_s)$ were calculated for various values of σ and C_1, C_2 pairs for a 5mM sample with $S = 60\text{mV/dec}$. The results are shown in Figure 4.4 and it can be seen that $\sigma(C_s)$ rises linearly with σ , and also increases as the concentration of the standards approach that of the sample. On the other hand as

$$\frac{C_2}{C_1} \rightarrow \infty, \sigma(C_s) \rightarrow \frac{\ln 10 C_s}{S} \sigma \quad 4.3-G$$

that is the limit of $\sigma(C_s)$ as C_2/C_1 tends to infinity is the same as the values given at the start of this section when there were no errors due to calibration.

As can be seen from the above considerations the vital parameter in the errors in analysis by bracketing standards is the concentration of the standards used. To make the results as accurate as possible the standards should be as close to the sample as possible. As can be seen

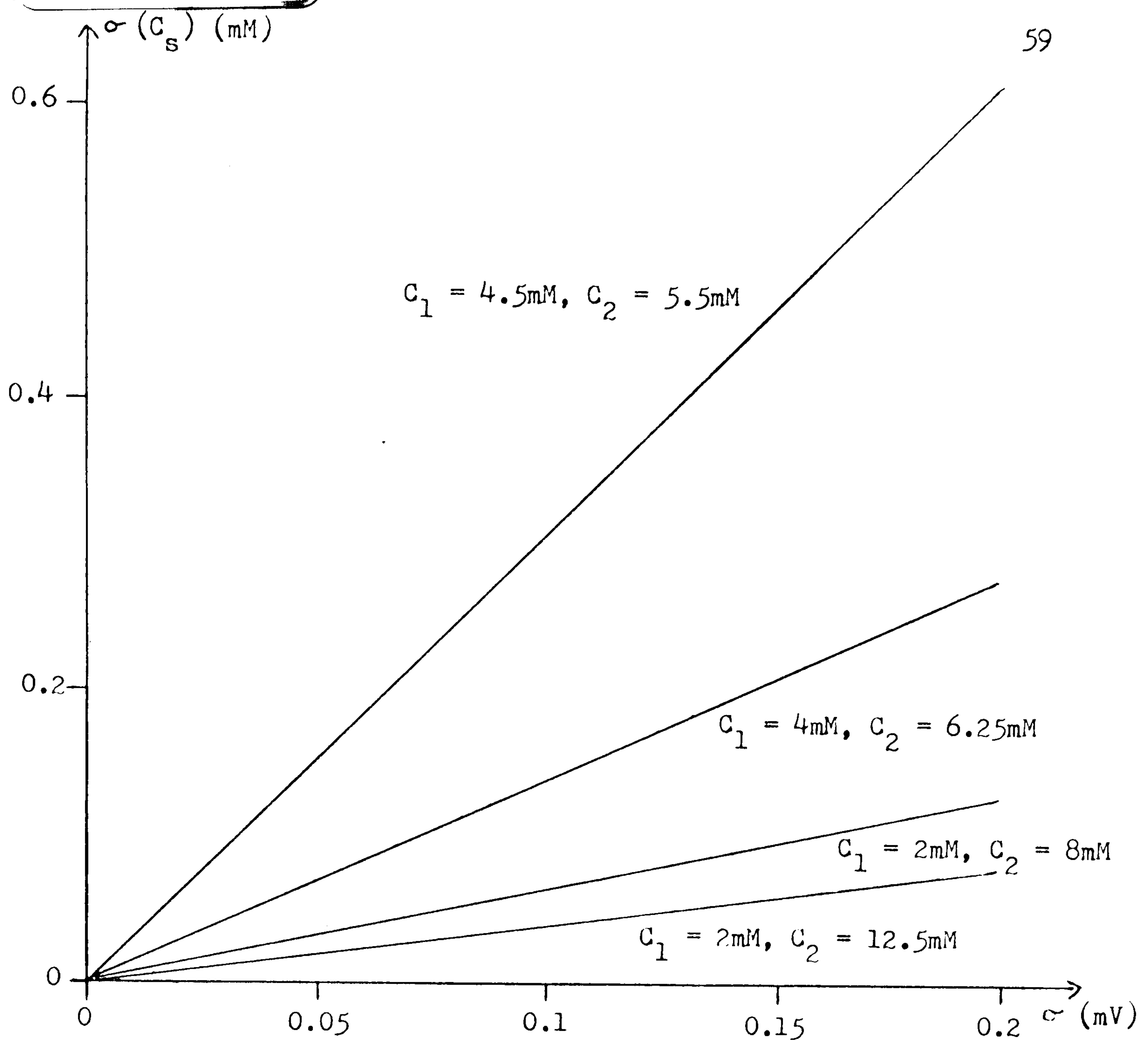


Figure 4.4 The standard deviation of the sample concentration ($\sigma(C_s)$) with the standard deviation of the signal (σ) with various standards ($C_s = 5\text{mM}$, $S = 60\text{mV/dec}$)

from Figure 4.4 for typical values of σ of 0.5 to 0.75mV (see Section 3.3) the ratio of C_2 to C_1 had to be at least 1.5 for a relative standard deviation of C_s of 2%. This is for a univalent electrode, for a divalent electrode the precision would be twice as bad.

To investigate these effects upon the accuracy and precision available using BRACKSTAN1 a series of analyses of 5mM calcium and potassium solutions were carried out using various standards, the results being shown in Table 4.5. The potassium analyses were carried out with 0.25M MgSO_4 ionic buffer, the calcium analyses with a 0.25M KCl buffer using the neutral carrier calcium electrode and in both cases $F_s = F_b = 2\text{ml/min}$.

Standards used (mM)	Potassium			Calcium		
	Mean	Relative Error	R.S.D.	Mean	Relative Error	R.S.D.
1,25	5.24mM	+4.8%	1.7%	5.15mM	+3.0%	3.1%
2,12.5	5.17mM	+3.4%	2.0%	5.12mM	+2.4%	3.7%
3,8	5.01mM	+0.2%	1.4%	5.08mM	+1.6%	3.2%
4,6	4.94mM	-1.2%	2.2%	4.96mM	-0.8%	3.7%
4.5,5.5	5.04mM	+0.8%	3.6%	4.99mM	-0.2%	4.9%

Table 4.5 The Accuracy and Precision of the Analysis of 5mM Samples using Various Standards

Sample Conc(mM)	Standards Suggested (mM)	Mean Found	Relative Error	R.S.D.
1.5	1&2,1&2,1&2,1&2,1&2	1.54mM	+2.7%	2.8%
4	3&5,3&5,3&5,3&5,3&5	4.03mM	+0.8%	0.9%
8	5&8,5&8,8&12,8&12,5&8	7.97mM	-0.4%	1.7%
18	20&30,12&20,12&20,12&20,12&20	17.7mM	-1.7%	2.4%
40	30&50,30&50,30&50,30&50,30&50	40.4mM	+1.0%	1.8%
80	50&75,4x Sample Overrange	78.4mM	-2.0%	-

Table 4.6 The standards suggested and the accuracy and precision of the results obtained by five replicate analyses of various potassium samples using BRACKSTAN2. Standards available were 1,2,3,5,8,12,20,30 50&75mM

Each analysis was performed five times to enable an estimate for the standard deviation to be found.

The results show the trend towards increasing accuracy and decreasing precision as the concentration of the standards approach that of the sample and that the precision of the potassium results was greater than that of the calcium results, as expected. The limits of BRACKSTAN1 are shown, this programme only being suitable for quite narrow ranges of standards, preferably with the ratio C_2/C_1 being around 1.5 to 2.

To analyse samples covering a large range BRACKSTAN2 was used, it being possible to cover up to two orders of magnitude using ten standards. A series of results for potassium samples are shown in Table 4.6, showing that for 28 out of the 30 samples the correct standards were chosen and that the accuracy and precision of the results were better than that obtainable using BRACKSTAN1 over such a high range.

4.4 Conclusions

It has been shown that bracketing standards can be a fairly accurate and precise method for the analysis of solutions containing only a compound of the species of interest. The concentration of the standards used was found to be of vital importance, with the optimum value of C_2/C_1 being around 1.5 to 2 with the typical noise levels associated with the measured electrode signals in this study. Unfortunately the analysis of real samples may be hampered by the presence of interfering ions or complexing agents. If their effect cannot be taken into account it would prove wise to use another method of analysis.

Using BRACKSTAN1 only samples within a narrow range of concentrations could be analysed, but the method was convenient and even placing only one sample between each standard led to a potential analysis rate of 30/hr. BRACKSTAN2 proved slower at 12 samples/hr and required a greater volume of sample since it passed the electrodes twice, but catered for a

wider range of sample concentrations. For instance using eleven standards with a ratio of C_2/C_1 equal to 2 a range of three orders of magnitude could be covered, although problems with the time response of the electrode between two standards of greatly differing concentration would be found. The setting up of solutions in the sampler proved quite time consuming but the manual effort involved in both methods would have been cut considerably by storing the standards in reservoirs and using a computer controlled valve system to switch between the standards and the samples.

5.1 Introduction

As well as bracketing standards, standard addition techniques have been used to overcome the problem of drift in the constant terms in the Nernst equation by recalibrating the electrode during each distinct analysis (see Section 1.4.2). Their main advantage over bracketing standards is that the multiple addition of standards may increase the precision of analyses if the number of additions is greater than the number of unknowns to be determined (a maximum of three; E_o , S and sample concentration) because of the reduction of the effect of random experimental errors. Some claims have also been made that standard additions give superior results for sample concentration when complexing agents or interfering ions are present; this is dealt with more fully in Sections 5.5 and 5.6.

The method of Gran, described in Section 1.4.2 for additions made in an open beaker, may also be applied to the standard addition technique using Autoanalyzer modules as shown in Figure 5.1 (109). In this case the potential of an electrode when a standard C_i is being added to the sample of concentration C_s is

$$E_i = E_o + S \log \frac{F_s C_s + F_i C_i}{F_s + F_i + F_b} \quad 5.1-A$$

providing the activity coefficient is constant for each addition. This may be rearranged to give

$$10^{E_i/S} = 10^{E_o/S} \frac{F_s C_s + F_i C_i}{F_s + F_i + F_b} \quad 5.1-B$$

and thus a plot of $10^{E_i/S}$ vs. C_i will give a straight line which intercepts the x-axis when -

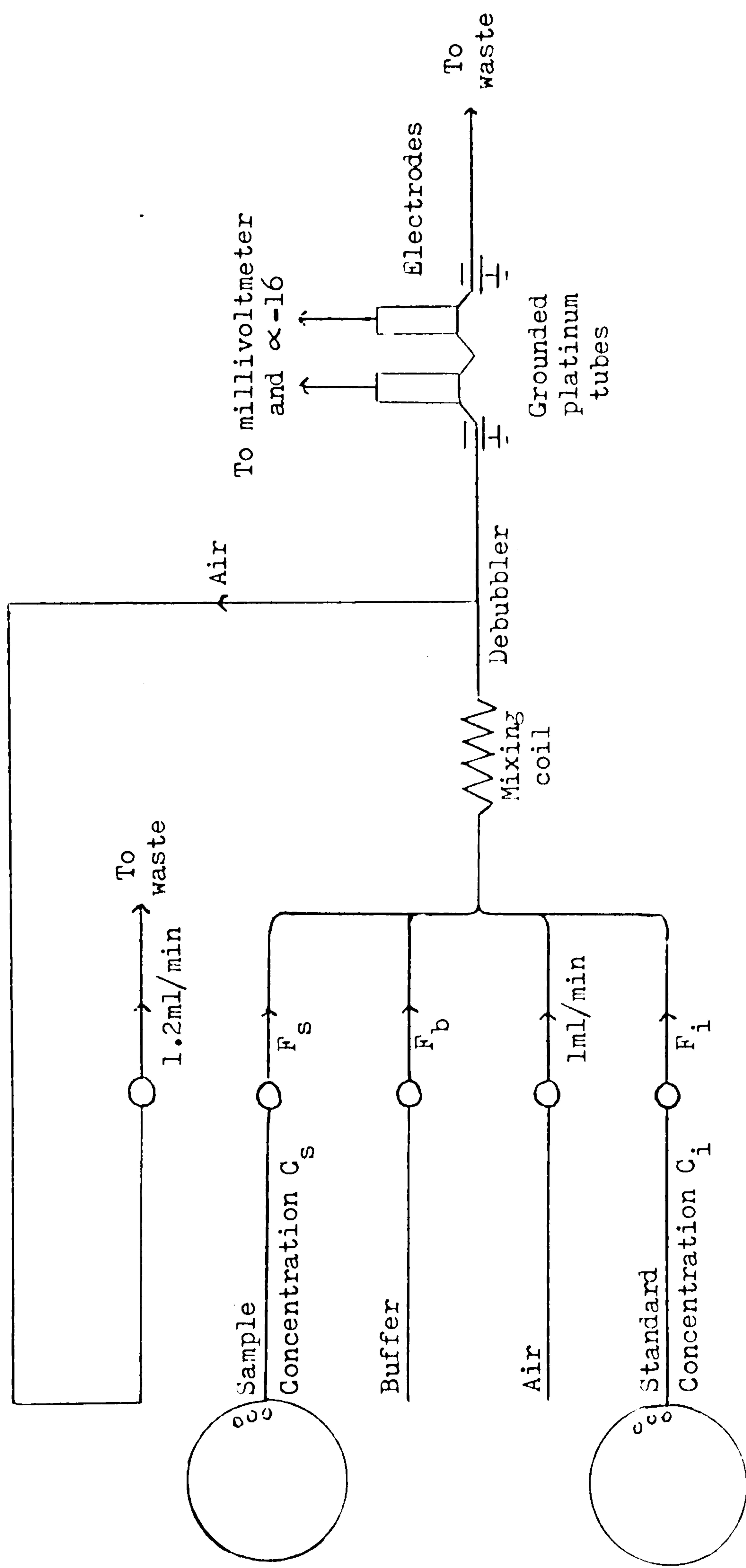


Figure 5.1 Flow stream for standard additions

$$C_i = - \frac{F_s C_s}{F_i} \quad 5.1-C$$

However this method for calculating sample concentration requires a predetermined value of the electrode slope to be entered and is thus sensitive to changes in S with time and concentration. If an incorrect value of the electrode slope S' is inserted equation 5.1-B becomes

$$10^{E_i/S'} = 10^{E_o/S'} \left[\frac{F_s C_s + F_i C_i}{F_s + F_i + F_b} \right]^{S'/S} \quad 5.1-D$$

and while a plot of $10^{E_i/S}$ vs. C_i will still give the correct intercept it will no longer be linear. Attempts to treat it as such will lead to errors, the magnitude of these depending on the proximity of the ratio S'/S to 1.

However techniques are available for handling standard addition data without the need for predetermining either electrode constant. Since in this case there are three unknowns a minimum of three readings are required, as opposed to two when using the Gran's plot. One of these techniques is to apply the rigorous least squares adjustment to non-linear equations as described by Wentworth (79) to the problem (55). This treatment does not depend upon a linear regression and thus may be used to take into account the errors in both the electrode potentials and the standard concentrations, but although an accurate value of S is not needed rough estimates of the values of E_o , S and C_s (E_o^o , S^o and C_s^o) are required.

Firstly an expression for D_i , the difference between the calculated electrode potential and the experimental potential is obtained -

$$D_i = E_o^o + S^o \log \frac{F_s C_s^o + F_i C_i}{F_s + F_i + F_b} - E_i \quad 5.1-E$$

Then the partial differentials of D_i with respect to the various parameters and variables are -

$$D_{E_o}^o = +1$$

$$D_{S_o}^o = \log \frac{F_s C_s^o + F_i C_i}{F_s + F_i + F_b}$$

$$D_{C_s}^o = \frac{S^o F_s}{F_s C_s^o + F_i C_i} \quad 5.1-F$$

$$D_{E_i} = -1$$

$$D_{C_i} = \frac{S^o F_i}{F_s C_s^o + F_i C_i}$$

A value of each of these differentials as well as L_i , a weighting factor, is then calculated for each (C_i, E_i) pair. L_i is given by -

$$L_i = 1/\sigma_o^2 (D_{E_i}^2 \sigma_{E_i}^2 + D_{C_i}^2 \sigma_{C_i}^2) \quad 5.1-G$$

where σ_o^2 is the variance of unit weight (an arbitrary constant) and $\sigma_{E_i}^2$ and $\sigma_{C_i}^2$ are the variances of E_i and C_i respectively.

Then values for the corrections to be made to E_o^o , S^o and C_s^o ($\Delta E_o^o, \Delta S^o$ and ΔC_s^o) can be calculated by solving the normal equations -

$$\begin{vmatrix} \sum D_{E_o}^o{}^2/L_i & \sum D_{E_o}^o D_{S_o}^o/L_i & \sum D_{E_o}^o D_{C_s}^o/L_i \\ \sum D_{S_o}^o D_{E_o}^o/L_i & \sum D_{S_o}^o{}^2/L_i & \sum D_{S_o}^o D_{C_s}^o/L_i \\ \sum D_{C_s}^o D_{E_o}^o/L_i & \sum D_{C_s}^o D_{S_o}^o/L_i & \sum D_{C_s}^o{}^2/L_i \end{vmatrix} \begin{vmatrix} \Delta E_o^o \\ \Delta S^o \\ \Delta C_s^o \end{vmatrix} = \begin{vmatrix} \sum D_{E_o}^o D_i/L_i \\ \sum D_{S_o}^o D_i/L_i \\ \sum D_{C_s}^o D_i/L_i \end{vmatrix}$$

M . N = P

5.1-H

Finally new estimates for E_0^0 , S^0 and C_s^0 are calculated by subtracting the relevant correction from the old value and the whole procedure repeated using the new values until it is judged that the corrections are so small that the estimates are close enough to the actual values. As can be seen this method is too complex to be considered for use in manual calculations, but it can be used for data processing by computer. It is compared with the Gran's plot in the next section.

5.2 Comparison of Gran's and Wentworth's Methods for Processing Data

Before setting up an on-line system to perform standard additions it was decided to compare the methods of Gran and Wentworth to see which proved superior. It has been shown that using a linear Gran's plot would cause errors if an incorrect value of S were to be used but there are also possible problems with the Wentworth method e.g. the rate of convergence, possible divergence and the criterion by which it is judged when to stop further iteration.

To perform the calculations for a Gran's plot two subroutines were written, GRAN which evaluated the sample concentration from the data, using LLS to find the best fit for the Gran's plot. GRAN took in values for n (the number of additions), F_s , F_i , S and the (C_i, E_i) pairs, calculated $10^{E_i/S}$ for each addition and called LLS to find the equation of the best fitting straight line through the Gran's plot, the percentage fit of this line and the standard error of the intercept on the x-axis. From this C_s and its standard error was calculated and passed to the main programme, along with the percentage fit.

LLS performed a linear regression of y upon x for n points by the method of least squares, giving values for a and b in the regression equation $y = a + bx$. LLS took in values for n and the (x, y) pairs, then calculated the sums of x , x^2 , y , y^2 and xy and the following -

$$S_{xx} = \sum x^2 - (\sum x)^2/n$$

$$S_{yy} = \sum y^2 - (\sum y)^2/n \quad 5.2-A$$

$$S_{xy} = \sum xy - \sum x \sum y/n$$

LLS then calculated the best estimates of a and b, which can be shown (110) to be -

$$b = \frac{S_{xy}}{S_{xx}}, \quad a = \sum y/n - b \sum x/n \quad 5.2-B$$

Next LLS calculated the percentage fit of the regression, which gave an indication of how good the regression is -

$$\text{Percentage Fit} = \frac{b^2 S_{xx}}{S_{yy}} \times 100\% \quad 5.2-C$$

Finally, of particular interest when using GRAN, LLS calculated the best estimate of the standard error in the prediction of the intercept on the x-axis, S.E.(I), where -

$$S.E. (I) = \frac{1}{b} \frac{S_{yy} - b^2 S_{xx}}{n-2} \sqrt{1 + 1/n + \frac{\sum y^2}{n^2 b^2 S_{xx}}} \quad 5.2-D$$

and returned values of a, b, percentage fit and S.E.(I) to the main programme, with an error flag set if the floating point range had been exceeded.

To investigate the effect of inserting an incorrect value of S into GRAN sets of theoretical electrode potentials for standard additions of 0, 5, 10, 15 and 20mM to a 5mM sample with $F_s = F_i = F_b$ were calculated with $S = 30$ and 60mV/dec. These potentials were inserted into a programme calling GRAN and the sample concentration found when various erroneous values of S were 'latched in'. The results, shown in Figure 5.2, indicate that the percentage error in concentration was around two and a half times the percentage error in the value of S. Therefore an error of 1mV/dec in the value of S would lead to a 4% error in C_s for a

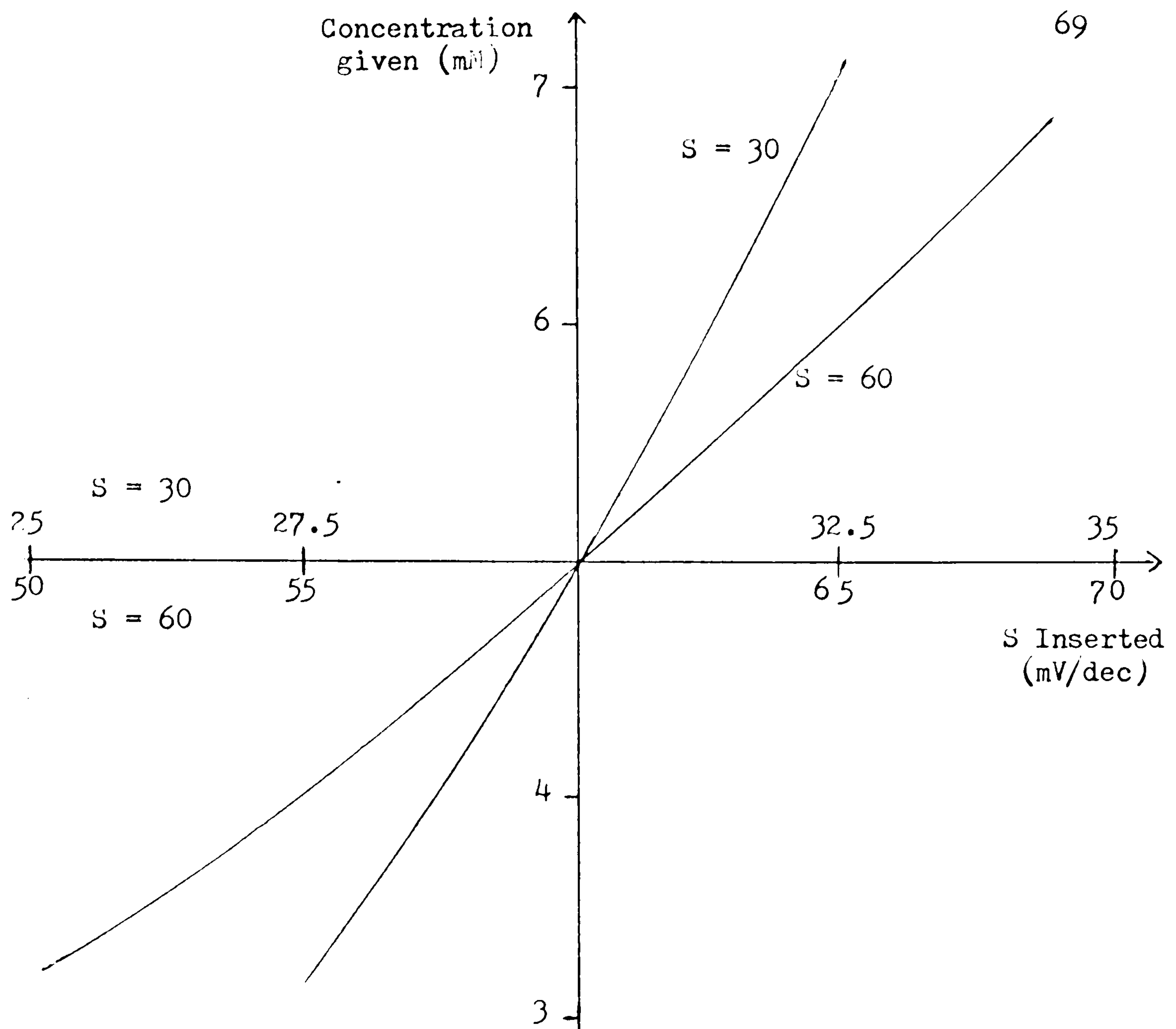


Figure 5.2 The sample concentration found by a Gran's Plot when incorrect values of S were used, using calculated electrode potentials ($C_s = 5\text{mM}$, $F_s = F_i = F_b$, $C_i = 0, 5, 10, 15, 20\text{mM}$)

univalent electrode and 8% for a divalent electrode.

These errors were judged to be potentially too high, but could have been reduced by using a curve-fitting routine. This would not only have proved very tedious to write in assembler language, but the fitting of an equation of the ' z 'th order would require at least ' $z+1$ ' additions to be made, increasing the number of additions needed to give the same reduction of experimental errors. Also the prediction of a value outside the range covered by the data, as is the case for Gran's plots, is inadvisable in curve-fitting since the regression curve may exhibit large changes in curvature outside this range and may even pass through maximum or minimum values.

Another possible way of reducing these errors was investigated, by locating the value of S which gave a maximum for the percentage fit. Attempts to do this were thwarted by the low rate of change of the percentage fit near the correct value of S - for instance for the data used in Figure 5.2 when $S = 61$ instead of 60mV/dec leading to an error of 4% the percentage fit was still greater than 99.9%.

Because of this problem it was decided to use Wentworth's method and STAD subroutine was written to perform the necessary calculations. STAD took in values for n , F_s , F_i , F_b , E_o^0 , S^0 , C_s^0 , each (C_i, E_i) pair and $\sigma_{E_i}^2$ and continued as described in Section 5.1. To determine the weighting for each point values for $\sigma_{C_i}^2$ and $\sigma_{E_i}^2$ were required, but the accuracy of these factors was not of great importance. A value of $\sigma_{C_i}^2 = 2.5 \times 10^{-5} C_i^2$ was taken from the literature (55) and a typical value of $\sigma_{E_i}^2$ could be obtained using VARY (see Section 2.6). Then so that the magnitude of the weighting factors was convenient σ_o^2 was set to such a value that $L_i = 1$ for the first pair of (C_i, E_i) values.

The normal equations (5.1-H) were then solved by inverting matrix M to $\bar{M}$ and obtaining N from $N = P \cdot \bar{M}$, then the resulting corrections were made. The ratio $C_s^0 / \Delta C_s^0$ was calculated and if less than a set value, k , the procedure was repeated. To show the effect of the value of k chosen the data used in the generation of Figure 5.2 was used with L_i set to 1 and E_o^0 and S^0 set to their correct values with $C_s^0 = 10$ and the percentage error in the concentration and the number of iterations required plotted against k in Figure 5.3. It was decided to set k to 10,000 since the error was acceptably low, 0.01%, and the number of iterations not excessively high.

With k set to 10,000 and the same data being used the effect of the initial value of C_s^0 upon the number of cycles required was investigated. Figure 5.4 shows that if C_s^0 was several times larger than the actual C_s STAD failed since it proved impossible to invert matrix M , although STAD worked for all values of C_s^0 less than C_s . However a large number

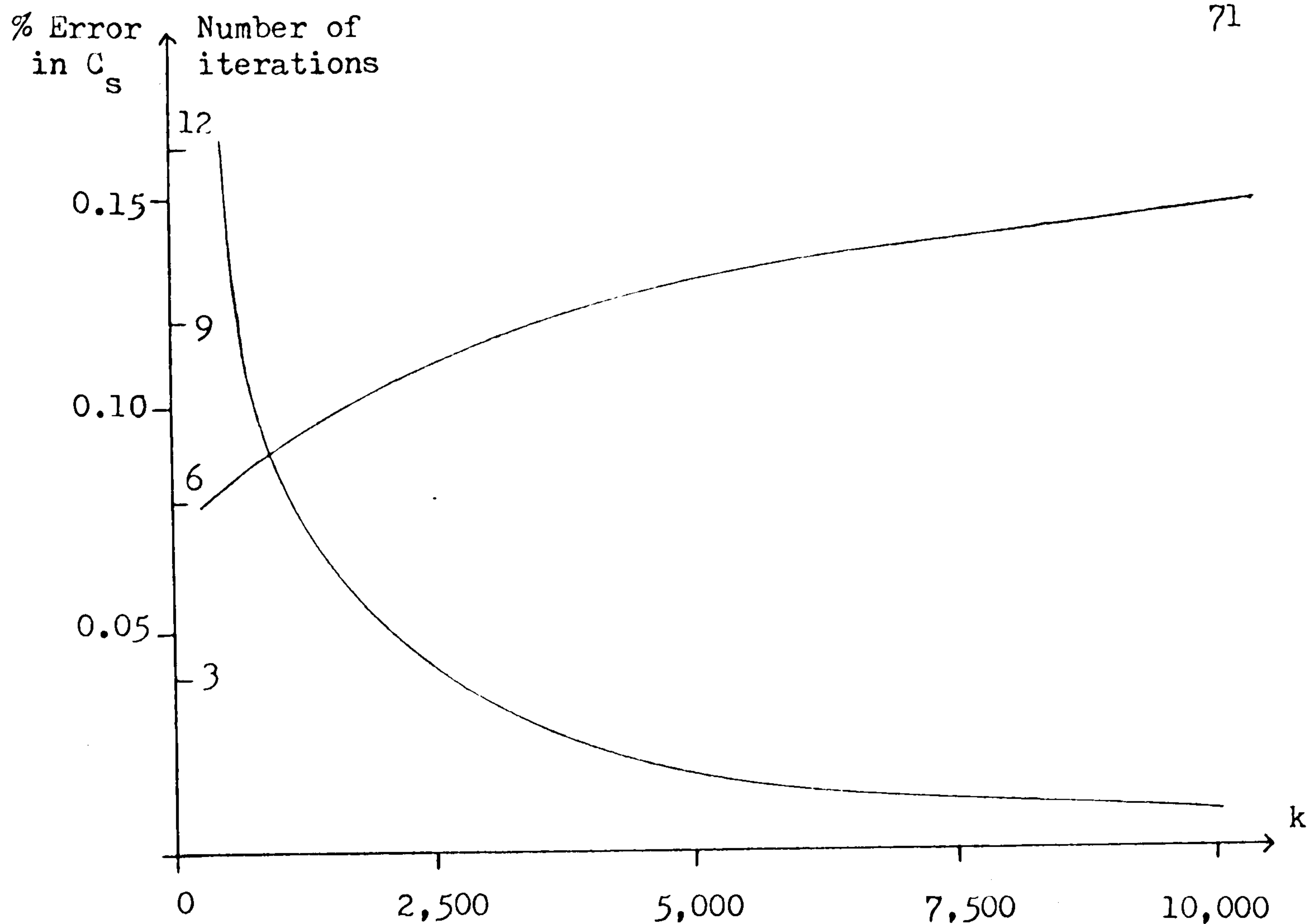


Figure 5.3 The number of iterations needed and the error in C_s with various values of k . ($C_s = 5\text{mM}$, $S = 60\text{mV/dec}$, $F_s = F_i = F_b$, $C_i = 0.5$, $10, 15, 20\text{mM}$, $C_s^0 = 10\text{mM}$, E_o^0 & S^0 were correct)

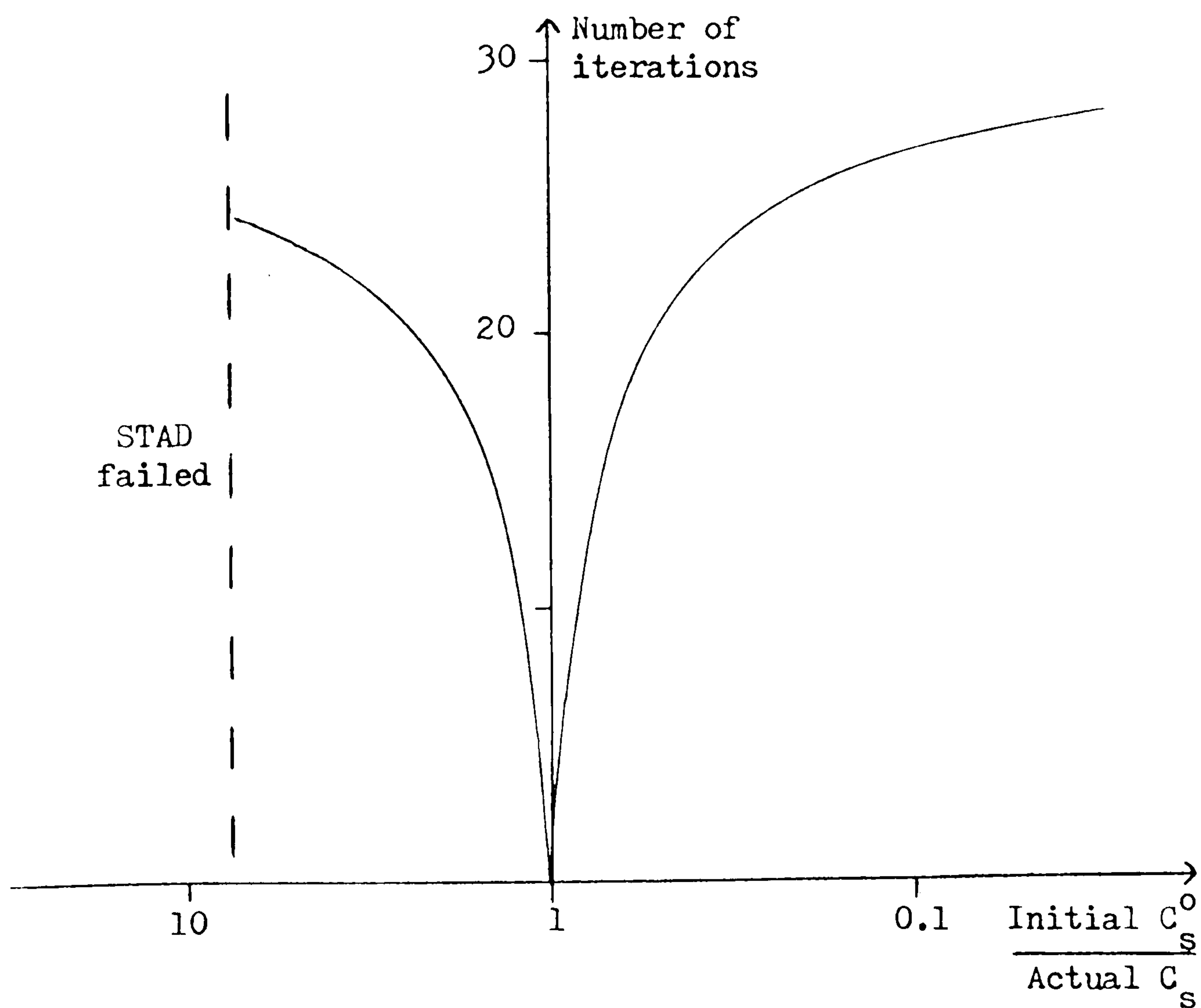


Figure 5.4 The number of iterations required with various values of C_s^0 (E_o^0 and S^0 being correct)

of iterations were necessary, up to 30 unless C_s^0 was a fairly good estimate. It was found that the values of S^0 and E_o^0 converged rather more rapidly than C_s^0 , which was unfortunate since good estimates of E_o and S were available from the electrode calibration or the previous analysis, while C_s would often be completely unknown. Attempts to speed up the convergence of C_s^0 by the methods suggested in (79) proved to be ineffective, but even with experimental electrode potentials STAD took no longer than a couple of seconds to run.

In summary the use of GRAN was not to be recommended for experimental purposes because of errors arising from using even only slightly inaccurate values of S . STAD, on the other hand, was relatively slow but only estimates of E_o , S and C_s were required and the answers were insensitive to the accuracy of the estimates, although it proved advisable to enter a low value for C_s^0 else the subroutine could fail completely.

5.3 On-line Standard Addition System

In order to take in the electrode potentials and to process the data from standard additions carried out in the system illustrated in Figure 5.1 the programme STANDAD was written. STANDAD, whose flowchart is given in Figure 5.5, allowed for the analysis of 'NSAMP' samples by the addition of 'NSTAN' similar standards to each sample.

The data acquisition scheme was similar to that of BRACKSTAN1 (see Section 4.2), using BREAK, PTSIN and MEAN subroutines to give the electrode potential on each addition. Therefore as well as values for NSAMP and NSTAN, values for NW1, NAV and NW2 had to be latched in for PTSIN to run and also the data required by STAD to evaluate the results (E_o^0 , S^0 , C_s^0 , each C_i value, $\sigma_{E_i}^2$, F_s , F_i and F_b).

After the acquisition of data the sample concentrations were determined by STAD. To improve convergence after the first calculation

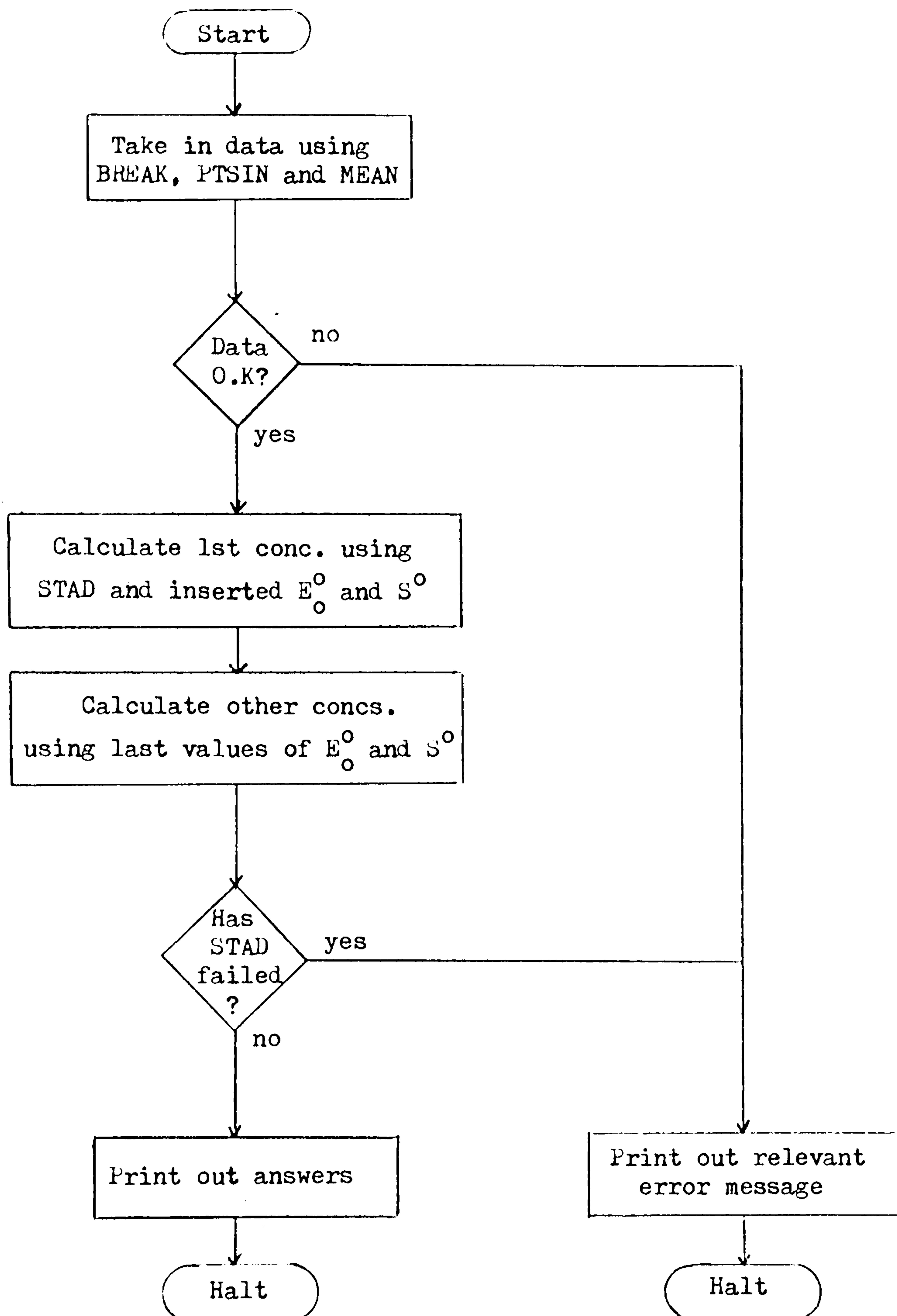


Figure 5.5 Flowchart of STANDAD

the values of E_o^o and S^o used were those provided by the last iteration. Then the sample concentrations were printed on the teletype and the last calculated values of E_o and S stored for use in the next running of the programme if the same electrode was to be used.

5.4 The Accuracy and Precision of Standard Additions

The accuracy obtainable from this technique depends on the accuracy of the values of the experimental parameters C_i , F_s , F_i and F_b and also on how closely the measured electrode potential follows the relationship of Equation 5.1-A. On the other hand, the precision depends on the random errors associated with the measured signal, the number of additions made and the concentration of the standards used.

To show how the accuracy of the method was affected by inaccuracies in the values of the flow rates and the standard concentrations and by accuracy of the electrode potential measured, a programme calling STAD was used with the perfect data described in the previous section. The inserted values of flow rates and concentrations were then varied from the original figures, as shown in Table 5.6 along with the resulting value of the sample concentration. Then the electrode potentials were calculated when measured on systems of different resolutions, when readings were taken before each potential change was complete and when E_o or the temperature was drifting. These potentials were then substituted into the programme, along with the correct values for the standards and the flow rates, and the sample concentrations calculated.

Table 5.6 shows that errors in C_i , F_s and F_i led to comparable errors in the result for C_s , but there were no errors arising from incorrect values of F_b or if the errors in measuring the flow rates were consistent. The error increased rapidly with decreasing resolution, but was small at the approximate level of the resolution of the ADC. The undesirability of drifts in E_o and temperature and the desirability of allowing the complete potential change are shown, but small levels of these may lead to acceptably small errors.

Another factor which influences the accuracy is how perfectly Nernstian the response of the electrode is over the range of concentrations

Conditions	Calculated C_s (mM)	Conditions	Calculated C_s (mM)
Perfect data	5.000		
C_2 1% high	5.016	C_2 5% high	5.076
C_3 1% high	5.019	C_3 5% high	5.080
C_4 1% high	5.020	C_4 5% high	5.092
C_5 1% high	5.022	C_5 5% high	5.096
C_2 - C_5 all 1% high	5.037	C_2 - C_5 all 5% high	5.153
F_s 1% high	4.948	F_s 5% high	4.761
F_i 1% high	5.054	F_i 5% high	5.252
F_b 1% high	5.000	F_b 5% high	5.000
F_s, F_i & F_b all 1% high	5.000	F_s, F_i & F_b all 5% high	5.000
0.05mV resolution	5.005	0.1mV resolution	4.989
0.5mV resolution	4.883	1mV resolution	4.807
Each step only 99% complete	5.035	Each step only 95% complete	5.202
Drift of E_o of 0.1mV/reading	4.895	Drift of E_o of 0.2mV/reading	4.783
Temperature rising (20-22°C)	4.913	Temperature falling (20-18°C)	5.154

Table 5.6 The error found in a 5mM sample due to various errors in standard concentrations, flow rates and electrode potentials.

being used. To minimise this effect a small range of standard concentrations could be used; this also would minimise changes in activity coefficient during the experiment which would invalidate Equation 5.1-A. However doing this will be shown to be detrimental to the precision of the measurement.

To investigate the precision the method of Gran was used for simplicity. The major reason for not using it in STANDAD was the poor accuracy of the

results if an inaccurate value of S was inserted. However there is no reason to believe that the method of evaluating the results affects the factors which determine the precision.

The best estimate of the standard error of C_s , $S.E.(C_s)$ is given by -

$$S.E.(C_s) = \frac{\partial C_s}{\partial I} S.E.(I) \quad 5.4-A$$

where $S.E.(I)$ was given by Equation 5.2-B, so that

$$S.E.(C_s) = \frac{F_i \sigma}{F_s b} \sqrt{1 + 1/n + 1/S_{xx}(\bar{x} + a/b)} \quad 5.4-B$$

where $\bar{x}$ is the mean of the x values and σ^2 is the variance around the regression -

$$\sigma^2 = \frac{\sum (y-Y)^2}{n-2} = \frac{S_{yy} - b^2 S_{xx}}{n-2} \quad 5.4-C$$

$(y-Y)$ being the difference between the actual y co-ordinate of a point and that predicted by the regression.

It appears from Equation 5.4-B that $S.E.(C_s)$ varies with the ratio F_i/F_s , but this is not the case since the term representing $S.E.(I)$ is inversely proportional to this ratio. Similarly σ is proportional to b so that $S.E.(C_s)$ does not depend on the gradient of the regression. Therefore the precision of the method depends on the number of additions made, the range of concentrations of the additions and the degree of scatter of the points around the regression.

When the number of additions is varied, within the same range of concentrations and with equal steps between each standard within each set of additions, both the $1/n$ and the $1/S_{xx}$ terms in Equation 5.4-B change. More importantly as n increases $S.E.(C_s)$ becomes closer to the correct standard error of C_s . To account for this the 95% confidence limits of C_s^t (the true value of the sample concentration) were used in the following considerations, these confidence limits being -

$$C_s \pm t S.E.(C_s) \quad 5.4-D$$

where t depends on the degrees of freedom that $S.E.(C_s)$ is based upon and the level of confidence required, its values being found in statistics tables. Using Equations 5.4-B and 5.4-D the confidence limits for n additions of between 0 and 20mM, with equal steps between standards, to a sample of 5mM potassium were calculated with $F_i = F_s$, for values of n from 3 to 9 and the results shown in Figure 5.7. The precision was poor if only three additions were made, but improved greatly for larger values of n . However when using STAD to evaluate results it must be remembered that calculating the electrode slope removes another degree of freedom, so an extra addition is required to achieve the precision indicated in Figure 5.7.

The range of standard concentrations affects the $(\bar{x}+a/b)^2/s_{xx}$ term of Equation 5.4-B. Since it is obvious that a better value of the intercept will be given if the first addition is of zero concentration, i.e. as close to the intercept as possible, the first addition was always of distilled water. To investigate the effect of the maximum concentration of the standards used the 95% confidence limits of C_s^t were calculated for $F_i = F_s$, $C_s = 5\text{mM}$, $n = 6$ for various ranges of additions, the concentrations increasing in equal steps within a set of additions, and the results are shown in Figure 5.8. It is shown that the precision increases with the maximum value of C_i . However using too large a range of standards may lead to a non-Nernstian response of the electrode, so that a compromise of insuring that C_i reaches at least twice C_s was used.

To calculate the absolute values of the confidence limits the scatter about the regression has to be evaluated. To do this the difference between the expected electrode potential and the actual potential for each addition was defined as DIF, which was taken to be constant for all additions, and it was assumed that the scatter had no effect on the calculated regression equation. Then -

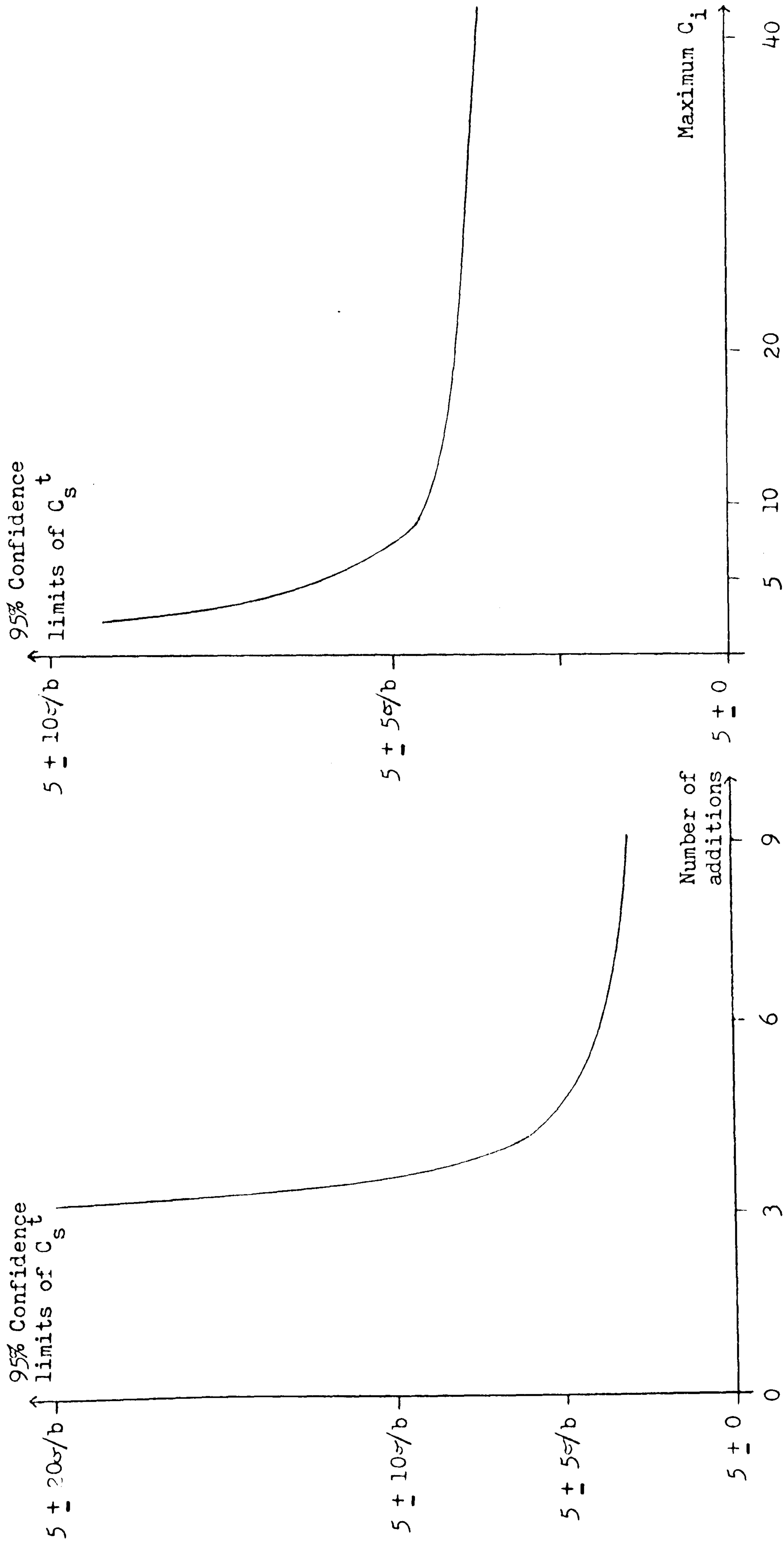


Figure 5.7 The variation of the 95% confidence limits of C_s^t with n .

Figure 5.8 The variation of the 95% confidence limits of C_s^t with the range of the additions.

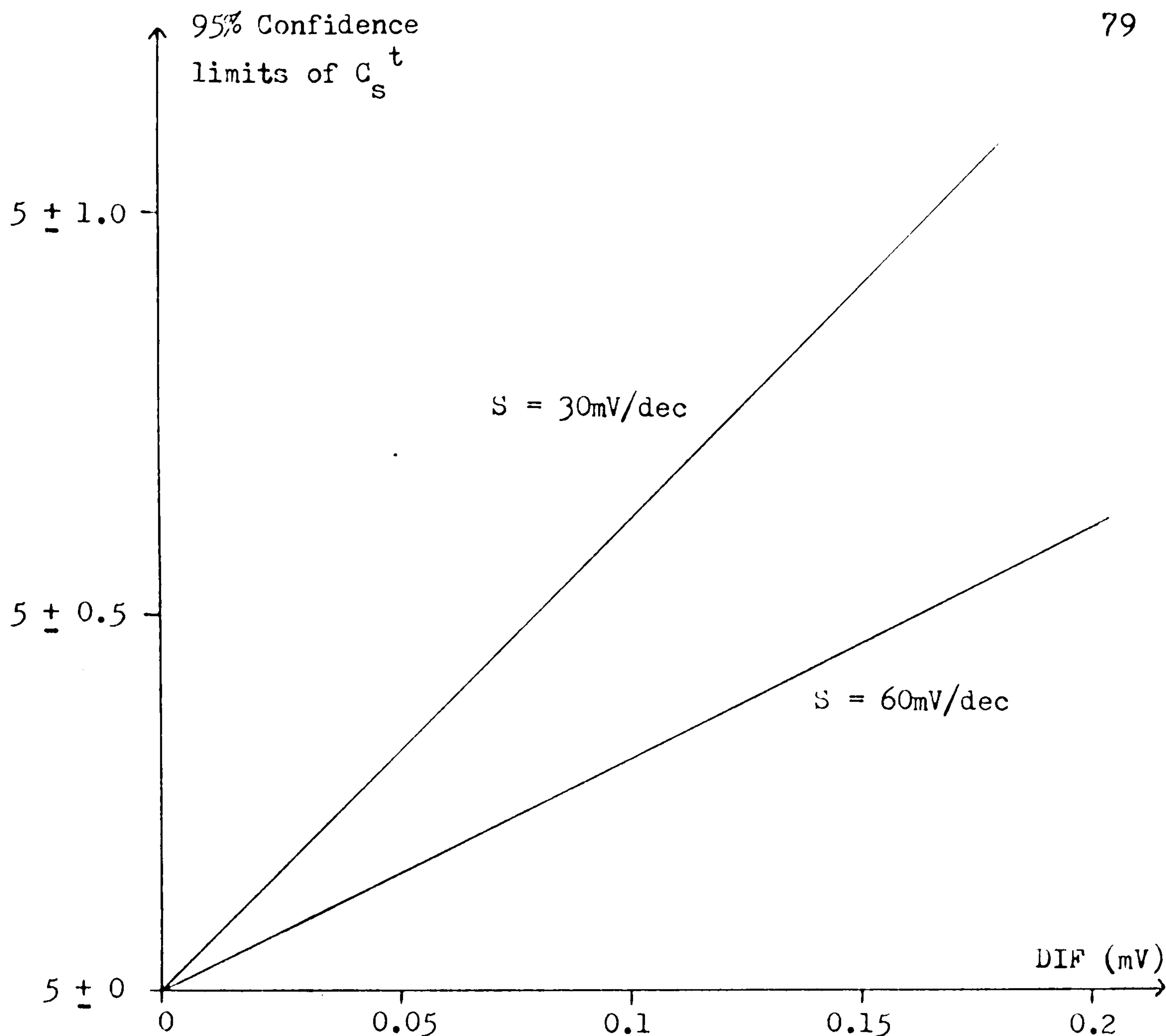


Figure 5.9 The variation of the 95% confidence limits of C_s^t with DIF.

$$\frac{\sigma^2}{b^2} = \frac{\sum(y-Y)^2}{b^2(n-2)} = \frac{(10^{(E_i + \text{DIF})/S} - 10^{E_i/S})^2}{b^2(n-2)}$$

$$\frac{\sigma^2}{b^2} = \frac{((x+a/b)(10^{\text{DIF}/S} - 1))^2}{n-2} \quad 5.4-F$$

The 95% confidence limits for C_s^t were then calculated for $F_s = F_i$, $n = 6$, $C_s = 5\text{mM}$ and $C_i = 0$ to 20mM for various values of DIF and S , the results being shown in Figure 5.9.

It can be seen that the precision is inversely proportional to DIF and that the errors for divalent ion electrodes are double those for univalent ion electrodes. The precision of the method seems to be rather poor, but it must be remembered that these figures were calculated for all

electrode potentials being different from the expected values by a constant amount. If all the readings were within 0.1mV of the expected value the precision would probably be much higher than that indicated by $DIF = 0.1mV$, since some of the points would lie nearer or actually on the regression. Also the method of presentation, using 95% confidence limits, gives higher values for errors than the more commonly used standard deviation or error.

To test the accuracy and precision of the method experimentally STANDAD was used to measure the concentrations of various potassium and calcium solutions between 1 and 10mM, using the respective electrodes. The flow rates F_s , F_i and F_b were all set to around 1.4ml/min, accurate values being obtained by the calibration of each tube. The concentrations of the additions were 0, 4, 8, 12, 16 and 20mM so that the errors shown in Figure 5.8 were minimised in all cases. The ionic buffers used were 0.5M $MgSO_4$ for the potassium determinations and 0.5M KCl for the calcium analyses. Each analysis was repeated six times to enable a value for the standard deviation to be calculated, these being shown in Table 5.10. The precision of the potassium results was higher than that of the calcium results, as was expected from the electrode slopes. However the accuracy and precision of the lower concentrations were poorer than the higher concentrations, especially the accuracy of the calcium results. This could be attributed to imperfect Nernstian behaviour over the relatively large concentration ranges or to the effects of interference by the ionic buffer. The analyses of the 1 and 3mM samples were repeated with 0.1mM ionic buffers and 0, 1, 2, 3, 4, 5 and 6mM additions to reduce these effects and the accuracy and precision were both considerably improved.

Once again the accuracy and precision obtainable were dependent on the slope of the electrode. The number and range of the additions had to be brought into consideration, along with the Nernstian response and the effect of interfering ions (which is dealt with in the next section), particularly those present in high concentrations in the ionic buffer.

Actual conc. (mM)	Potassium				Calcium			
	Mean conc. found (mM)	Relative error	Standard deviation (mM)	Relative S.D.	Mean conc. found (mM)	Relative error	Standard deviation (mM)	Relative S.D.
1.0	1.06	+6.0%	0.045	4.2%	1.18	+18.0%	0.072	6.1%
3.0	3.08	+2.7%	0.051	1.7%	3.17	+5.7%	0.130	4.1%
5.0	4.92	-1.6%	0.079	1.6%	5.09	+1.8%	0.127	2.5%
8.0	7.99	-0.1%	0.072	0.9%	8.09	+1.1%	0.266	3.3%
10.0	10.08	+0.8%	0.127	1.2%	9.89	-1.1%	0.289	2.9%
1.0 ¹	0.98	-2.0%	0.027	2.8%	1.03	+3.0%	0.025	2.4%
3.0 ¹	3.06	+2.0%	0.064	2.1%	3.02	+0.7%	0.112	3.7%

Table 5.10 The accuracy and precision of some standard addition results
(¹ performed with more dilute standards and buffer, see text)

5.5 The Effect of Interferents on Standard Additions

Standard addition techniques are widely used in analytical chemistry to lessen the effect of interfering species upon the result of the analysis, proving very effective when the signal due to the interference is constant both before and after the addition. However this is not the case with ion selective electrodes, whose potential E_i is given by -

$$E_i = E_o + S \log (a_i + \sum K_{ij} a_j^{n/m}) \quad 5.5-A$$

where a_i is the activity of the primary ion of charge n , a_j the activity of the interfering ion of charge m and K_{ij} the selectivity coefficient. Thus the electrode potential vs. log activity of primary ion curve is curved, as would be the standard addition plot. Also considering a Gran's plot when interfering ions are present in the sample stream, the intercept of the curve on the x-axis will be when -

$$C_i = - \frac{F_s (C_s + \sum K_{ij} C_j^{n/m})}{F_i} \quad 5.5-B$$

To show the effect of various levels of interferences upon an analysis of a 5mM sample with $S = 60\text{mV/dec}$, $F_s = F_i = F_b$ and additions of 0, 4, 8, 12, 16 and 20mM, sets of theoretical electrode potentials were calculated for $\sum K_{ij} C_j^{n/m} = 0, 10^{-5}, 10^{-4}$ and 10^{-3} . These potentials were processed by STAD to give values for the sample concentration, which are shown in Table 5.11. The results obtained were fairly accurate, being better than those that would be obtained by bracketing standards with aqueous standards, which are also the values predicted by Equation 5.5-B for standard additions. To investigate the reason for this unexpected accuracy Gran's plots were drawn for $\sum K_{ij} C_j^{n/m} = 0$ and 10^{-3} , using the values of S calculated by STAD (S_{opt}). As can be seen, with interferents present the Gran's plot was curved but especially so in the area of negative C_i , so that the best fit straight line through the experimental points gave

$\sum K_{ij} C_j^{n/m}$	Concentration of sample	
	Standard additions	Bracketing standards
0	5.00mM	5.00mM
10^{-5}	4.998mM	5.01mM
10^{-4}	4.981mM	5.10mM
10^{-3}	4.804mM	6.00mM

Table 5.11 Effect of interfering ions upon the analysis of a 5mM sample by standard additions and bracketing standards, using calculated data.

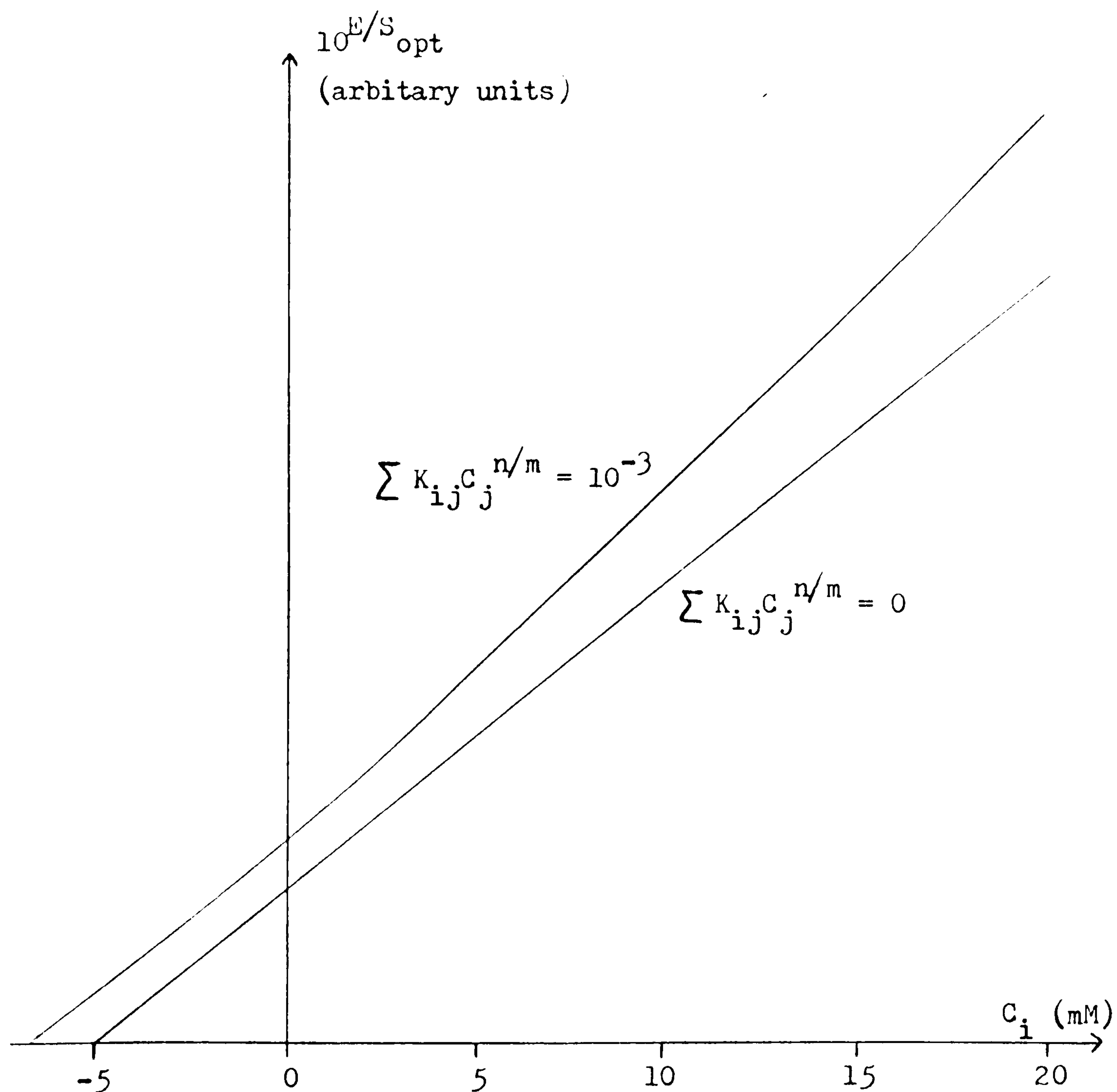


Figure 5.12 Effect of interfering ions upon a Gran's plot of a 5mM sample.

Magnesium Concentration	Calcium concentration found	
	Standard additions	Bracketing standards
1mM	5.07mM	4.94mM
10mM	4.97mM	5.12mM
100mM	4.86mM	5.81mM

Table 5.13 Experimental analysis of a 5mM calcium solution containing various magnesium concentrations by standard additions and bracketing standards.

an intercept nearer that of the Gran's plot with no interfering ions present than that of the curve.

This standard addition was repeated experimentally for 5mM calcium samples containing 1, 10 and 100mM magnesium using STANDAD, these solutions also being analysed by bracketing standards using BRACKSTAN1 and 4 and 6mM standards. The results are shown as a mean of three analyses in Table 5.13 and confirm the greater accuracy of the standard addition method.

5.6 The Effect of Complexing Agents on Standard Additions

The effects of complexing agents upon the results of standard additions can be split into two depending on the strength of the complex, whether or not the complexing of the species of interest can be taken as complete or only partial.

An example of total complexation is the reaction between calcium and EDTA. To investigate the effects of this a series of 5mM calcium samples were analysed using STANDAD as in Section 5.4, except that varying amounts of EDTA were added to the samples instead of magnesium. It can be seen from Table 5.14 that when the calcium concentration exceeded

EDTA conc.	Calcium found	EDTA conc.	Calcium found
0mM	4.86mM	5mM	-0.77mM
1mM	4.09mM	10mM	-4.32mM
2mM	3.12mM	15mM	-6.35mM
3mM	1.99mM	20mM	STAD failed
4mM	0.81mM	25mM	STAD failed

Table 5.14 Concentration of calcium found by standard additions on a 5mM sample with various concentrations of EDTA present.

Bicarbonate concentration	Calcium found by STANDAD	Calcium found by BRACKSTAN1	Uncomplexed calcium conc.
5mM	4.97mM	4.89mM	4.77mM
10mM	5.09mM	4.55mM	4.56mM
50mM	4.82mM	3.29mM	3.37mM
100mM	4.73mM	2.70mM	2.53mM

Table 5.15 Concentration of calcium found by standard additions and bracketing standards on a 5mM sample with bicarbonate present, along with calculated uncomplexed calcium concentrations.

that of the EDTA the concentration of the uncomplexed calcium was given. When the EDTA concentration was higher than that of the calcium sample there was no change in potential for the first additions, then when all the EDTA had reacted a normal type of increase was observed. Treating this data gave meaningless negative answers for the concentration, but if the points of zero concentration could have been ignored the concentration of the free complexing agent in the sample would have been given. Finally when the EDTA concentration neared or exceeded the highest calcium concentration obtained during the additions there was no change in or little change in the electrode potential and STAD subroutine failed.

When weaker complexes are present in the sample, analysis by standard additions will give the total concentration of the complexed and uncomplexed ions if the proportion of complexed ions remains constant throughout the additions. This can be seen from Table 5.15 for the analysis of calcium when bicarbonate ions were present, bicarbonate forming a fairly weak 1:1 complex with calcium ($K=6.5$). The standard addition results were close to the total calcium concentration, while those obtained by bracketing standards gave fairly good agreement with the calculated values for uncomplexed calcium (the analysis figures being quoted as the mean of three results). However as the bicarbonate concentration increased the difference between the standard addition results and the true concentration increased. This can be attributed to the proportion of complexed ions changing. Thus as the concentration of the complexing agent and the strength of the complex increase the concentration found by standard additions will decrease, until as with the case of EDTA the uncomplexed concentration is found.

5.7 Conclusions

When compared with the results in Chapter 4, it may be seen that the accuracy and precision obtained by standard additions were little better than those obtained by bracketing standards, although the effect of random errors should have been reduced by multiple additions. However using standard additions the concentration is calculated by extrapolation back from a range of data, so the effect of errors in these points upon the precision of the analysis is greater than in the case of bracketing standards when the result is obtained from interpolation from two points either side of the unknown. Also the results depended on any error in the measurement of the flow rate or any change in flow rates with time, which was not the case with bracketing standards.

In addition, standard addition methods were comparatively slow, with

six additions a rate of 10 samples/hour was achieved; only catered for a fairly low range of sample concentrations and were fairly tedious to set up, although again this could have been improved if the standards were stored in reservoirs and their flow controlled by the computer by means of a series of valves.

However the great advantage of standard additions when compared with bracketing standards was that the effect of interfering ions and complexing species were lower. As long as a fair percentage of the complexing species remained free in solution and the complex was fairly weak, the results given would be the total concentration of the complexed and uncomplexed ion. Although the presence of interferents caused the standard addition plot to become curved and to give incorrect intercepts for the analysis, treatment of the curve as a straight line gave answers nearer the true concentration.

CHAPTER SIX ANALYSIS BY GRADIENT TITRATION

6.1 Dual and Single Cup Gradient Titrations

The basic principle of any titration system is that the amount of titrant or sample is varied until the equivalence point is detected. Most automated titrators are of the 'batch type' (see Section 1.5), but these generally require a considerable amount of operator attention and routine analyses may be more conveniently carried out by a 'gradient titration', where the variable amount of titrant is obtained by means of a concentration gradient being developed. In this chapter an automated gradient titration system using the α -16 for control and data processing is described.

The use of concentration gradients for changing the composition of the eluent in liquid chromatography was first described in 1954 (111), using various mixing systems, but it was later shown that they could be more conveniently and consistently developed by the use of peristaltic pumps (112). The first gradient titrations were carried out using the scheme illustrated in Figure 6.1 (113), using an Autoanalyzer pump and Autoanalyzer sampler cups as the gradient generation and titration vessels. The concentration of the gradient at time t (C_b), assuming instantaneous and perfect mixing in the sampler cup, was given by (113) -

$$C_b = C_a + (C_{b0} - C_a) \left[\frac{(F_a - F_b)t}{V_{b0}} + 1 \right]^{F_a/F_b - F_a} \quad 6.1-A$$

If F_b was made equal to $2F_a$ the gradient concentration was a linear with respect to time until vessel B emptied -

$$C_b = C_{b0} + \frac{C_a - C_{b0}}{V_{b0}} \times F_a t \quad 6.1-B$$

So for a titration with the stoichiometry



the sample concentration C_s was given by -

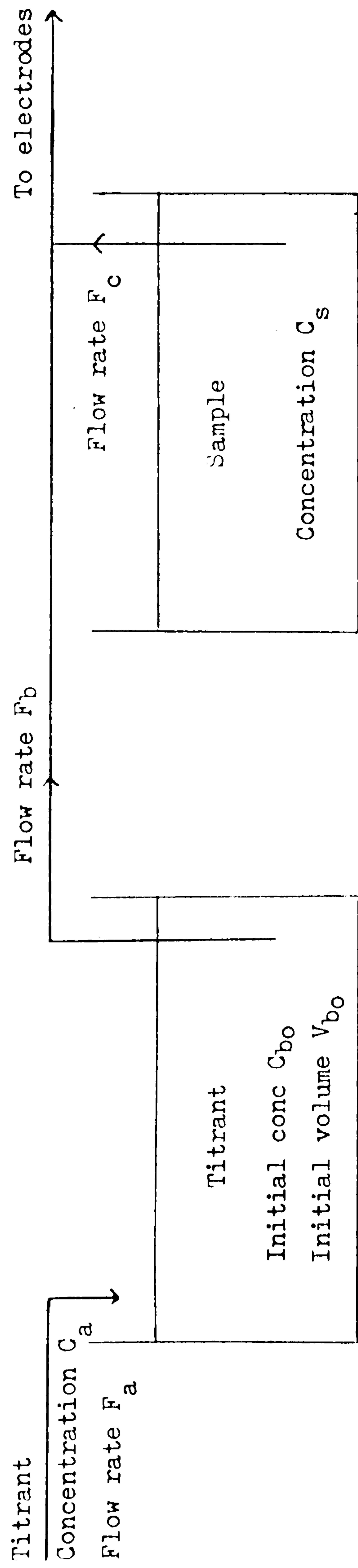


Figure 6.1 Two Cup Gradient Titration

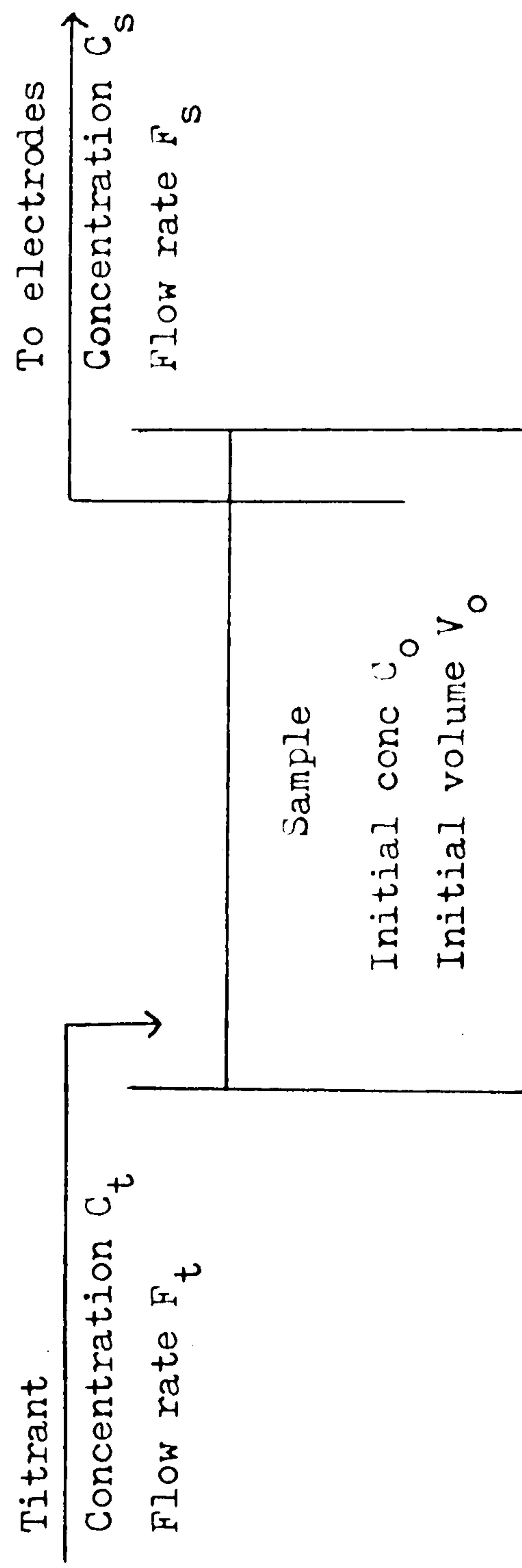


Figure 6.2 Single Cup Gradient Titration

$$C_s = \frac{nF_a F_b}{F_c V_{b_0}} (C_a - C_{b_0}) t_e \quad 6.1-D$$

where t_e was the time for the end-point to be reached.

Generally it proved difficult to automate this system. Either the use of a modified Technicon sampler or the use of two sample turntables for the gradient formation and the titration were suggested, but both of these proved difficult to synchronize exactly and also the flow rates changed slowly over a period of time, so F_b was not exactly twice F_a . Therefore a similar, but more experimentally convenient arrangement was suggested (60) in which the titration of the sample was carried out in the sample cup itself by adding titrant at a constant rate, monitoring the progress of the reaction by pumping a stream from the cup, as shown in Figure 6.2. In this case no titration gradient was developed, the technique being more comparable to a conventional titration, but it has been termed the 'Single Cup gradient titration' and the true gradient titration the 'Two Cup gradient titration'.

The single cup method uses only one sampler, thus being cheaper and avoiding timing problems, and because of this was used for the work in this chapter. However unfortunately no single cup titration can have a linear calibration plot, as shown below.

Considering the single cup titration illustrated in Figure 6.2 with a rapid reaction of the stoichiometry of Equation 6.1-C, assuming perfect mixing, C_s at a given time t is given by

$$\begin{aligned} C_s &= \frac{\text{Amount of titrand in cup}}{\text{Volume of solution in cup}} \\ &= \frac{C_o V_o - nF_t C_t t - F_s \int_0^t C_s dt}{V_o + (F_t - F_s)t} \end{aligned} \quad 6.1-E$$

Differentiating this with respect to time and simplifying -

$$\frac{dC_s}{dt} = - \frac{nF_t C_t + F_t C_s}{V_o + (F_t - F_s)t} \quad 6.1-F$$

Solving this differential equation with boundary conditions $t = 0$,

$C_s = C_o$ and $t = t_e$, $C_s = 0$ -

$$\int_0^{t_e} \frac{dt}{V_o + (F_t - F_s)t} = - \int_{C_s}^0 \frac{dC_s}{nF_t C_t + C_s F_t} \quad 6.1-G$$

$$\text{if } F_t \neq F_s, \quad t_e = \frac{V_o}{F_t - F_s} \left[\left[\frac{nC_t + C_o}{nC_t} \right]^{(F_t - F_s)/F_t} - 1 \right] \quad 6.1-H$$

Therefore a linear calibration could only be obtained if F_s was zero, which means that no sample could be removed from the cups for the titration to be monitored. However a straight line should be obtained if

$$\left[\frac{F_t - F_s}{V_o} t_e + 1 \right]^{F_t/(F_t - F_s)} \quad \text{vs} \quad C_o \quad 6.1-J$$

were to be plotted, with gradient $1/nC_t$ and passing through the origin.

A condition of solving 6.1-G was that $F_t \neq F_s$. If this is ^{not} the case Equation 6.1-H becomes

$$\int_0^{t_e} \frac{dt}{V} = - \int_{C_o}^0 \frac{dC_s}{nF_t C_t + F_t C_s} \quad 6.1-K$$

and the equivalence point is at -

$$t_e = \frac{V_o}{F_t} \ln \frac{nC_t + C_o}{nC_t} \quad 6.1-L$$

Again this cannot lead to a linear calibration unless

$$\left(\exp F_t t_e / V_o \right) - 1 \quad \text{vs.} \quad C_o \quad 6.1-M$$

were to be plotted, again having gradient $1/nC_t$ and passing through the origin.

Figure 6.3 shows some theoretical calibration plots of single cup

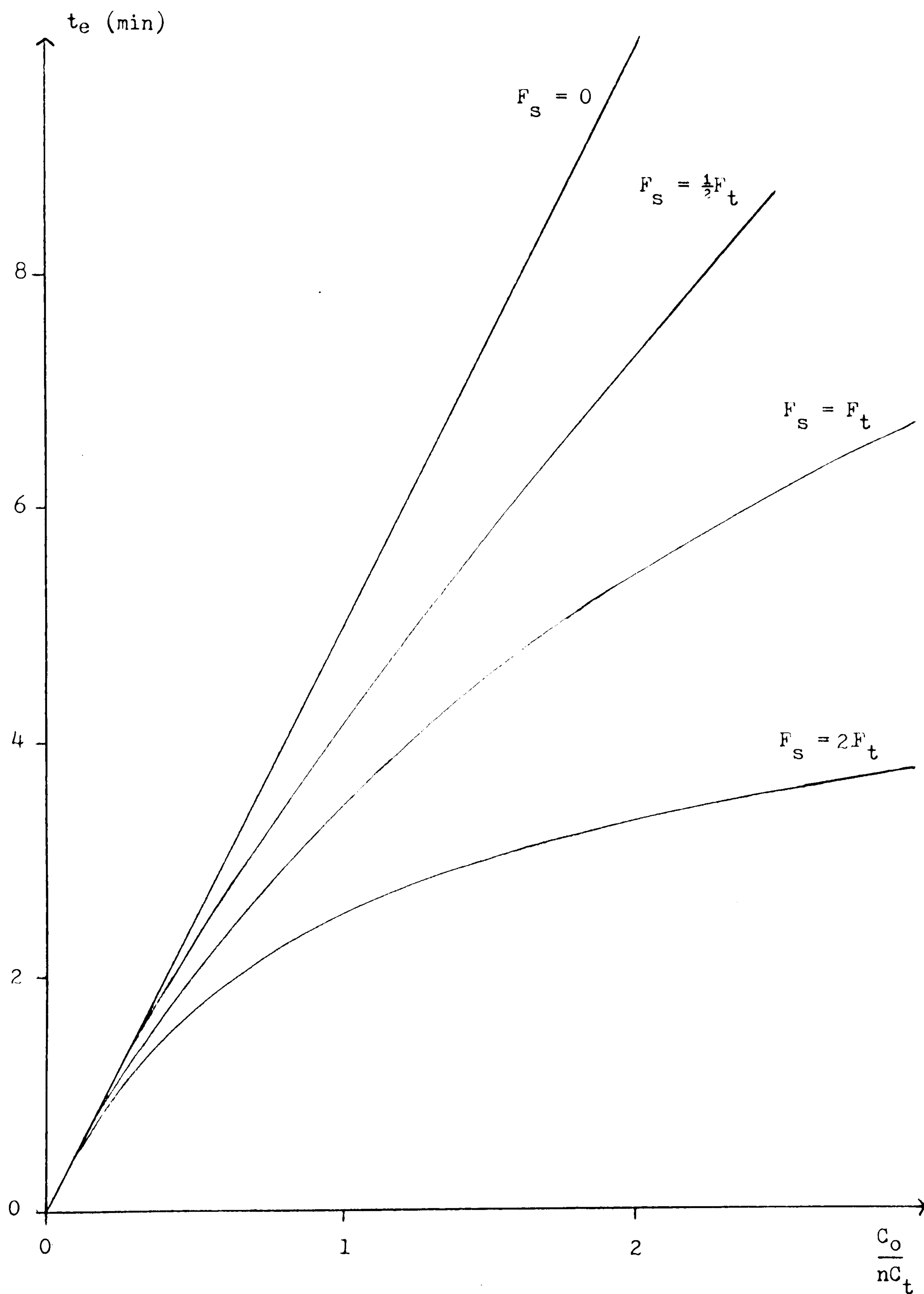


Figure 6.3 Theoretical calibration curves for single cup gradient titrations with $V_o=8\text{ml}$ and $F_t=1.6\text{ml/min}$

titrations with typical values of $V_o = 8\text{ml}$ and $F_s = 1.6\text{ml/min}$ inserted. As was to be expected the smaller the flow rate to the electrode becomes in comparison with the flow of the titrant the more nearly linear the calibration, as less sample is being removed from the cup instead of being titrated. Also the gradients of the calibrations decrease as the ratio C_o/nC_t increases, again since more of the sample species has been removed for sensing.

To minimise errors in determination of sample concentration leading from errors in assessing the titration time the gradient of the calibration curve should be as high as possible. This implies using a system where F_s is lower than F_t , over a range where the ratio C_o/nC_t is also fairly low, but this presents some problems. In order to detect the end-point reliably and reproducibly the response of the electrodes must be as rapid as possible. With F_s low the time response may be inadequate, unless the flow past the electrodes is increased by mixing the sample stream with a stream of distilled water or a buffer, but this leads to a loss in the sensitivity of the method. Also the volume of the sample used has to be limited to much less than the 10ml of the sample cup used to prevent the possibility of overflow.

These problems may be overcome by setting $F_s = F_t$ (allowing for differences in the diameter of the pump tubing used) and using a concentration range where the ratio C_o/nC_t is less than 1. The calibration curve obtained with this system is fairly steep over the whole range, a fair flow of sample to the electrodes may be maintained and there are no problems with overflow from or emptying of the sample cup, since the volume of the sample remains almost constant.

6.2 Calibration of Gradient Titration Systems

Before setting up an on-line single cup gradient titration system, it was necessary to ascertain whether precalibration would be required or whether the sample concentration could be determined directly from the titration time. Also to simplify data processing it would prove advantageous if any calibration needed was linear, since LLS (see Section 5.2) could be used to give a least squares best fit.

Verification of the applicability of Equation 6.1-B to the gradients produced by the two cup methods has been carried out by measuring the titrant concentration produced at various times by atomic absorption spectroscopy and by potentiometry (114). This showed that with the solution in the cup being agitated by a rapid stream of gas mixing was close to perfect and that the effects of dispersion in the flow stream were negligible. This is also probably true for single cup titrations, but they also require the titration reaction to be rapid for the titration to follow the course predicted by Equation 6.1-E.

Therefore titration times determined experimentally should be close to the theoretical times. This proved to be true with solid state membrane electrode sensors (113), when the equivalence points could be located visually from the 'breaks' in potential in the titration curve. However when liquid membrane electrodes were used as sensors the titration became more rounded due to the slower response of this type of electrode and accurate manual detection became more difficult. To overcome this problem and to enable automation of the technique the starts and end-points of the titrations were determined by signal differentiation, either from the locations of the maximum and minimum values of dE/dt or the zero crossing points of d^2E/dt^2 .

The use of liquid membrane sensors with signal differentiation unfortunately gave calibrations differing greatly from the theoretical,

for both single cup and double cup titrations (60,115). Little importance was attached to these deviations, since the calibrations were found to be reproducible and sample concentration was anyway to be found manually from a calibration graph, but they were attributed to imperfect gradient formation (60) and poor mixing, flow rates differing from given values and slow electrode response (115). It has been shown that gradient formation and mixing are near perfect and errors due to the wrong values being used for flow rates can be eliminated by accurate calibration of the pump tubes. Therefore the deviations can be better explained by the sluggish response of the electrode, the fact that the maximum and minimum slope of the titration curve does not indicate exactly the start and the end of the titration and the damping characteristics of the signal differentiator used.

To investigate the calibrations of single cup gradient titrations the off-line portion of the apparatus illustrated in Figure 6.4 was used. Because the signal from the second differential of the titration curve proved to be too noisy to easily and accurately locate the zero crossing points, the titration time was determined from the first differential. The sampler timer cam could be set to change samples any number of times within a six minute cycle. Maximum titration times used in this study were usually three or six minutes, although some work was carried out with 90sec titrations. The mixing of the solution was carried out by passing a rapid stream of air through the sample, care being taken to ensure that no bubbles entered the sampling stream to the electrodes. To minimise the risk of sample splashing out of the cup, a maximum volume of 8ml of sample in the cup at any one time was advisable.

The first system investigated was the titration of calcium chloride with EDTA at pH9, using a calcium electrode sensor. The titrant used was 10mM EDTA, F_s was nominally 1.2ml/min and F_t was varied with nominal values of 0.6, 1.2 and 2.4ml/min, the actual values being determined by accurate calibration of the tubes used. The samples, of volume 5ml,

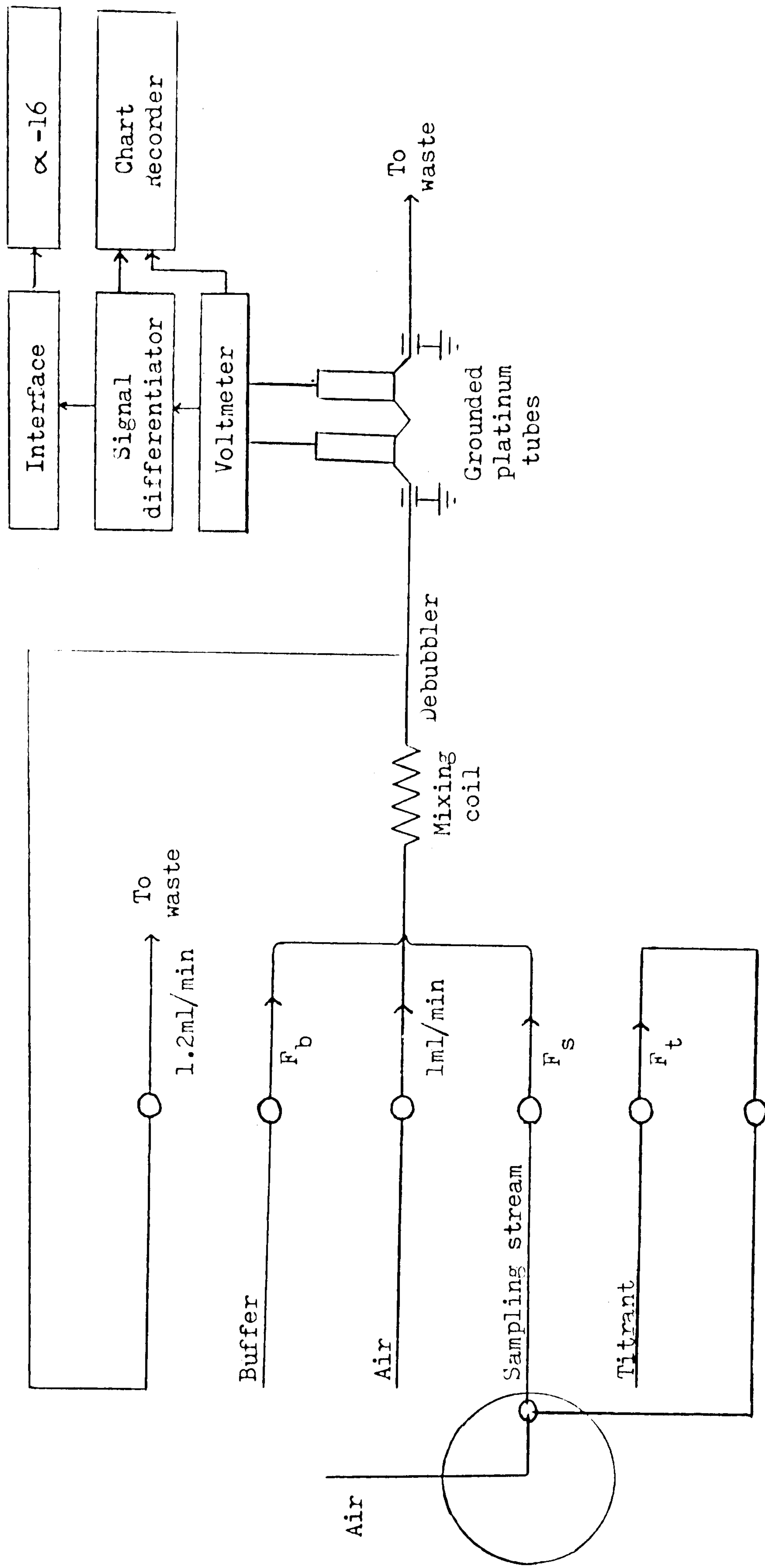


Figure 6.4 Flow stream for single cup gradient titrations

ranged in concentration from 1 to 10mM. An ammonia / ammonium chloride pH9 buffer was mixed with the sampling stream at a rate of 2ml/min to minimise any interference with the electrode response by hydrogen ions and to cut down noise due to variations in streaming potential.

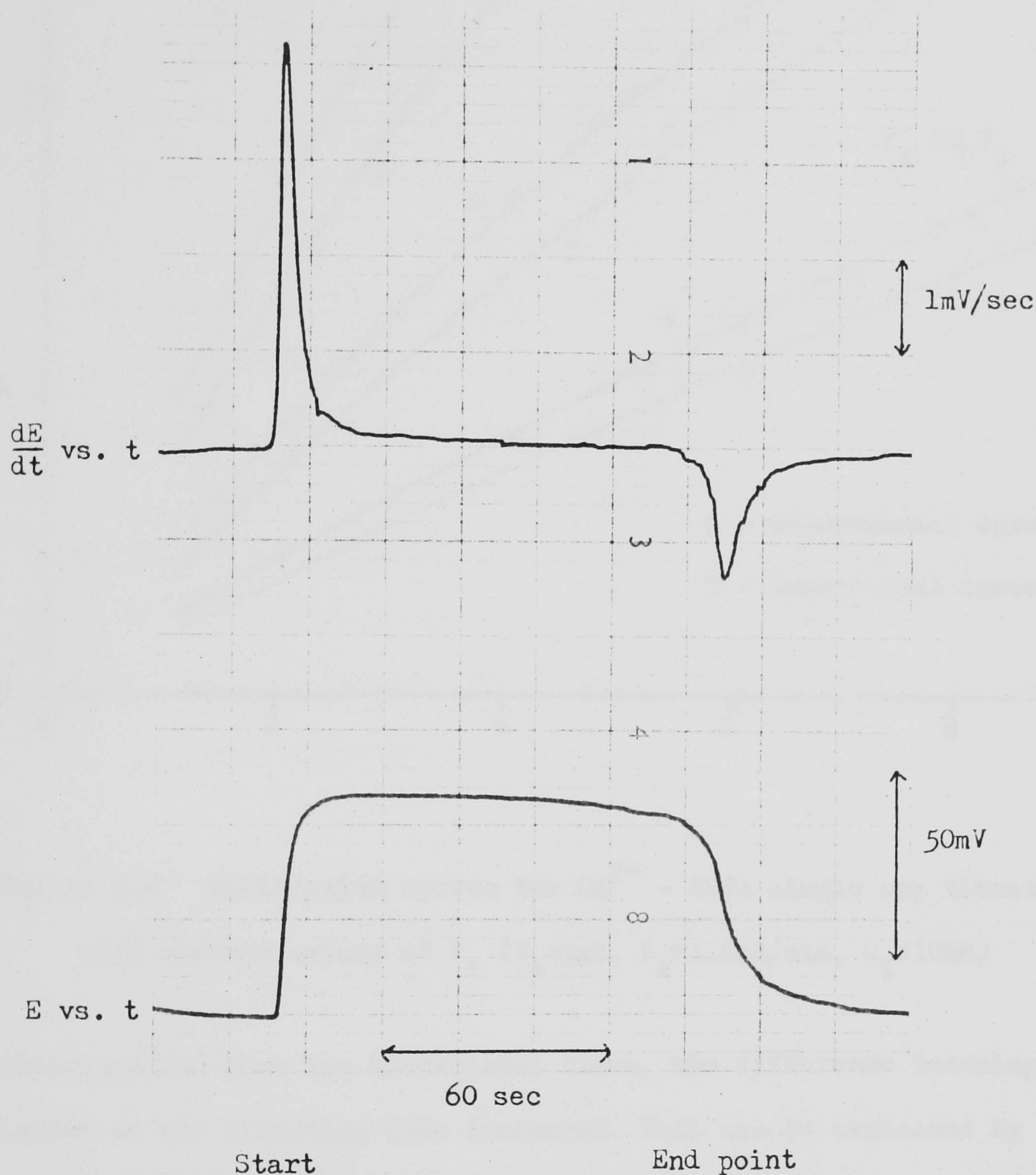


Figure 6.5 Calcium - EDTA titration monitored as potential vs. time and first differential vs. time ($F_s = F_t = 1.2\text{ml/min}$, $C_o = 5\text{mM}$, $V_o = 5\text{ml}$ and $C_t = 10\text{mM}$)

A typical example of the titration curves obtained is shown in Figure 6.5. The theoretical calibration curves and those actually obtained experimentally are shown in Figure 6.6. The actual titration times were

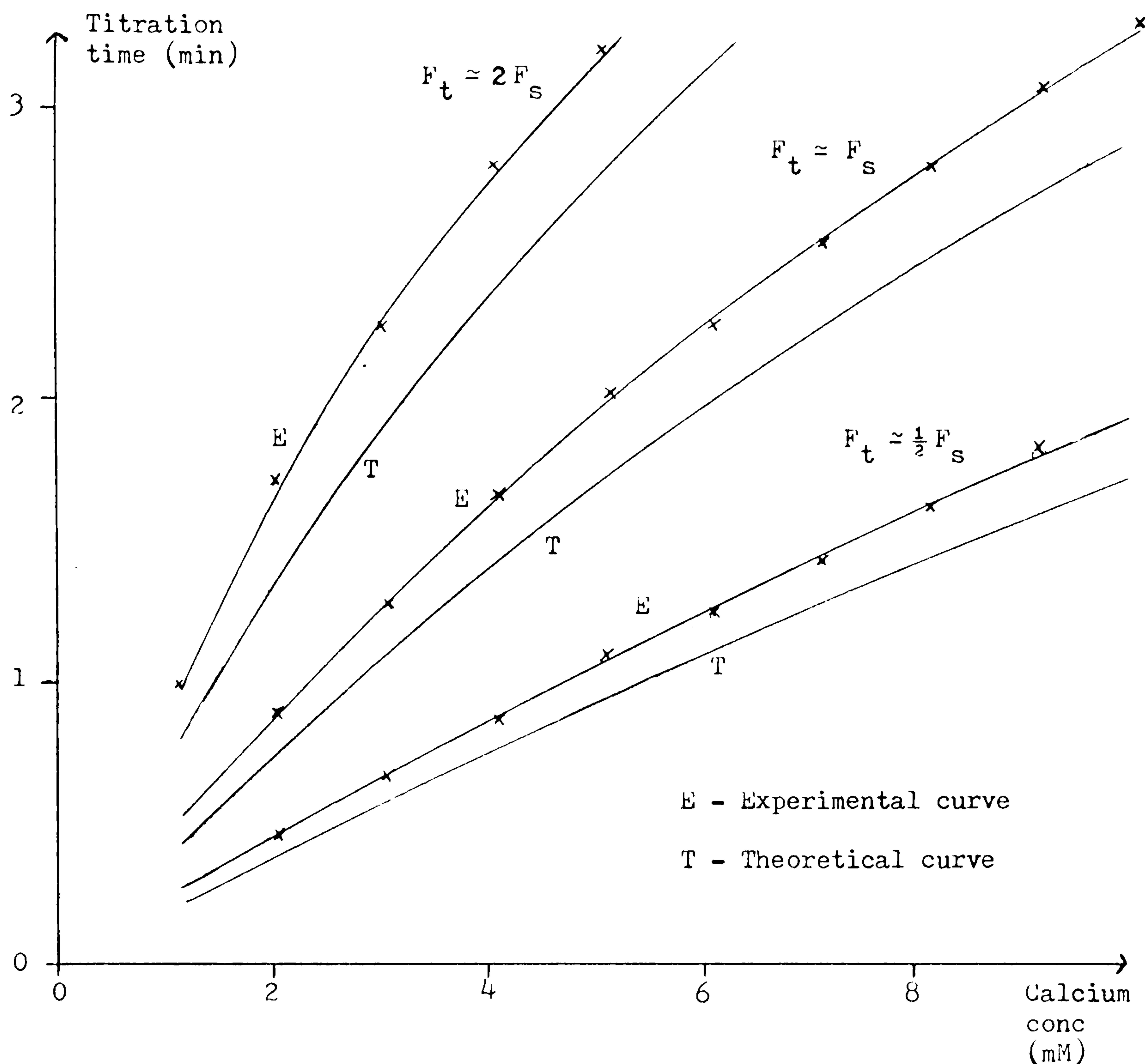


Figure 6.6 Calibration curves for Ca^{2+} - EDTA single cup titrations with various values of F_t ($V_o = 5\text{ml}$, $F_s \approx 1.2\text{ml/min}$, $C_t = 10\text{mM}$)

always greater than the theoretical times, the difference becoming larger as the titration time increased. This can be explained by examination of the titration curve. The introduction of the calcium sample led to a rapid increase in calcium ion activity from zero to that of the sample. Because the rate of change of concentration was high and the electrode was responding to an increase in concentration, the change was rapidly followed by the electrode and the maximum value of dE/dt gave a relatively good indication of the start of the titration. As the

titration proceeded the calcium concentration fell slowly until the end point was approached, when the potential dropped more rapidly and a minimum in dE/dt was observed. However this drop was relatively sluggish when compared with the rise, since the rate of change in concentration was lower and the electrode was responding to a decrease in concentration. This suggested that the minimum differential value occurred after the end point had passed. This was probably exacerbated by the damping of the input to the signal differentiator, which tended to damp out the smaller peak due to the end point more than the larger peak due to the start of the titration, so that the output from the differentiator indicated the minimum slope after it had in fact occurred.

In order to investigate the relationship between the actual titration time (t_{act}) and the calculated theoretical titration time (t_{th}) a graph of $t_{act} - t_{th}$ vs. t_{th} was plotted for the calcium titration systems (see Figure 6.7). The difference between t_{act} and t_{th} rises in a linear fashion within experimental error (most of the points lie within 1 or 2sec of the best fit line), so that

$$t_{act} - t_{th} = m + n t_{th} \quad 6.2-A$$

where m and n are constants. It follows from this that there was a linear relationship between t_{th} and t_{act} -

$$t_{th} = a + b t_{act} \quad 6.2-B$$

a and b being constants. This means that if graphs of the functions given in Equations 6.1-J and 6.1-M are plotted, with t_{act} substituted for t_e straight lines would not be produced since

$$\left[\frac{F_t - F_s}{V_o} t_{th} + 1 \right] \frac{F_t}{F_t - F_s} = \left[\frac{F_t - F_s}{V_o} (a + b t_{act}) + 1 \right] \frac{F_t}{F_t - F_s}$$

which is not a linear function with respect to -

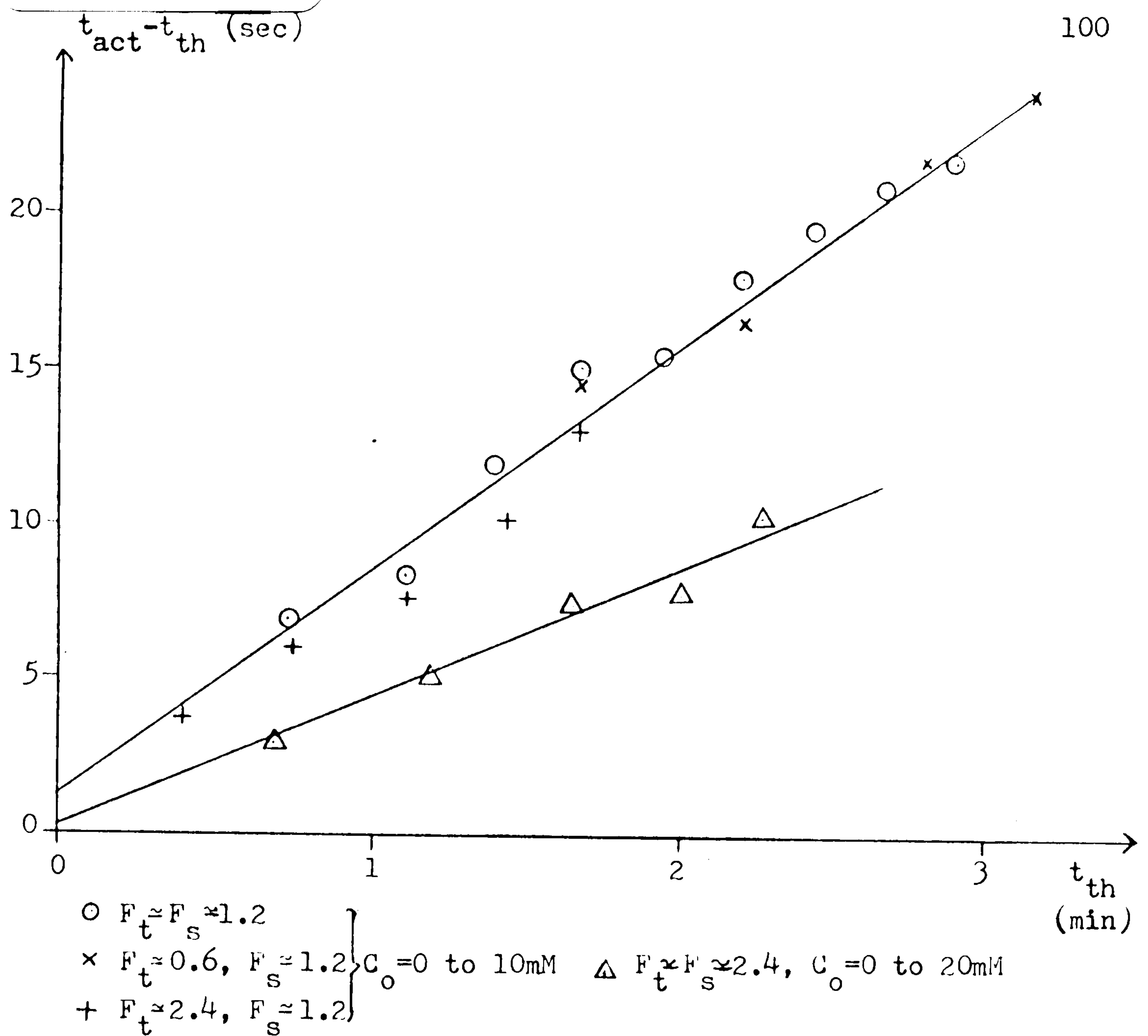


Figure 6.7 Difference between t_{act} and t_{th} vs. t_{th} for several Ca^{2+} - EDTA titrations ($V_o = 5ml, C_t = 10mM$)

$$\left[\frac{F_t - F_s}{V_o} t_{act} + 1 \right]^{F_t / (F_t - F_s)}$$

and similarly for the expression when $F_s = F_t$.

If the flow rate past the electrodes were to be increased, the time response would be faster and the difference between t_{act} and t_{th} less marked. This was carried out for the calcium - EDTA titration with $F_s \approx F_t \approx 2.4ml/min$ and $F_b \approx 4ml/min$, doubling the flow rate past the electrodes, using 10mM EDTA titrant and calcium samples of 4 to 20mM. Figure 6.7 shows that the difference between t_{th} and t_{act} was nearly halved. However due to the increase in noise due to variations in streaming potential, there was a great increase in the noise of the differential signal and the flow rates which would be required to make

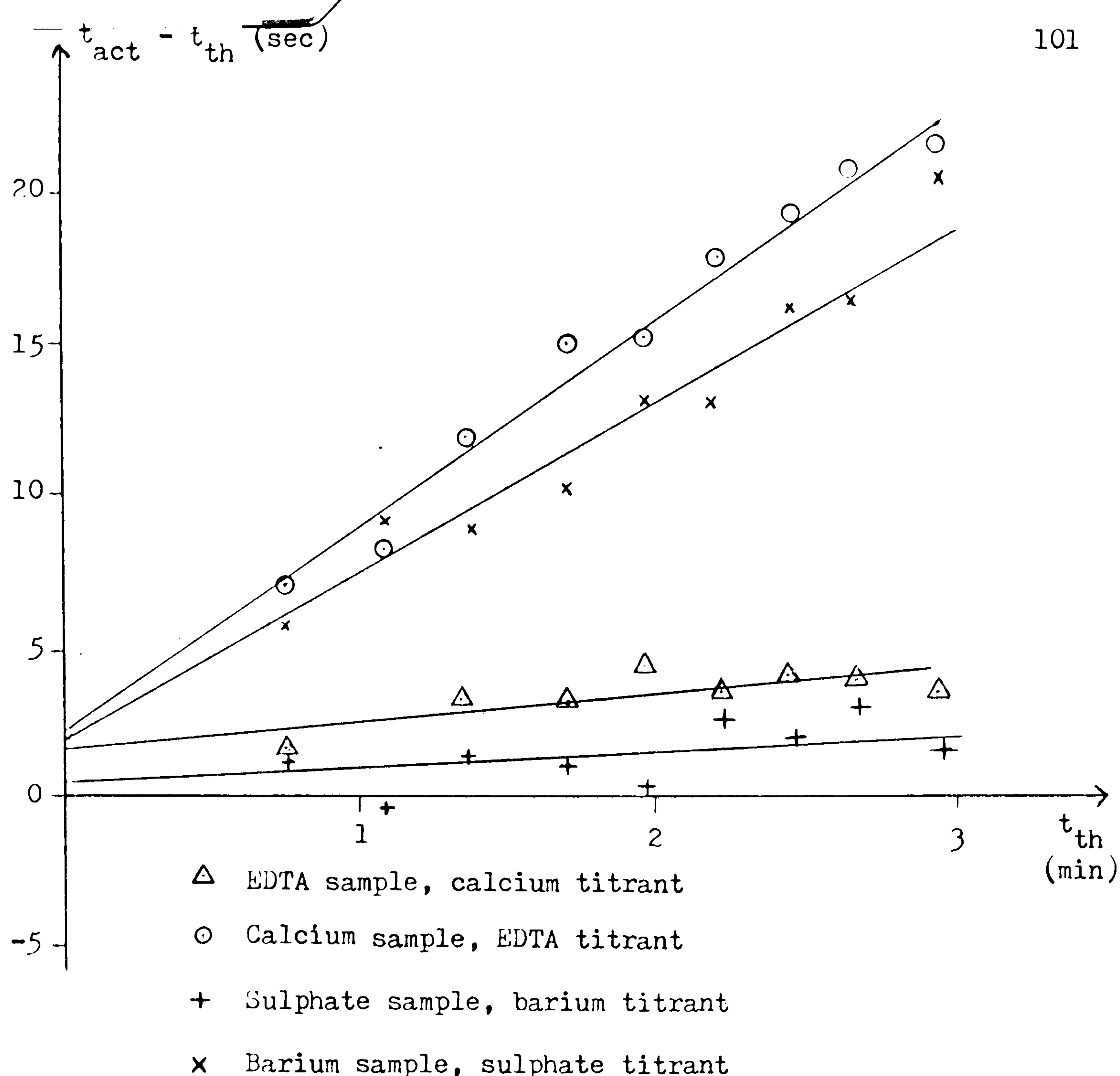


Figure 6.8 Difference between t_{act} and t_{th} vs. t_{th} for a titration system with $V_o = 5\text{ml}$, $F_s \approx F_t \approx 1.2\text{ml/min}$, $C_t = 10\text{mM}$ and $C_o = 0$ to 10mM .

differences between t_{act} and t_{th} insignificant would not be feasible, so these differences could not be eliminated in this way.

The applicability of Equation 6.2-A to other titrations was investigated by performing more calcium - EDTA titrations, with EDTA as the sample and calcium as the titrant, and some barium - sulphate titrations, with barium used as both sample and as titrant. The system used was the same as in the last experiment, except that F_t was set to 1.2ml/min in all cases and a barium selective electrode was used as a sensor in the barium - sulphate titrations. The results, shown in Figure 6.8, indicated that a linear relationship existed between t_{th} and t_{act} for all the titrations performed. The titration curve of the titration

of a barium sample with sulphate titrant was similar to that of the titration of a calcium sample with EDTA and the differences between t_{act} and t_{th} were also similar for both systems. However when the metal ions were used as titrants, the values of $(t_{act} - t_{th})$ were much lower, but the reasons for this could again be found by examining the titration curves. To position the titration the metal ion had to be passed to the electrodes first, then at the start of the titration the concentration of the sensed metal fell rapidly to zero. The electrodes did not respond as fast to this decrease in concentration as rapidly as the start of the metal ion sample titrations and a smaller and broader peak in dE/dt resulted, the minimum value probably occurring after the true start of the titration. The concentration of the metal ion then remained at zero until the end point, when it rose relatively slowly, again producing a small, broad peak in dE/dt , the maximum value again probably occurring after the end point. Thus the actual titration time evaluated from the difference in the positions of the peaks in dE/dt was much closer to the theoretical value.

Therefore it has been shown for single cup gradient titrations with liquid membrane electrodes and with end point detection by signal differentiation that it was necessary to calibrate the systems with known standards before the analysis of unknown samples. No function of the actual titration time was found that was linear with respect to the sample concentration, but for the titrations investigated it was found that the actual titration time was a linear function of the expected titration time. Therefore the on-line gradient titration system could be calibrated with the relationship between t_{th} and t_{act} found by LLS (see Section 5.2), then titrations of samples performed, a value for t_{th} calculated from the measured value of t_{act} and the sample concentration evaluated from t_{th} using Equation 6.1-H or 6.1-L.

6.3 On-line Single Cup Gradient Titration System

The programme 'TITRATE' was written to take in data and evaluate results from single cup titrations carried out in the system illustrated in Figure 6.4. TITRATE consisted of two parts, Part 1 performed the calibration as suggested in the last section and stored up to five of these calibrations for use by Part 2, which performed the analyses.

As it will be shown later, the precision of the results was improved if the maximum titration time for the concentration range of interest was increased. If this was done much time may have been wasted if the sampler was changing samples at a constant rate when samples near the bottom of the concentration range were being titrated, since the titration time was short. To overcome this and to improve the sampling rate the sampler was advanced under computer control, using SAMPLE subroutine (see Section 2.5).

The data from a titration was acquired by 'PEAKS' subroutine. This subroutine input data from the ADC using DATEX4, since the signal from the differentiator often exceeded the input range available when taking more than four conversions per second. When PEAKS was called it waited for 'PTDEAD' conversions to take place (to allow for the dead time of the flow system after the changing of the sample), then took in up to a maximum of 'NPT' points from the ADC, sent the difference in position of the two peaks in the signal to the main programme and returned. If the input to the ADC was overloaded at any time or if two peaks were not detected, the routine returned immediately with a flag set to indicate this.

Several algorithms were tried to enable the location of the peaks in real-time. One of the simpler algorithms used was found to give the best results, rarely detecting false peaks due to noise and locating the position of the true peaks well. The start of the first peak was detected when four successive inputs were greater than 80_{16} ADC units

for a maximum or less than -80_{16} ADC units for a minimum. These levels were about twice the peak to peak noise levels usually observed, but it was necessary that the ADC reading was adjusted to zero when the inputs to the differentiator were shorted. Then the position of the maximum or minimum datum input was stored, before the end of the first peak being recognized when the input passed zero. The second peak was detected in the same way as the first, but the end of the peak was taken as when six successive points had been in the reverse direction after the maximum or minimum, so that the subroutine could return before the input returned to zero.

To test the reliability of PEAKS it was used to take in data from several calcium - EDTA titrations, with $C_t = 10\text{mM}$ and $F_s = F_t = 1.2\text{ml/min}$. Each titration was performed eight times, to give a total of seventy two titrations and PEAKS detected both peaks in all cases, with no obvious false readings. The initial volumes used (3ml, 5ml and 8ml) corresponded to an average sampling rate of 40, 25 and 15 samples per hour using TITRATE. The results are shown in Table 6.9 and indicate that the precision generally increased with increasing titration time, since the standard deviations were fairly constant, around 1 to 1.25 seconds. This suggested that precision is higher at the higher end of the concentration range and with lower sampling rates.

The flowcharts of TITRATE Parts 1 and 2 are shown in Figure 6.10. Part 1 took in the data from 'NSTD' titrations of standards, then theoretical titration times for all the standards were calculated using Equations 6.1-H or 6.1-L and the best linear fit of t_{th} vs t_{act} was calculated, printed on the teletype along with the percentage fit, and stored in one of five locations. Since the plot of t_{th} vs. t_{act} was only carried out for making relatively small adjustments to the titration times, the percentage fit of the regression was acceptable as low as 90% with little effect on the accuracy, in contrast to the situation with standard additions.

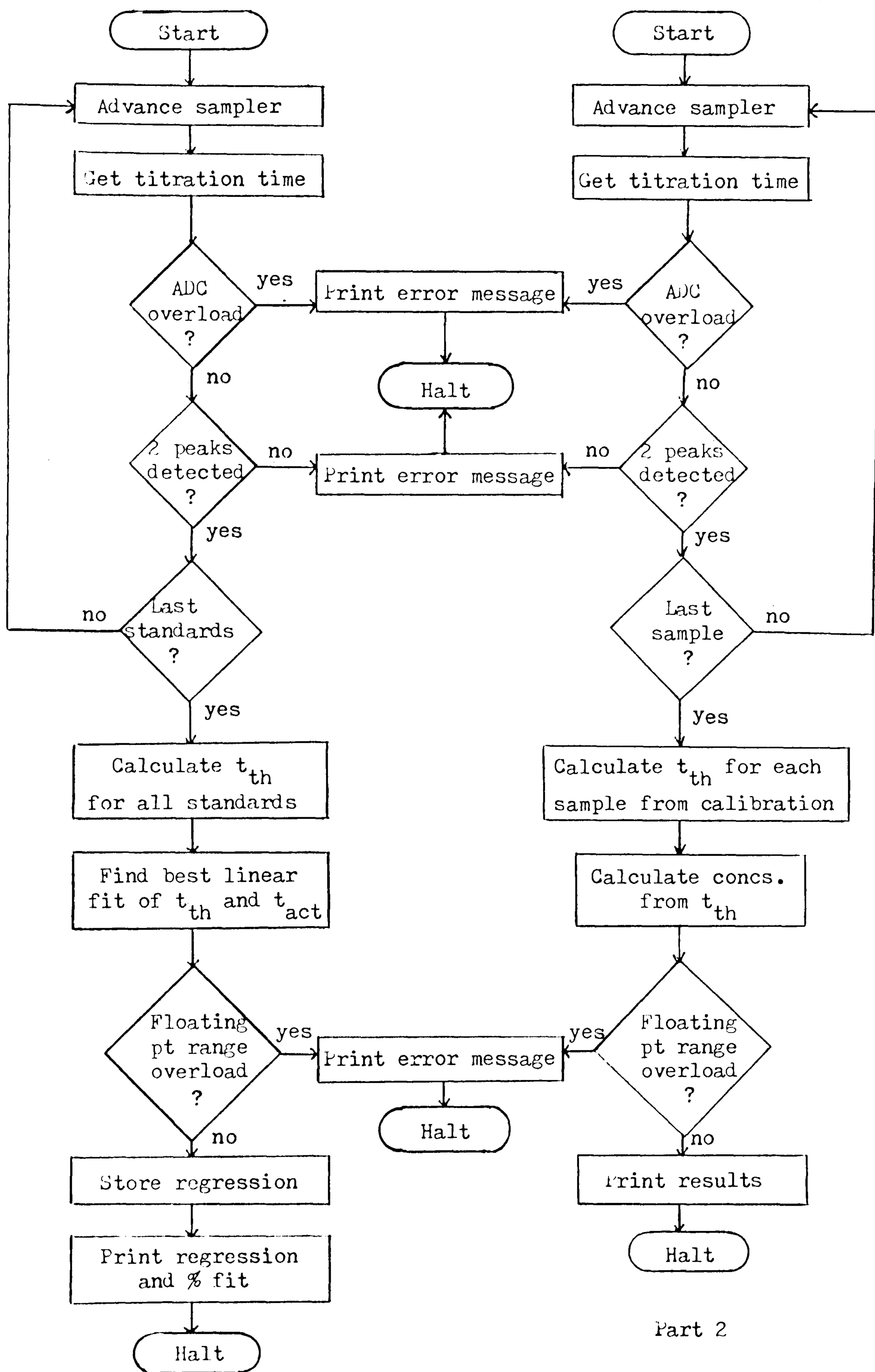
Calcium concentration	Initial volume of sample		
	3ml	5ml	8ml
3mM t_{act} (mean)	43.2sec	73.5sec	115.0sec
t_{act} (standard deviation)	1.5sec	0.7sec	1.4sec
t_{act} (relative s.d.)	3.5%	0.9%	1.2%
5mM t_{act} (mean)	68.8sec	112.5sec	182.4sec
t_{act} (standard deviation)	1.0sec	1.5sec	1.1sec
t_{act} (relative s.d.)	1.4%	1.3%	0.6%
8mM t_{act} (mean)	99.9sec	164.1sec	263.2sec
t_{act} (standard deviation)	1.8sec	1.7sec	1.6sec
t_{act} (relative s.d.)	1.8%	1.0%	0.6%

Table 6.9 Titration times for calcium - EDTA titrations measured by PEAKS

TITRATE part 2 took in the data from 'NSAMP' titrations of samples, calculating their concentrations using the calibration stored in Part 1 and printing the results on the teletype. Data was acquired in the same way as Part 1, the values of t_{act} converted to t_{th} for each sample, then the concentrations calculated.

6.4 The Accuracy and Precision of Gradient Titrations

The precision and accuracy of analyses using this sytem depended on the errors associated with the precalibration of a titration, the errors in the calculation of concentration from the value of t_{th} due to errors in F_s , F_t , V_o and C_t and experimental errors in the determination of t_{act} .



Part 1

Part 2

Figure 6.10 Flowchart of TITRATE

Parts 1 and 2

The errors arising from precalibration depended on the applicability of a linear regression to the calibration, the number of standards used, the accuracy of the parameters entered into TITRATE and the frequency of calibration. It was thought advisable to check whether the relationship between t_{th} and t_{act} was linear for a given titration before using the on-line system, although a non-linear function would be shown up by a poor percentage fit of the calibration. To increase the precision of the calibration a fair number of standards were used, usually six or eight, covering the whole of the concentration range of interest, and the values of F_s and F_t were accurately determined just before calibration. Thus errors from these sources were minimised.

It has been shown that the calibration obtained depended on the time response of the electrode used. This characteristic may in turn change with the age of the membrane used. Also the flow rates obtained from the pump tubes may vary with time, so the accuracy of the analyses may decrease as the interval after calibration increases. To investigate this, the calibrations of the calcium-EDTA and the barium-sulphate titrations with $F_s = F_t = 1.2 \text{ ml/min}$ were carried out at weekly intervals over four weeks using TITRATE Part 1 using previously unused electrodes and pump tubes. The results appear in Table 6.11, showing the changes in calibration to be small within the four week period. However with a calcium membrane which was very aged the calibration differed greatly, because of the poorer response time. Therefore it was judged best to calibrate any titration system in use for any length of time at least weekly, so that any deterioration in the response of the membrane or change in the flow rate of the tubes could be spotted and replacements made.

To study further the effects on accuracy of flow rates changing with time and also the effect of inserting incorrect values of V_o and C_t in Part 2 of TITRATE, a further programme 'ERROR' was written. This calculated a value for the titration time for a 5mM sample with a set of

Titration	Week of use of electrode	Calibration	Percentage fit
Ca^{2+} -EDTA	1	$t_{th} = 0.002 + 0.882t_{act}$	98%
"	2	$t_{th} = -0.012 + 0.874t_{act}$	97%
"	3	$t_{th} = 0.098 + 0.877t_{act}$	94%
"	4	$t_{th} = 0.162 + 0.890t_{act}$	96%
"	12	$t_{th} = -0.720 + 0.825t_{act}$	89%
SO_4^{2-} - Ba^{2+}	1	$t_{th} = -0.042 + 1.011t_{act}$	99%
"	2	$t_{th} = 0.061 + 1.012t_{act}$	97%
"	3	$t_{th} = 0.087 + 1.006t_{act}$	97%
"	4	$t_{th} = 0.029 + 1.025t_{act}$	95%

Table 6.11 The variation of some Single Cup Titration Calibrations with time ($V_o=5\text{ml}$, $F_s=F_t=1.2\text{ml/min}$, $C_t=10\text{mM}$ and $C_o=1,2,4,5,7,8,10\text{mM}$)

Conditions	Calculated $C_o(\text{mM})$	Conditions	Calculated $C_o(\text{mM})$
Perfect data	5.000		
C_t 10% low	5.555	F_s 10% low	5.126
1% low	5.051	1% low	5.012
1% high	4.950	1% high	4.988
10% high	4.545	10% high	4.879
V_o 10% low	4.404	F_t 10% low	4.879
1% low	4.940	1% low	4.988
1% high	5.061	1% high	5.012
10% high	5.621	10% high	5.126

Table 6.12 The errors occurring when C_t , V_o , F_s or F_t differ from the values inserted in TITRATE Part 2, calculated using ERROR

of values for F_s , F_t , V_o and C_t including a value differing from the basic set of $F_s = F_t = 1.2\text{ml/min}$, $V_o = 5\text{ml}$ and $C_t = 10\text{mM}$. This corresponded to the titration time which would be obtained assuming $t_{th} = t_{act}$ and no experimental errors in determining t_{act} , if the flow rates, sample volume or titrant concentration varied from the values inserted in TITRATE Part 2. An erroneous value of C_o was then calculated from this titration time with the basic set of values. Table 6.12 shows that large errors occurred if C_t or V_o varied from the inserted value, but these errors were consistent with those which would be obtained in conventional titrimetry and C_t and V_o can anyway be reproduced quite accurately. However the errors associated with variations in flow rates were smaller, showing that a change of 1% leads to an error of only around 0.25%.

It must be stressed that these errors have been calculated for the values changing after calibration. If the same incorrect values had been inserted in TITRATE Part 1 their effect would have been to some extent taken into the calibration and errors would have been smaller.

Experimental errors in the determination of t_{act} have already been covered, being shown in Table 6.9 to be generally between 1 and 2% for a calcium - EDTA titration. The same titrations were repeated after precalibration with TITRATE Part 1 to give a set of eight values for each calcium concentration at each sample volume, the results being summarised in Table 6.13. Generally errors were higher than those in Table 6.9 since errors due to precalibration were added to errors in determining t_{act} . Again the precision of the method increased with increasing titration time, with a higher sample volume and concentration, so a balance between the precision and analysis rate had to be decided upon. However even with the 3mM samples the relative standard deviations were less than 2% so long as the sample volume was greater than 5ml.

Therefore it has been shown that it was possible to achieve fairly accurate and reproducible results using the gradient titration technique

Calcium concentration		Initial volume of sample		
		3ml	5ml	8ml
3mM	Concentration found	3.13mM	3.05mM	3.01mM
	Standard deviation	0.10mM	0.06mM	0.03mM
	Relative s.d.	3.4%	1.8%	1.1%
5mM	Concentration found	5.12mM	4.96mM	5.03mM
	Standard deviation	0.10mM	0.08mM	0.07mM
	Relative s.d.	1.9%	1.6%	1.1%
8mM	Concentration found	7.88mM	8.03mM	7.96mM
	Standard deviation	0.14mM	0.11mM	0.07mM
	Relative s.d.	1.8%	1.4%	0.9%

Table 6.13 Precision and accuracy of some calcium - EDTA titrations

$$(C_t = 10\text{mM}, F_s \approx F_t \approx 1.2\text{ml/min})$$

when compared with bracketing standards and standard additions. However the errors were greater than those which can be obtained with conventional potentiometric titrations, because of the need for precalibration and because of the need for absolute reproducibility of the electrode response with time.

6.5 The Effect of Interferents on Gradient Titrations

Gradient titrations naturally suffer from the same chemical interferences as other titrations e.g. calcium cannot be titrated with EDTA if magnesium or any other metal ion forming a strong complex with EDTA is present. However the possible effects of other ions interfering

Magnesium concentration	Calibration	Percentage fit	Error if aqueous standards were to be used
0	$t_{th} = -0.007 + 0.896t_{act}$	97%	0
$10^{-3}M$	$t_{th} = 0.000 + 0.900t_{act}$	98%	-0.8%
$10^{-2}M$	$t_{th} = 0.004 + 0.886t_{act}$	98%	+0.3%
$10^{-1}M$	$t_{th} = -0.016 + 0.851t_{act}$	93%	+5.3%

Table 6.14 Calibrations of Ca^{2+} -EGTA titration in the presence of magnesium ions ($V_o = 5ml$, $F_t \simeq F_s \simeq 1.2ml/min$, $C_t = 10mM$, $C_o = 0$ to $10mM$)

with electrode behaviour have to be considered. It has been shown that interfering ions have only a small effect on the location of the end point of conventional potentiometric titrations, where the electrode potential is allowed to reach an equilibrium value before another addition is made (65). However in the case of gradient titrations the the location of the maximum and minimum values of the first differential depended upon the time response of the electrode and this may change with the concentration of interfering ions.

For instance, it has been shown that the presence of magnesium ions increases the response time of the calcium ion exchange electrode appreciably, although the initial response was still very rapid (54). To check the effect of magnesium ions upon a calcium titration using this sensor a series of calcium - EGTA titrations were carried out with and without magnesium ions present. This titrant gives a much more stable complex with calcium ($K = 10^{11}$) than with magnesium ($K = 1.6 \times 10^5$), so can be taken to complex calcium selectively in the presence of magnesium at the concentrations used. Therefore any interference was due to differences in response times.

The calibration did change with increasing magnesium concentration, the titration time lengthening (Table 6.14). However the errors caused by

using the calibration with aqueous standards for samples containing magnesium, calculated for a 5mM sample, are quite small, being within experimental error until the magnesium concentration reached 0.1M, a twenty fold excess. Even at this concentration the effect was small when compared with bracketing standards and similar to that obtained with standard additions (see Section 5.5).

Much of the effect of the interference by magnesium ions was reduced by using the neutral carrier calcium electrode, which displayed a better selectivity and exhibited smaller differences in response time when magnesium ions were present. However it has been shown that ions interfering with electrode behaviour have little effect upon gradient titrations until the response of the electrode is considerably slowed by their presence. In this respect gradient titrations are at a disadvantage with respect to conventional potentiometric titrations where any difference in response time has little or no effect on the accuracy.

6.6 Conclusions

The system described here generally provided more accurate and precise results than those obtained with bracketing standards and standard additions, with a precision of 1 - 1.5% obtainable. Also the range of species which could be determined was considerably higher, since the titrant or a titration product could be monitored instead of the titrand. The sampling rate obtained was also quite high, being around 25 samples/hour with a 5ml sample.

However the detection limits were probably poorer than direct potentiometric methods since there had to be a fairly substantial jump in electrode potential for the titration to be monitored properly. Also the range of concentrations that could be analysed without changing the system was only an order of magnitude, much lower than BRACKETAN2, but

higher than with BRACKSTAN1 or STANDAD but the precision of the analysis worsened as the sample concentration dropped within the range being used, this not usually being the case with direct potentiometric methods.

However the largest problem with the gradient titration method was that the response of the electrode had to be reproducible to give accurate results, and this was not always the case in the presence of interfering ions or as time passed. Frequent recalibration of the system minimised this problem but if the calibration was found to have changed appreciably results from previous analyses might have to have been scrapped.

7.1 Introduction

As standards for domestic and industrial water supplies and for the allowable levels of pollution of surface water have been increasingly established, the number of analyses needed to monitor these standards has led to the widespread introduction of instrumental and automated methods of analysis in preference to conventional gravimetric and volumetric techniques. Especially common are colorimetric methods, atomic absorption spectroscopy and atomic emission spectroscopy, although polarography has also been used.

The use of ion selective electrodes in recommended standard procedures has been limited to the determination of pH and fluoride concentration by direct potentiometry and of chloride by titration with silver ions (116). At present the recommended procedures are generally atomic absorption or emission for metals and colorimetric or titrimetric methods for non-metals. In the determination of metals ion selective electrodes provide few advantages over spectroscopic methods whose precision, linear range and selectivity cannot usually be matched potentiometrically. However ion selective electrodes could be used if activity rather than concentration were the parameter of interest and also, because of their size and the relatively small size of the associated instrumentation, could prove more useful if measurements in the field were to be made. For anions ion selective electrodes may display distinct advantages over the colorimetric and titrimetric methods now recommended with respect to linear range, selectivity and convenience.

In addition dissolved gases may be readily determined with gas electrodes and the detection of some detergents is possible with surfactant electrodes. The wide possible uses of ion selective electrodes

has been emphasised by a recent book (117) which suggests procedures for the non-automated analysis of about thirty species potentiometrically. In this chapter the analysis of seven water samples taken from domestic supplies in various parts of the country for calcium, total water hardness, sulphate, nitrate and total carbonic species (CO_2 , HCO_3^- , CO_3^{2-} & H_2CO_3) using the techniques described in the last three chapters is covered.

7.2 Calcium and Total Hardness

Calcium and magnesium are usually the metals present in the highest concentrations in natural waters, their combined concentration being termed the 'total hardness' of the water. The evaluation of this factor is of utmost importance because of its effect upon many industrial processes and upon soaps used domestically. At present this is carried out either by the separate determinations of the elements spectroscopically or by compleximetric titration.

Determination of these elements with ion selective electrodes has the same major drawback as compleximetric titrations i.e. that the magnesium concentration cannot be determined directly, only as the difference between the calcium concentration and the total water hardness. Another problem is the high range of possible concentrations - soft water may have less than 0.5mM hardness while hard water may be up to 5mM. However here the determination of calcium and total water hardness by bracketing standards, standard additions and gradient titrations is covered, the magnesium concentrations being found by difference.

7.2.1 Bracketing Standards

To determine both the calcium and total water hardness of the samples BRACKSTAN2 was used, because of the wide possible range of concentrations, with the calcium neutral carrier or the water hardness electrode (see

Table 2.1). The apparatus shown in Figure 4.1 was used with $F_s \simeq F_b \simeq 2.5 \text{ ml/min}$ and the buffer being a pH9 $\text{NH}_3/\text{NH}_4\text{Cl}$ solution of 0.25M ionic strength. Eight standards (0.2, 0.4, 0.6, 1.0, 1.5, 2.5, 4 and 6mM calcium) were available for both analyses. The results are shown in Table 7.1 as a mean of two analyses, along with the results obtained by Atomic Emission from an Inductively Coupled Plasma. It can be seen in both cases that the results obtained potentiometrically were considerably lower. To test whether these differences were significant a paired comparison test was used. The ratios of the analyses of each sample were calculated, then the mean and the standard deviation of these ratios ($\bar{r}$ and σ_r , respectively). If the 95% confidence limits of the ratio did not include unity it could be concluded that one set of results was biased with respect to the other. The 95% confidence limits were

$$\bar{r} \pm \frac{t \sigma_r}{\sqrt{n}} \quad 7.2-A$$

where n was the number of ratios and t being found from statistical tables. In this case with seven ratios these limits were given by

$$\bar{r} \pm 0.925 \sigma_r \quad 7.2-B$$

Taking the ratios of the results by bracketing standards to the results by plasma emission, there were significant differences in the calcium results ($\bar{r} = 0.85, \sigma_r = 0.11$) and in the total hardness results ($\bar{r} = 0.87, \sigma_r = 0.06$), with the potentiometric results being about 15% lower. This was probably due to the complexation of the calcium and magnesium ions, especially by sulphate ions which form quite strong complexes with these metals ($K = 204$ for calcium and 169 for magnesium), so for example if 2mM calcium and 1mM sulphate were present about 12% of the calcium would be complexed. Thus the presence of sulphate in natural waters seems to preclude the use of bracketing standards to obtain accurate results for measuring the metal concentrations as opposed to

Sample	Calcium (mM)			Total Hardness (mM)				
	I.C.P.	Bracketing standards	Standard additions	Gradient titration	I.C.P.	Bracketing standards	Standard additions	Gradient titrations
A	0.46	0.42	0.49	0.48	0.68	0.61	0.67	0.64
B	1.23	1.19	1.22	1.26	1.64	1.52	1.50	1.60
C	3.65	3.35	3.49	3.71	5.06	4.86	5.27	5.20
D	1.88	1.62	1.87	1.85	2.43	2.07	2.50	2.42
E	2.75	2.36	2.62	2.65	2.95	2.43	2.89	3.06
F	0.42	0.25	0.44	0.40	0.67	0.53	0.64	0.68
G	2.71	2.43	2.59	2.74	3.06	2.70	2.97	3.17

Table 7.1 Calcium and total hardness of the water samples by various methods

Sample	I.C.P.	Bracketing standards	Standard additions	Gradient titrations
A	0.22	0.19	0.18	0.18
B	0.41	0.33	0.28	0.34
C	1.41	1.51	1.36	1.49
D	0.55	0.45	0.63	0.57
E	0.20	0.07	0.27	0.51
F	0.25	0.28	0.20	0.18
G	0.32	0.27	0.48	0.43

Table 7.2 Magnesium concentrations of the samples (mM) by the I.C.P. and by the difference of the total hardness and calcium results obtained with ion selective electrodes

their activities.

The results for magnesium obtained by difference are shown in Table 7.2. Here again the results by bracketing standards are generally lower than those from the plasma ($\bar{r} = 0.84$) but the variation was far greater ($\sigma_r = 0.25$). This was caused by the large error resulting from a fairly small magnesium concentration being found by the difference in two larger concentrations.

7.2.2 Standard Additions

Because of the wide range of concentrations, the samples were divided into three categories according to their total hardness: soft (under 1mM), reasonably hard and hard (1 to 3mM) and very hard (over 3mM).

STANDAD was used, setting the flow rates F_s , F_i and F_b in Figure 5.1 to

around 2ml/min and using the same buffer and electrodes as in the last section. Additions of 0, 0.25, 0.5, 1, 1.5 and 2mM were made for soft waters; 0, 1, 2, 3, 4 and 6mM for the hard and reasonably hard samples and 0, 1, 3, 5, 7 and 10mM for the hard sample.

Again the results, shown as a mean of two analyses, for both the calcium concentration ($\bar{r} = 0.99, \sigma_r = 0.04$) and the total hardness ($\bar{r} = 0.98, \sigma_r = 0.04$) were lower overall than those obtained by the I.C.P. In this case however the difference was only around 2% and was not statistically significant, showing the much lower effect of complexing agents upon these analyses by standard additions. The results for magnesium ($\bar{r} = 1.11, \sigma_r = 0.41$) again showed great variation.

7.2.3 Gradient Titrations

TITRATE was used to perform the single cup titrations of total hardness with EDTA, monitored by the water hardness electrode, and of calcium with EGTA, monitored by the calcium neutral carrier electrode. EGTA was used because of its selectivity towards the complexing of calcium in the presence of magnesium (see Section 6.5), so there was no chemical interference by the magnesium present in the determination of calcium. The flow rates F_t , F_s and F_b in Figure 6.4 were all set to around 1.2ml/min, the titrant concentration was 5mM, the sample volume 5ml and the buffer was that used in the last two sections. The system was calibrated with 0.5, 1, 1.5, 2, 3, 4 and 5mM standards of mixed calcium/magnesium for the water hardness titration and calcium for the calcium titration.

The calibrations were similar for both titrations ($t_{th} = -0.011 + 0.940t_{act}$ for water hardness and $t_{th} = 0.023 + 0.935t_{act}$ for calcium) and were of the type expected by the considerations of Section 6.2. The results showed the greatest agreement of all with the I.C.P. figures ($\bar{r} = 0.999, \sigma_r = 0.033$ for calcium and $\bar{r} = 1.004$ and $\sigma_r = 0.036$ for total hardness), showing the effects of interfering ions and complexing agents to be negligible. Yet again the magnesium results obtained by difference showed great differences from the I.C.P. results, although

the values of $\bar{r} = 1.19$ and $\sigma_r = 0.63$ were greatly affected by the very poor agreement of the analysis of sample E.

7.2.4 Conclusions

As forecast the major problem proved to be the determination of magnesium, especially in the samples used which contained little magnesium compared with calcium. However the results for calcium and total hardness were much more acceptable. The use of bracketing standards gave the free metal ion concentration at the pH of the buffer used, which is probably of little use. However if no ionic or pH buffer were to be used the technique would provide information on the activity of the sample, which may be of more use than the concentration of the sample, especially in biological surveys. However in such cases great care would have to be taken with the samples since the activity may change with temperature or with carbon dioxide dissolving in or being lost from the sample.

The more normally quoted metal concentration was obtained by standard additions and by gradient titrations, the titration technique giving higher analysis rates and slightly superior results. Moreover it is possible that both magnesium and calcium could be determined directly by titration to two consecutive equivalence points by the method of Christiansen et al. (84) if a calcium electrode with a lower detection limit were to be used.

7.3 Sulphate

Sulphate is one of the major anions present in water samples, but its analysis by instrumental methods, rather than by gravimetric or volumetric determination with barium can prove troublesome. Turbimetric titration with barium (116) or amperometric titration with lead (117) can be used, but both can cause problems and are difficult to automate. Addition of a known amount of excess barium and determination of the barium remaining in solution is possible, but involves sample pretreatment including filtration.

Sulphate selective electrodes have been described e.g. (118) but their performance has generally been poor and sulphate can be determined more accurately and precisely by potentiometric titration. Titration with lead using a solid-state lead electrode has been recommended (117), but the sample must be diluted at least 1:1 with methanol since lead sulphate is slightly soluble in water and calibration of the titration system with standards closely matching the sample is necessary since there is evidence that the reaction is not perfectly stoichiometric, with the stoichiometry changing with concentration. Also the detection limit of this method (around 100ppm or 1mM) is poor and is higher than the concentrations of sulphate occurring in soft and even some hard waters.

To overcome these problems barium was used as titrant for the determination of sulphate by gradient titration. Barium sulphate is highly insoluble ($pK_{sp} = 10$) and since it is widely used in gravimetric analyses, its formation must be assumed to be stoichiometric. This titration was probably not recommended because of the poor reputation of the barium electrode, but in this work it was found to perform very satisfactorily as a titration monitor, at least, over a fair period of time (see Section 6.4). Also it was found to respond to barium solutions to below $10^{-5}M$ and so proved suitable for determining sulphate down to 0.1mM or 10ppm, an order of magnitude better than the lead titration and was thus capable of measuring the sulphate content of all but the softest of water samples.

TITRATE was used for the analysis, with the flow stream described in Section 7.2.3. for the determination of calcium and **total** hardness, with a 0.25M NaCl buffer. The system was calibrated over two concentration ranges, 0 to 1mM with a 1mM titrant and 0 to 5mM with a 5mM titrant. The calibrations were very similar and again showed good agreement with those obtained in Section 6.2. The results (Table 7.3) show excellent

Sample	Sulphate concentration (mM)	
	Conventional Titration	Gradient titration
A	0.23	0.23
B	0.94	0.91
C	4.05	4.25
D	1.12	1.09
E	1.15	1.15
F	0.35	0.34
G	1.09	1.11

Table 7.3 Sulphate analyses

agreement with results obtained by a conventional titration (119) ($\bar{r} = 0.997$, $\sigma_r = 0.036$), showing that possible interference by calcium or magnesium ions with the electrode response was negligible.

In conclusion the on-line gradient titration with barium proved to give good results, there being no evidence of interference from other ions commonly present in water samples and the method being relatively sensitive and very convenient. However because the range of sample concentrations was higher than one order of magnitude, two systems had to be used with different titrant concentrations - 1mM for 0 to 1mM samples and 5mM for 1 to 5mM samples.

7.4 Nitrate

Nitrate is present naturally in water samples in small amounts, but its presence in larger quantities may be injurious to health, as well as perhaps indicating contamination by sewage. The maximum permissible levels vary according to the source of information, but are generally around 50ppm (0.8mM).

The recommended methods of analysis (116,119) are all relatively complex, are subject to interferences from the other anions present in water samples and have limited linear ranges. Ultraviolet absorption spectrometry at 220nm may be used for concentrations of up to 50ppm, but this method suffers from interferences if the concentration of chloride or bicarbonate ions is greater than 10ppm and 5ppm respectively and also if dissolved organics or suspended particles are present. A direct colorimetric technique exists with the reaction of nitrate with brucine to give a yellow complex. However the absorption only obeys Beer's law in the range 0.5 to 10ppm. Also the formation of the complex is sensitive to the heat given off by the reaction and so the treated sample has to be kept at a controlled, known temperature for a precise time before the analysis. Indirect methods of analysis also exist, based on the reduction of nitrate to either nitrite or ammonium.

The nitrate selective electrode used here displayed few of the above disadvantages, being simple to use, having a linear range from 0.05mM (3ppm) to 0.1M and responding down to 0.005mM (0.3ppm). The major interfering ions (perchlorate, iodide, chlorate and bromide) were unlikely to be present in significant quantities and the selectivity coefficients for chloride and sulphate were 5×10^{-3} and $< 5 \times 10^{-4}$ respectively. Also complexes with nitrates tend to be very weak so that bracketing standards with an ionic buffer would give the total nitrate concentration.

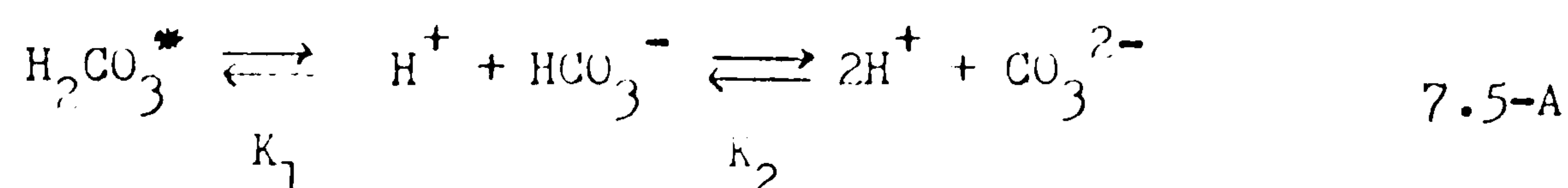
Sample	Nitrate concentration (mM)	
	Emission method	Bracketing standards
A	0.025	< 0.05
B	0.206	0.224
C	0.401	0.393
D	0.354	0.367
E	0.368	0.374
F	0.080	0.073
G	0.391	0.368

Table 7.4 Nitrate analyses

BRACKSTAN2 was used for the analysis, with $F_s \simeq 2.5\text{ml/min}$ and $F_b \simeq 1\text{ml/min}$ (see Figure 4.1) with the buffer being $0.2\text{M K}_2\text{SO}_4$ slightly acidified with sulphuric acid. Eight standards (0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8 and 1.5mM) were available, representing the lower linear limit of the electrode's response and the likely maximum concentration to be reached. The results are shown in Table 7.4 along with the results from a molecular emission spectroscopy method. This consisted of reducing the nitrate to ammonium with titanium (III) sulphate, adding sodium hydroxide to the solution, passing the ammonia produced into a hydrogen/oxygen flame diluted with nitrogen and integrating the resulting NH emission at 336.0nm over 2mins. The sets of results gave good agreement with $\bar{r} = 0.996$ and $\sigma_r = 0.058$.

7.5 Total Carbonic Species

As was the case with calcium and magnesium ions, the analysis of the total carbonic species (H_2CO_3^* , CO_2 , HCO_3^- and CO_3^{2-}), otherwise known as the carbonate or temporary hardness, is of great importance in determining the suitability of waters for industrial, agricultural and domestic use. The existence of the four species in equilibrium is best described by the equation



where H_2CO_3^* describes both the carbonic acid and the dissolved CO_2 content of the sample and K_1 and K_2 are dissociation constants ($\text{p}K_1 = 6.3$ and $\text{p}K_2 = 10.3$). It can be shown from these figures that below pH 6 H_2CO_3^* is the species present in the highest concentration, between pH 6 and 11 it is HCO_3^- and above pH 11 CO_3^{2-} is the major component (120).

Usually the analysis of carbonate hardness is carried out by manual titrimetric methods or by an automatic inorganic carbon analyser, which strips the acidified sample of CO_2 and measures the quantity evolved by an instrumental technique such as infra-red spectroscopy or gas chromatography. The carbon dioxide gas electrode provides a simple way of measuring the free carbon dioxide concentration of a sample, but this is generally not the parameter of interest, although acidification of the sample to convert all the carbonic species to H_2CO_3^* is possible.

An automated method for determining both the carbonate and non-carbonate hardnesses simultaneously with a water hardness electrode has been described by Sekerka and Lechner (121). The electrode was calibrated by a single standard and then immersed in the sample to give a concentration which was assumed to be the non-carbonate hardness. A single known dilution was then carried out and the second concentration found taken to

represent the total water hardness.

There is much to criticise in this method, not only the poor experimental technique in using only one standard for calibration and making only one known dilution, but also some of the assumptions made. While it is probable that the known dilution gave a good approximation to the total water hardness, as standard additions did in Section 7.2, the direct potentiometric reading was unlikely to give the non-carbonate hardness. The degree of complexation would vary with the pH as the species present varied, the complexation due to carbonic species would be very unlikely to be 100% and other anions present would also complex the metals.

It is unlikely that carbonic species other than bicarbonate would be present in the solution or form complexes with the metal ions, thus only the complexes with bicarbonate are considered here. The log K values for calcium and magnesium complexes are similar, being 0.81 and 0.77 respectively, both complexes being of a 1:1 stoichiometry. An example of the true degree of complexation can be seen in Table 5.15, in a 5mM solution of both calcium and bicarbonate only 4.6% of the calcium would be complexed and at the lower concentrations normally found in water samples the proportion would be lower. Also there are usually other anions present which may complex the metal ions, indeed it was pointed out in Section 7.2 that sulphate forms much stronger complexes with calcium and magnesium than bicarbonate. Thus the good correlation between results for carbonate hardness obtained by this method and by an EDTA titration after boiling must be viewed with some suspicion.

In this work the concentration of total carbonic species was measured by difference, measuring the calcium content of the samples at pH 8 and 12 by standard additions. The proportion of the total carbonic species (C_t) present as bicarbonate and carbonate ions are (120) -

$$\frac{[\text{HCO}_3^-]}{C_t} = \left[\frac{[\text{H}^+]}{K_1} + \frac{K_2}{[\text{H}^+]} + 1 \right]^{-1} \quad 7.5-B$$

$$\frac{[\text{CO}_3^{2-}]}{C_t} = \frac{K_2}{[\text{H}^+]} \frac{[\text{HCO}_3^-]}{C_t} \quad 7.5-C$$

At pH 8 only 0.4% of the carbonic species was present as carbonate and 98% as bicarbonate, thus the standard additions determination gave the total calcium content. At pH 12 bicarbonate formed 1.9% and carbonate 98% of the total carbonic species in solution. Thus the solubility product of calcium carbonate ($K_{sp} = 6 \times 10^{-9}$) was exceeded and the precipitation of the carbonic species as calcium carbonate nearly complete, the standard addition giving the residual calcium concentration.

Formation of magnesium carbonate was unlikely, since it is more soluble ($K_{sp} = 3 \times 10^{-5}$) and since magnesium was much more likely to be precipitated as the hydroxide ($K_{sp} = 3 \times 10^{-12}$). However the presence of other metals which form less soluble carbonates than calcium interfered, although these metals were unlikely to be present in quantities comparable with calcium. There were no other anions which were likely to precipitate calcium at the increased pH and the difference in complex formation (e.g. with hydroxide, which gives a 1:1 complex with calcium with $K = 4$) was accounted for by using standard additions.

The calcium determinations were carried out as in Section 7.2.2, using pH 8 and pH 12 buffers. In some cases where the total carbonic species concentration was greater than the calcium concentration, there was not enough calcium present in the sample for all the carbonate to be precipitated. To overcome this the buffer solution was made approximately equal in calcium concentration to the sample, so that precipitation of carbonate was completed.

The concentration of the total carbonic species was also determined by a potentiometric titration technique described fully in (120). The pH of

Sample	Calcium conc. at pH 8	Calcium conc. of pH 12 buffer	Calcium conc. at pH 12	Carbonic species conc. by difference	Carbonic species conc. by titration
A	0.48	-	0.18	0.30	0.29
B	1.24	-	0.53	0.71	0.69
C	3.64	-	2.13	1.51	1.57
D	1.91	2.5	1.49	2.92	3.06
E	2.64	2.5	1.52	3.62	3.70
F	0.44	0.5	0.59	0.35	0.36
G	2.48	2.5	0.56	4.42	4.31

Table 7.5 Total Carbonic Species Analyses

(All concentrations shown are mM)

the sample was taken and then the sample titrated with standard hydrochloric acid to find the ' H_2CO_3^* end-point', the pH around 4.3 at which there was an inflection in the titration curve due to a minimum in the buffering capacity of the carbonic species when the bicarbonate had been converted to H_2CO_3^* . The concentration indicated by this titration is known as the 'alkalinity' of the sample and C_t was given by -

$$C_t = \left[1 + \frac{K_2}{[\text{H}^+]} + \frac{[\text{H}^+]}{K_1} \right] \left[\frac{\text{Alkalinity} - K_w/[\text{H}^+] + [\text{H}^+]}{1 + 2K_2/[\text{H}^+]} \right] \quad 7.5-D$$

where K_w is the dissociation constant of water and $[\text{H}^+]$ the original hydrogen ion concentration of the sample.

The results, shown in Table 7.5 as a mean of three analyses, again show good agreement between the standard method and the technique using ion-selective electrodes and show the validity of the precipitation technique. However in some cases the determination of the calcium concentration at pH 12 was inconvenient using the standard additions system available, since unless the approximate level of the total carbonic species was known the choice of the correct standards and the concentration of the calcium in the buffer was difficult without preliminary analyses being carried out. On the other hand the method proved more convenient than the titrimetric method, whose end-point was difficult to determine accurately, and was less susceptible to interference, the titration giving poor results if anions of other weak acids were present and could prove to be a useful alternative to other methods of analysis.

7.6 Conclusions

In this chapter the bracketing standards, standard additions and gradient titration systems have been shown to give results for water analyses compatible with those obtained by other methods, such as atomic emission spectroscopy and conventional titrimetry. It has also been shown that ion selective electrodes could find a wider use in these analyses.

In the determination of calcium and total water hardness standard additions and gradient titrations gave results comparable with those obtained spectroscopically, although the use of ion selective electrodes would probably only be preferred on the grounds of cost or if activity, not concentration, were the parameter of interest.

However in the determination of anions covered in this chapter the use of ion selective electrodes showed great advantages when compared with standard methods. Nitrate was determined accurately by simple direct methods, the barium electrode proved to be an excellent end-point detector for the titration of sulphate and if the method for determining total carbonic species was less convenient than the other two, it shows some advantages over other methods.

8.1 Introduction

The use of ion selective electrodes for the analysis of biological fluids has been the subject of much research reported in the literature and even the subject of some books (9, 10 & 15). The main reason for this work has undoubtedly been the possibility of measuring activities rather than concentrations - activity being a more important parameter since the behaviour of ions in generating a membrane potential depends on their activity in biological systems as well as in ion selective electrodes. Sample preparation may be easier as well, for example whole blood may be used instead of serum and also miniaturisation of the sensors has made possible 'in vivo' measurements, for continuous monitoring if necessary, rather than the taking of periodic samples for 'in vitro' analysis.

However the use of ion selective electrodes for routine clinical analysis seems to be restricted, except for the determination of pH. The main cations of interest in biological fluids are sodium, potassium and calcium and while suitable liquid membrane electrodes for potassium and calcium have existed for some time a suitable sodium electrode has not been available until recently (36) - the sodium glass electrode generally giving too slow a response. Also the standard method of flame photometry for these elements has proved to be satisfactory over many years, so there has been little reason for change, especially since a change in the interpretation of the results would be necessary at the same time.

Thus in this chapter, as well as testing the on-line bracketing standards systems for whole blood, serum and urine analyses, the difference between the results obtained with ion selective electrodes and spectroscopic methods is emphasised.

8.2 Potassium in whole Blood and Serum

The concentration of potassium in blood is normally in the range of 3 to 6mM, while the intracellular concentration is considerably greater, above 100mM. The existence and maintenance of this gradient across the cell membrane, and also the gradient of the sodium concentration, is responsible for the potential across the cell membrane which is essential for the functioning of living tissues. Thus any disturbance of the potassium concentration in blood can cause disorders of nerve and muscle tissue. The effect on cardiac muscle is particularly important and so a periodic check on potassium levels is advisable during surgery or other treatment where it is likely to be affected.

Before the introduction of the valinomycin exchanger this determination was difficult using ion selective electrodes, because the relatively poor selectivity of potassium glass electrodes towards sodium proved inadequate for analysing potassium in the presence of typical levels of sodium of 140mM. However the neutral carrier electrode has a selectivity of around 10^{-4} for potassium over sodium, which is easily adequate, and it has been used for the analysis of serum since shortly after its introduction (123).

The chemical and physical state of potassium in blood and serum and the nature of the solution itself leads to possible differences in the results of analyses with ion selective electrodes and the analyses (usually of serum) with such methods as flame photometry. As noted above the sodium concentration is relatively high, leading to an activity coefficient considerably less than unity. Thus performing bracketing standards analyses with aqueous standards of known activity will give the activity of the solution, which will be considerably lower than its concentration. Also complexation of the metal ion by organic and inorganic ions or binding by proteins will tend to lower the activity

with respect to the total concentration. However between 3 and 7% of the serum volume consists of non-aqueous solids (124), this tending to increase ion selective electrode readings, which relate solely to the activity of the aqueous phase.

In order to correlate ion selective electrode readings with total concentration readings, measurements of the free ion concentration, rather than activity have been made. The use of an ionic strength adjustment buffer is inadvisable since it may affect equilibria within the sample, and anyway the high initial ionic strength of the sample would make it difficult to equalise the activity coefficients of both sample and standard. However as the ionic composition of serum is relatively constant it is possible to use standards made up to a similar ionic strength (conveniently with 140mM NaCl). Since the activity coefficient should now be virtually identical in both standards and sample it can be ignored and the results quoted as free ion concentrations.

To investigate the analysis of whole blood and serum four samples of each were analysed using BRACKSTAN1. This was suitable despite the fairly wide range of possible concentrations because in clinical practice 0.2mM would be about the smallest change that would be regarded as significant. F_s (see Figure 4.1) was set to 3.4ml/min and F_b zero. For the determination of activities the standards used were KCl solutions of concentration 2.1 and 5.0mM, their activities calculated from the Debye-Huckel equation being 1.994 and 4.625mM respectively. For the determination of free ion concentration the standards were 3 and 6mM KCl in 140mM NaCl. The results of the analyses are shown in Table 8.1 as the average of triplicate trials. The potassium concentration of the serum samples was also measured by flame photometry, but unfortunately the small volume of the whole blood samples precluded their conversion to serum for spectroscopic analysis.

Sample	Type	Potassium Activity	Free Potassium Concentration	Concentration by Flame photometry
A	Whole Blood	3.63	4.91	-
B	"	2.77	3.90	-
C	"	3.21	4.32	-
D	"	3.27	4.27	-
E	Serum	3.45	4.66	4.63
F	"	3.50	4.74	4.58
G	"	2.83	3.76	3.52
H	"	3.62	5.02	4.82

Table 8.1 Potassium Analyses of Whole Blood and Serum
(All concentrations in mM)

The whole blood analyses display a fairly constant activity - concentration ratio of 0.75 ± 0.02 , showing the validity of assuming the ionic strength of the serum samples to be fairly constant and that the activity coefficient is close to that predicted by Covington (125) - 0.748. Unfortunately no comparisons with other methods were possible, but other workers have suggested that there is a systematic bias between the free ion concentration as determined by ion selective electrodes and the total concentration as determined by flame photometry, the ion selective electrode readings being higher (126,127). Since the electrodes also gave higher readings in whole blood than in serum from the same sample, it is probable that this is at least partly due to a residual liquid junction potential caused by the red blood cells. However because this potential seems to be fairly constant at +0.6mV it can be easily accounted for, but this does not explain all of the difference. Thus the effect of the solids in blood reducing the effective volume of the solution seems to outweigh the effect of potassium ions being complexed or bound by protein.

The serum analyses again showed a constant activity - concentration ratio of 0.74 ± 0.01 . When compared with the results obtained by flame photometry the concentrations were slightly higher, but the results obtained potentiometrically were probably better guides to the serum concentration, being the concentration of uncomplexed potassium in the aqueous phase, instead of the total concentration in the whole of the serum, and the differences were only about 0.2mM, only just significant.

8.3 Calcium in Whole Blood and Serum

The physiological importance of calcium is broader and more complex than that of potassium, there being many examples of processes which are known to depend critically upon the calcium levels of blood, including bone formation and resorption and nerve, muscle, heart and brain function. Also abnormal calcium levels may be recorded during the investigation of disease e.g. parathyroid disorders (128). Again the use of ion selective electrodes to measure the calcium activity or free ion concentration was only made possible with the development of liquid membrane electrodes, glass membranes not being sufficiently selective and having a poor response time. Since then many papers have been published on their use, the first probably being the lengthy work of Moore (129), but again their use has not become widespread.

There are some problems with the determination of calcium levels in whole blood and serum. The whole clinical range of non-bound calcium is about 1.10 to 1.35mM, much lower than that of potassium, and the ion is divalent, thus the electrode slope is only half that of the potassium electrode. Therefore the whole range is covered by only a small change in electrode potential, about 2.5mV, necessitating a stable electrode system for accurate analyses.

The various forms in which calcium can exist in body fluids are much the same as potassium. Anionic complexation is more likely than with potassium and the complexation by proteins, shown to be negligible for potassium, is again possible. The effect of the non-aqueous solids present in the solution should be much the same, but the activity coefficient may be very different. Covington (125) showed that the activity coefficient of calcium ions in a serum-like environment of sodium, potassium and chloride ions was similar to that of calcium

Sample	Type	Calcium Activity	Free Calcium Concentration	Concentration by Flame Photometry
A	Whole Blood	0.47	1.24	-
B	"	0.39	1.12	-
C	"	0.49	1.31	-
D	"	0.45	1.16	-
E	Serum	0.43	1.19	2.68
F	"	0.43	1.26	2.95
G	"	0.45	1.16	2.40
H	"	0.46	1.28	2.62

Table 8.2 Calcium Analyses of Whole Blood and Serum (All concentrations in mEq)

in a solution of CaCl_2 only of the same total ionic strength, assuming normal calcium levels. The activity coefficient was thus estimated to be 0.347.

With the small range of concentrations to be measured BRACKSTAN1 proved suitable for the analysis of the whole blood and serum samples, the flow rates used being the same as for the potassium analyses. For the determination of activities the standards used were CaCl_2 solutions of concentration 0.4 & 0.55mM, their calculated activities being 0.370 and 0.501mM. For the determination of the free ion concentration the standards were 1.1 and 1.35mM CaCl_2 in 140mM NaCl. The results are shown as a mean of three replicate analyses in Table 8.2, again only the serum samples being analysed by flame photometry.

The whole blood and serum samples show activity - concentration ratios of 0.37 ± 0.03 and 0.36 ± 0.03 respectively. While the mean values differ from Covington's calculated value the range of the ratios include it and there is insufficient evidence for a difference.

Although there are no comparisons with other techniques for the whole blood samples, there were large differences between the values of free ion concentration and the total calcium as given by flame photometry, the total concentration figures being around twice as high. This means that the reducing of the volume of the serum by the solid content had a much lower effect than the complexation of the calcium ions by inorganic and organic anions and by protein. This is in keeping with the results of Moore (129) who found the ionised calcium to be on average only 46% of the total calcium, 14% being complexed and 40% being bound by protein. No correlation was found between the serum ionised and the total calcium; the free ion concentration being fairly constant, while the total calcium varied considerably, increasing as the amount of protein in the serum increased. Therefore the serum total calcium, which is routinely analysed in most hospitals, is not

a measure of the physiologically active calcium ion, but an indirect measurement of serum protein. This is an area where ion selective electrodes could be used to great advantage.

However because of the degree of complexation of the calcium ions, readings of their concentration should be taken at 37°C - normal body temperature - since the proportion complexed may change with temperature. It has been shown that there is a small but significant difference between analyses carried out at room temperature and at 37°C , room temperature results being about 0.04mM lower (129). This effect is less marked with potassium analyses since the degree of complexation is much lower.

8.4 Potassium and Calcium in Urine

The excretion of potassium in urine usually amounts to 50 - 300 millimoles per day, while that of calcium is much lower - 3 to 8 millimoles per day. However as with all urinary constituents the actual concentration can change considerably with the volume of the urine excreted. The analysis of urine is usually carried out to calculate the day by day electrolytic balance of a patient, hence total concentration is more often required than the activity or the free ion concentration.

While the factors considered in discussing the state of calcium and potassium in blood can in principle be applied to urine, there are some major differences. Protein is generally absent from urine and so protein binding of calcium is unlikely, but the anionic content of urine varies considerably more than that of serum, so the amount of complexed calcium differs greatly from sample to sample. This is also affected by change in the pH of the urine, which may vary between 4.6 and 7.2, changing the concentration of such ions as bicarbonate, hydroxide and phosphate. Finally the ionic strength of urine can vary

enormously , with 100 to 400mM representing the range of concentrations possible under reasonably normal circumstances, so the activity coefficients of both calcium and potassium can take a variety of different values.

Hence the measurement of activity is of little importance and it is impossible to make up standards in a fixed sodium chloride matrix in order to measure the free ion concentration of a sample. Therefore it was decided to attempt to measure the free ion concentrations of seven urine samples by using an ionic strength buffer, of a higher than usual concentration because of the possible high ionic strength of the sample, and to compare these with the total concentrations obtained by flame photometry. A 2M NaCl buffer was chosen, to minimise changes in the complexing of calcium, chloride forming only a very weak complex with calcium and sodium not being significantly complexed by any of the anions likely to be present. Both flow rates F_s and F_b (see Figure 4.1) were set to 2ml/min so that the concentration of the solution passing the electrodes was just over 1M, although the 1:1 dilution of the sample with respect to calcium may again have affected its complexation. Because of the large range of the concentrations likely to be encountered BRACKSTAN2 was used, with standards of 20 to 200mM KCl for the potassium analysis and 0.5 to 4mM for the calcium analysis. The results are given in Table 8.3 as the mean of two analyses.

The potassium results show good agreement - using the paired comparison test described in Section 7.2 $\bar{r} = 0.99$ and $\sigma_r = 0.03$. Thus the complexation of potassium in urine is negligible and this method provides an alternative to spectroscopic techniques, the electrode performing satisfactorily even in the high sodium concentration. However the method was slower, since BRACKSTAN2 had to be used. In contrast the calcium results were generally lower than the flame

sample	Potassium Concentration		Calcium Concentration	
	ISE	Flame photometry	ISE	Flame photometry
A	53.5	56.1	0.67	0.82
B	57.9	58.5	1.42	1.49
C	72.0	70.8	1.47	1.62
D	44.6	44.5	3.18	3.63
E	82.2	85.0	2.31	2.39
F	54.0	52.0	1.34	1.61
G	68.0	70.2	1.78	2.00

Table 8.3 Analyses of some Urine samples (All concentrations in mEq)

photometry results ($\bar{r} = 0.89$) and the ratio of the electrode results to the flame photometer results varied rather more ($\sigma_r = 0.06$), due to the degree of complexation varying. Therefore the method was not suitable for the determination of the calcium concentration, but the free ion concentration or the activity could be determined.

8.5 Conclusions

In this chapter BRACKSTAN1 and BRACKSTAN2 have been shown to work satisfactorily for the analysis of blood, serum and urine samples. For whole blood and serum samples the narrow limits of the concentrations meant that BRACKSTAN1 could be used, leading to a sampling rate of 30/hr. The urine samples required the use of BRACKSTAN2 and so sampling was slower - around 12/hr.

Since the activity coefficient for potassium in blood samples is very much like that in saline and there is no significant complexation, there is no great advantage in the use of ion selective electrodes to measure the potassium free ion concentration, rather than the total potassium concentration as given by flame photometry. Potentiometric readings tend to be slightly higher than flame photometry measurements because of the solids present in the solution and as such give a truer measure of the solution concentration, but this effect is small and is barely significant.

The determination of calcium in serum and whole blood samples potentiometrically is potentially more important. Much of the calcium is complexed by anions or bound by proteins and only ion selective electrodes can give the concentration of the physiologically active calcium ion. Interesting results may be obtained if the calcium concentration were to be measured by standard additions or gradient titrations. Standard additions should give a concentration nearer the total calcium content, depending upon the strength of the complexes

and the kinetics of their dissociation. One problem would be keeping the activity coefficient constant during the additions, using an ionic buffer would be inadvisable and the ionic strength of the serum itself may not be sufficient. Gradient titrations with EDTA or EGTA would also give a result nearer the total calcium concentration.

The use of ion selective electrodes to determine the total calcium and potassium concentrations of urine showed little or no advantage over flame photometry. While potassium results gave good agreement the calcium measurements were lower because of complexation and the variable levels of the concentrations to be measured and the ionic strength of the samples posed problems. Only if the activities or the free ion concentration of the samples were to be measured would the potentiometric method prove superior.

CHAPTER NINE CONCLUSIONS

This short chapter is a summary of the conclusions drawn from the previous chapters and includes suggestions for improvements. Some of the properties of ion selective electrodes in flow streams have been investigated, followed by the use of an on-line minicomputer to monitor electrode potentials, process data and perform a certain amount of experimental control using the analytical techniques of bracketing standards, standard additions and gradient titrations. To test the above systems various analyses of water and biological samples were performed.

It was shown that to optimize the performance of ion selective electrodes in flowing streams, especially the time response and noise levels, careful design was necessary. The time response of electrodes in some flow streams described has been plainly inadequate e.g. (55), but by varying the flow cell design and the flow rates used the time to effective full response (taken as 99.5% of the full potential change) for the immobilised liquid membrane electrodes used in this work was less than 30sec for an increase in activity from 10^{-3} to 10^{-2} M. Most of the changes in activity in this work, where an equilibrium potential was in fact to be measured, were smaller than this decade step, so that the response would actually be correspondingly faster. Noise levels were found to be due to variations in streaming potentials and to high frequency pick-up. These were reduced by grounding the flow stream and applying an ionic buffer and by screening the electrodes.

Bracketing standards was capable of giving fairly accurate and precise results for the activity or free ion concentration of samples, providing interfering ions were absent and the ratio of the concentrations of the standards used was in the range 1.5 - 2. BRACKSTAN1 provided rapid analyses, upwards of 30 samples/hr, as long as the unknown

concentrations lay between narrow limits. BRACKSTAN2 was much slower and less convenient, but covered a much greater range of concentrations.

The precision and accuracy of standard additions were little better than that of bracketing standards, but the effects of complexing agents and interfering ions were considerably lower. Again the results depended upon the concentration of the standards used and so the concentration of the sample had to be roughly known in order to choose the correct additions. This could have been found with a preliminary procedure similar to BRACKSTAN2, but this would have slowed the analysis rate, which was only 10 samples/hr anyway.

The use of single cup gradient titrations combined the best accuracy and precision, a rapid analysis rate (typically 25 samples/hr) and a fair range of possible sample concentrations (an order of magnitude). However frequent calibration of the system was necessary and unnoticed change in the response characteristics of the electrode meant that results would have to be discarded.

The analyses of water and biological samples showed applications of the above systems in the analysis of real samples. It was concluded that the use of ion selective electrodes showed some advantages over standard methods in these analyses, especially in the determination of anions such as nitrate, sulphate and bicarbonate in water and in the evaluation of calcium in blood samples.

Unfortunately the only experimental control performed by the computer in this work was the advancing of the sampler in TITRATE. The use of latching valves to switch the standards being used in bracketing standards or standard additions would have been advantageous, eliminating the need for putting standards in the sampler cups by storing them in reservoirs and perhaps allowing computer intervention during the course of an experiment to change the standards being used to optimize their range. Further examples of the possible use of

computer control would be the on-line coulometric generation of titrant for gradient titration and control of the flow rates by means of a pump driven by a stepping motor.

However almost total automation of the analytical techniques was achieved. The only manual tasks necessary were the insertion of the samples and standards in the sampler and the initiation of the relevant programme. The apparatus could then be left to run without operator attention and the results would be evaluated and printed out automatically.

Improvements in the input of data and its processing could have been achieved by the use of interrupts from the interface to the computer to inform the programme that a data point had been taken. This would have allowed the real-time processing of data instead of using the computer as a clock to time data inputs. Also the use of a different interface system taking in data at a much greater rate could have been advantageous in the detection of titration end-points, rather than the interface used, which only took in points at the rate of 4 per second. Rather more modern computer designs might have allowed programming in high level languages, the writing of programmes for the α -16 in assembler language proved extremely tedious and time-consuming, especially when relatively complex data processing was necessary e.g. in STAD.

Naturally the minicomputer could now be replaced by a microprocessor and the associated memory - the microprocessor being able to perform all the functions of control suggested above and the processing of data. While it may prove slightly slower than the minicomputer it would still be extremely rapid by human terms and of course would be much cheaper than a minicomputer.

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