APPLICATIONS OF COMPLEXOMETRY AND POLAROGRAPHY TO CONTINUOUS ANALYSIS

by

MAUNG SOE WIN, B.Sc. (Gen. Honours) (Rangoon)

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of the University of London

Department of Chemistry,
Imperial College of Science and Technology,
London, S.W.7.

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ABSTRACT

A rapid visual complexometric titrimetric method for the determination of calcium in the presence of magnesium has been developed. The method is directly applicable to the analysis of natural waters.(Section I)

The use of the anodic waves of complexones in inorganic polarographic analysis is described in Section II. A study of the fundamental polarographic behaviour of the complexone EGTA has been made. It is found to give a diffusion-controlled, one-step, two-electron, reversible anodic polarographic wave which is attributed to the formation of the mercury (II) complex. The half-wave potential of the wave is pH dependent and from thence the absence of other mercury complexes is indicated and the stability constant of the mercury (II) complex determined. Current-potential relationship of the wave fits in perfectly with theory. Analytical application is illustrated by the development of an indirect polarographic method and an amperometric titrimetric method again for the analysis of calcium in the presence of magnesium. Polarographic maxima of complexones and masking agents for magnesium in the presence of calcium has been dealt with in reasonable detail during the process. The concept of mixed titrants is also introduced. The application of a.c. polarography of complexones for analytical purposes is found to be feasible especially for trace analysis. In Section III the development of a continuous polarographic analyser for the simultaneous analysis of both calcium and magnesium employing complexometric principles illustrate the concept of continuous analysis via complexometry using electrochemical sensors which provides a foundation for widespread applications. Finally the use of some electrochemical sensors other than the D.M.E, namely, a hanging mercury drop electrode, a tubular platinum electrode and a mercury-plated tubular platinum electrode, has been investigated with special emphasis on the andoic oxidation of complexones.

ACKNOWLEDGEMENTS

The work recorded in this thesis was carried out in the Chemistry Department of Imperial College of Science and Technology between October 1965 and November 1968. It is entirely original except where due reference is made and no part has previously been submitted for any other degree.

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MAUNG SOE WIN

CONTENTS

SECTION I

COMPLEXOMETRIC METHODS OF ANALYSIS

SECTION II

USE OF COMPLEXONES IN POLAROGRAPHIC ANALYSIS

SECTION III

CONTINUOUS ANALYSIS VIA COMPLEXOMETRY USING ELECTROCHEMICAL SENSORS

INTRODUCTION

An amazingly large number of chemical analyses are performed each year in our highly industrialised and developing society. Billions of such analyses are carried out each year in the United States alone to monitor industrial processes and their products, to aid in research efforts in most branches of science to ensure physical and social welfare of the people through clinical, pollution control, forensic, food and drug administrative control laboratories, etc. Amazingly too a large proportion of these analyses are still performed manually by skilled scientists and technicians. There is, however, a trend during the past few years towards automation of chemical analysis necessitated by shortage of skilled personnel, for example in the overloaded clinical laboratories and also in an effort to keep pace with the rapid increase in automation in industry which has occurred during the present decade.

Studies on the applications of two very well-known analytical principles, namely, complexometry and polarography (voltammetry) to continuous analysis have been dealt with in this thesis. The theory and advantages of these two principles as applied to continuous analysis will be discussed in the relevant sections. For the moment, however, the relatively new term, continuous analysis, has to be defined. It is convenient to classify automated chemical analysis into two groups:

1. Continuous (automated) analysis, and
2. Repetitive (automated) analysis

For the purposes of this thesis, the author has agreed with Blaedel[1] in defining continuous analysis as that analytical procedure where sample processing, sensing and measurement are done as a steadily flowing stream. If the sample does not come in as a flowing stream (as from a chemical process stream) but as a batch it is converted to one by pumping, prior to analysis. Repetitive analysis on the other hand, has been defined to mean an analysis where the sample is processed and measured individually as a single batch whose identity is maintained throughout the processing and measurement.

Features of a continuous analysis system

The features of a continuous analysis system can be seen in Figure 1.

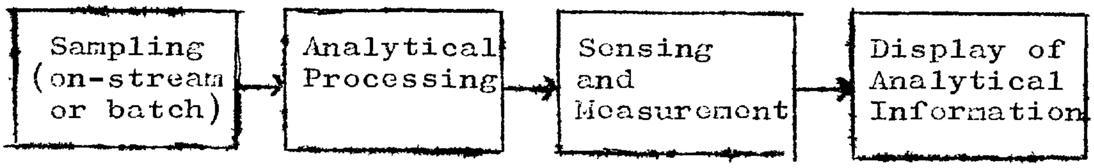


Figure 1. Features of a continuous analysis system

These processes are discussed individually in the following sections.

Sampling

Continuous analysis is by definition a wet chemical analysis technique and can therefore handle liquid or gas samples only, even though some limited applications to solid samples have been reported and shall be described later. Basically therefore, all samples for continuous analysis must be in liquid or gaseous form, either on-stream as from process streams or in batches as for instance in the case of blood samples in clinical laboratories. Sampling from process streams is simply done by having plug-in points at various positions in the process system where analysis is desired; complete manifolds of continuous analytical units are then "plugged-in" at these points and electrically or electronically valving systems devised so that analysis is done either continuously, or at predetermined intervals or whenever actuated by some external signal. On-stream sampling has the obvious advantage of minimising or totally eliminating handling of materials. This becomes important in sampling gas streams which is very difficult, or in handling materials which are liquids in the process streams but gases at room temperature or where materials are processed at high temperatures and solidify on sampling. Great ingenuity and skill are required for manual sampling of such materials as otherwise appreciably large errors can be introduced, as for example when fractionation of the samples takes place on vapourisation or solidification in the above cases. It becomes important again in the cases of materials which are very toxic, corrosive or otherwise dangerous to handle, and also in the cases of materials which cannot be exposed to oxygen, sunlight, etc. since an alteration of the sample composition can be affected. An even more obvious case is in physiological studies where some analytical information on the state of a living organ is desired. An example will be in determining the "availability of materials" in blood streams. Any sampling out of the system will be either totally pointless, or very tedious procedures will have to be followed for meaningful results, and analysis in situ will be preferred. Müller[2], as early as 1947, had suggested the possible use of a platinum micro-electrode as a voltammetric indicator electrode for such studies, since the limiting current found on the current-voltage curve obtained with such a "by-pass electrode" is a function of both the rate of flow and of the concentration of electroactive material.

In the case of liquid batch samples, it is only necessary to pump the samples out from a series of sample cups and programming the sampling in such a way that a new sample is introduced, after every complete sequence of analytical operations has been performed on the previous sample.

As already stated above, continuous analysis can handle only liquid or gaseous samples, and if the original material to be analysed exists in solid form, it will have to be made into a solution. Up to the present time this dissolution step has not been automated quite widely yet, thus for example, an analyst may be needed in an iron ore or steel analysis to weigh out and dissolve the sample. Since in continuous analysis, analysis frequencies in the region of 20 to 60 samples per hour are usual, the time-consuming dissolution step becomes the limiting factor as regards speed of analysis. As one can imagine, putting the solid dissolution step on a continuous basis is not an easy matter to accomplish, however, some steps have been taken towards this goal. Barabas[3] has applied the technique of anodic dissolution from solid samples. It consists in applying a fixed current for a pre-set period of time to the solid sample in the form of a rod, which acts as an anode against an inert graphite cathode. As the current shuts off automatically, at the end of the pre-set period, a solenoid valve located at the bottom of the electrolysis cell opens to release the liquid which descends by gravity into a sample cup placed on a rotating sampler plate. Precision tests on such a technique shows it to be very satisfactory and analysis of phosphorous in copper[3], and of manganese, phosphorous and nitrogen in steels[4] have been carried out by this technique. Docherty[5] has developed an automatic sampling technique for water-soluble compound fertilisers. A sample is obtained by passing a funnel ("Geco" sampler) rapidly through the fertiliser stream. The sample is rather large and is passed through a device ("Tyler" sample divider) which returns 15/16 ths to the process and retains 1/16 for analysis. This sample is used to fill the hopper of an automatic dispensing balance (Mettler DWA 10 C30) which weighs out about 10 g. accurately and rejects the remainder; a smaller sample weight though desirable is impossible since each granule weighs about 20 mg. and grinding before weighing is also unsatisfactory because the product does not flow freely except in granular form. The 10 g. sample is dissolved in 150 ml. of water in a high speed homogeniser (SolidPrep" unit) and passed to the analytical system. The range of applications of such dissolution techniques is obviously very limited yet, but nevertheless they are very welcome as pioneers in this field.

Analytical Processing

Analytical processing consists of preparing the sample for sensing and measurement. For example, a sample may have to be allowed to react with a colour-forming reagent, heated or allowed to stand to develop the colour in order to ready it for measurement in a spectrophotometer. Processing also includes separation or preconcentration steps which form an important part of the analytical method, especially in trace analysis. These steps separate the desired component from possible interfering substances and concentrate it into a smaller volume. Techniques such as the various forms of chromatography, electrophoresis, electrolytic separations, gas-liquid and liquid-liquid extractions, ion-exchange, etc. belong to this category.

In order to enjoy full benefit from the speed with which the sensing and measurement step in a continuous analysis may be performed, it becomes necessary to put as many associated operations on a continuous basis. Nowadays, continuous processing units range from simple mixing coils, time-delay coils and heating baths to more sophisticated instruments like digestion units for continuous Kjeldahl digestion. A wide variety of continuous separation and preconcentration units have also been manufactured or reported. A very good survey of these had been made by Blaedel[6]. These include centrifuges, gas chromatographs and apparatus for ion-exchange, paper, and thin layer chromatography, dialysis, digestion; distillation, electrochromatography, paper electrophoresis, electrolysis, gas-liquid extraction, liquid-

liquid extraction, and filtration.

Sensing and Measurement

Sensing and measurement are the heart of the analytical process, and consist of detecting a certain property in the processed sample which can be correlated with the composition of the components in the sample either qualitatively and/or quantitatively. A wide variety of properties - physical, chemical, biological etc. - that give such information has yielded to desperate efforts by analytical research chemists and today, sensors have been developed which measure properties ranging from magnetic properties to enzymic activity. In principle, all types of analytical sensing are applicable to continuous analysis. Some are however much more easily adapted than others, depending on whether the measured property is insignificantly or critically affected by the flowing state of the processed sample during measurement. This is the reason why optical sensors are in a much higher state of development than electrical sensors, since light absorption or emission (fluorescence) properties are hardly affected by a dynamic measurement, whereas electrochemical phenomena such as voltammetry are highly dependent on the mass transfer process (diffusion, electrical migration, convection, etc.) and therefore critically dependent on whether the measurement is made in a static or moving condition. Sensors suitable for continuous analysis reported in the literature had been surveyed by Blaedel et. al.[6] Even though a wide range of sensors have been investigated, the actual widespread use and acceptance has been the optical sensors. Such a large number of applications has been made in the field of visible spectrophotometry that a complete survey would be extremely difficult; some general articles are available.[7-9] Commercial flame photometers and fluorimeters, for direct use in continuous analysis, have also been manufactured. Applications of continuous infrared and ultraviolet spectrophotometry and emission spectroscopy to the determination of specific substances are also numerous. As yet the application of newer analytical spectroscopic techniques as atomic absorption and atomic fluorescence spectroscopy to continuous analysis is still rather limited; however there is no reason why they should not develop rapidly in the near future as the process of putting these techniques on a continuous basis should not involve much more than in flame photometry. Thus in one instance application of the technique of atomic fluorescence spectroscopy to automatic analysis had been reported by West.[10]

Other types of continuous sensors reported include sensors based on radioactivity, thermal, physical and miscellaneous properties such as the paramagnetism of oxygen and mass spectrometry. Thermal sensors measure either the thermal conductivity or the heat of reaction. Physical properties such as density, optical rotation, refractive index, etc. have also been used as continuous sensors.

So far, only sensors other than electrical or electrochemical sensors have been described. Electrical or electrochemical sensors which have been applied to continuous analysis include amperometric, conductometric, coulometric; coulometric titration, dielectric constant, galvanic, potentiometric, potentiometric titrations and voltammetric sensing. Detailed discussions on these shall be given in appropriate sections. (Sections II and III)

Display of analytical information

Discussion under this section will also include computation steps as in integration of chromatographic peaks, computer interpretation etc. Work in these connections are the concern of electronic, computer and automation engineers, rather than for the chemist. However, in dealing with the rapid piling of data, as in continuous analysis, the analytical chemist has to utilise the above facilities in order to keep abreast with the accuracy, speed and convenience of operation which he has achieved in the sampling, processing and sensing/measurement steps.

An understanding of the basic principle of display of analytical information may be facilitated by a schematic representation of general chemical instrumentation (Figure 2).[11]

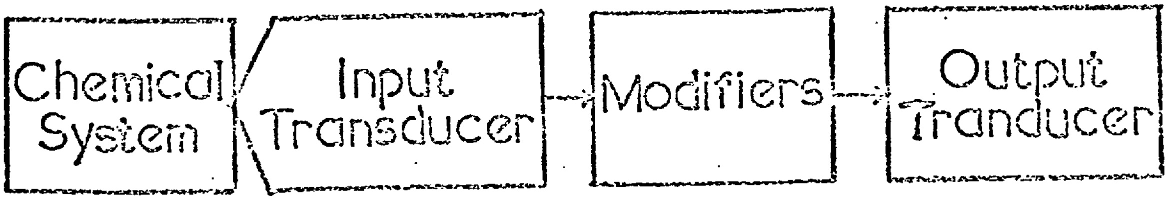


Figure 2. Schematic of generalized chemical instrumentation

In instrumentation, a transducer, literally meaning to lead across, is a device which responds to a stimulus in one realm with a response in a different realm. Here transducers are classified relative to electrical (and electronic) instrumentation, as being input transducers if they respond electrically to non-electrical stimuli, and output transducers if they produce a mechanical, optical, chemical, temperature or other non-electrical response when electrically stimulated. Modifiers perform tasks within a given realm. Amplifiers, filters and attenuators are examples of electronic modifiers. Typical optical modifiers will be prisms, lenses, mirrors and light filters. Thus to give a simple example, a potentiometric indicator electrode (input transducer) exhibits an electrical potential which is related to the activities of appropriate chemical species in the solution surrounding the electrode (chemical system). This electrical signal may then be amplified (modifier) and the amplified electrical signal is used to give a mechanical deflection of a meter (output transducer). The function of the display of analytical information starts after the input transducer or the modifier step when an electrical signal becomes available. The display of analytical information can then be achieved by means of an output transducer which in the above case is the meter deflection. For continuous automated analysis however, the displaying system has to be more elaborate. The following points are relevant. The display of analytical data should be

(1) at a stage if at all possible where no further computation step is required, and can be interpreted as such.

(2) in a form easily interpretable by the user of the analytical service.

(3) in a form which can be easily co-ordinated with such modern aids as computers.

To illustrate the first point, in a chromatographic method, the display normally obtained will be the chromatographic peaks which need to be integrated (computing step) to be able to interpret in concentration terms. Where a non-linear dependence of response upon sample size is used for analysis, calibration with a single standard sample and direct readout cannot be achieved; calibration must then be made with several standard samples and some computation steps and devices must again be used to correlate response into concentration.

The second point will best be illustrated by a description of the Technicon AutoAnalyzer SMA-12 data displaying system.[12,13] The SMA-12 is an AutoAnalyzer system which analyses for twelve components in a single blood serum sample and displays the results on a <u>single</u> recorder. The chart is perforated along its vertical axis at regular intervals to cover each group of twelve analyses. It is pre-calibrated in the terms of the tests - sodium from 110 to 160 milli-equivalents, potassium from 0 to 10 milli-equivalents, total protein from 0 to 10 milligrams, and so on. The area covering the normal values is shaded so that a glance at the chart tells whether a result falls within or close to the normal range. The chart, with the patient's name, ward number and date recorded on it, is merely torn off the roll and sent to the requesting clinician to be directly attached to the patient's chart. This kind of a displaying system is advantageous since the analytical laboratory does not have to read or manipulate (computations, data entry etc.) the results at all, but rather each clinician or or other clients may read and interpret on their own without any possibility whatsoever of introducing transcriptive or clerical errors.

The importance of the third and last point, namely, the easy adaptability of the data displaying system to coordinate with such modern aids as computers cannot be overemphasized. Here again, the advantage of speed achieved by continuous automated analysis must not be let down by the speed of communication and data processing. As mobile and field units provide dynamic analysis results continuously on air and water pollutants on a city-wide or even country-wide scale, it is necessary to monitor all available information at a central control which will also process the results and provide for early warning alarms etc. Possibilities of analytical data recording systems for analog to digital conversions and then fed directly to computers as for process control or storage on punched or magnetic tape or punched cards are highly desirable.

Characteristics of a continuous analysis system

The characteristics of a continuous analysis system shall be discussed in three sections:

(1) Kinetic parameters

(2) Type of calibration

(3) Sensitivity, errors and precision.

Kinetic parameters in continuous analysis

Unlike batch automated analysis, continuous analysis possesses kinetic parameters. Noise level and drift are associated with steady-state measurements, and in connection with this, Thiers and Oglesby[14] have made a study on the precision, accuracy and inherent error of automatic continuous flow methods. A very thorough and pioneering study of the kinetic parameters associated with the transient state has also been made by Thiers and co-workers.[15] The study includes time required to change from baseline steady state to sample steady state and vice versa, characteristics of this change, time interval between samples, ratio of sampling and washing time, fraction of steady state reached in any given sampling time and interaction between samples. Some relevant portions of these will be described here since the authors have managed to produce and define a very useful parameter for continuous flow analysis, namely the half-wash time ($W_{\frac{1}{2}}$). This parameter, measurement of which can be made on any continuous-flow analytical system, can be used to express quantitatively the characteristics of that system. Thus, improvements in methodology can be expressed as comparisons between $W_{\frac{1}{2}}$ before and after the improvements. Comparisons between instruments performing the same determinations can similarly be made, and monitoring of one instrument over a period of time can be very conveniently done, all in terms of this parameter or its derivatives. This parameter in fact seems to provide an extremely sensitive indication of malfunction of continuous-flow analytical systems. In short, this term, half-wash time, provides a comparative, evaluative and descriptive parameter which continuous analytical systems are badly lacking.

Nomenclature of the variables involved in continuous analysis will first be made, with the help of Figure 3.

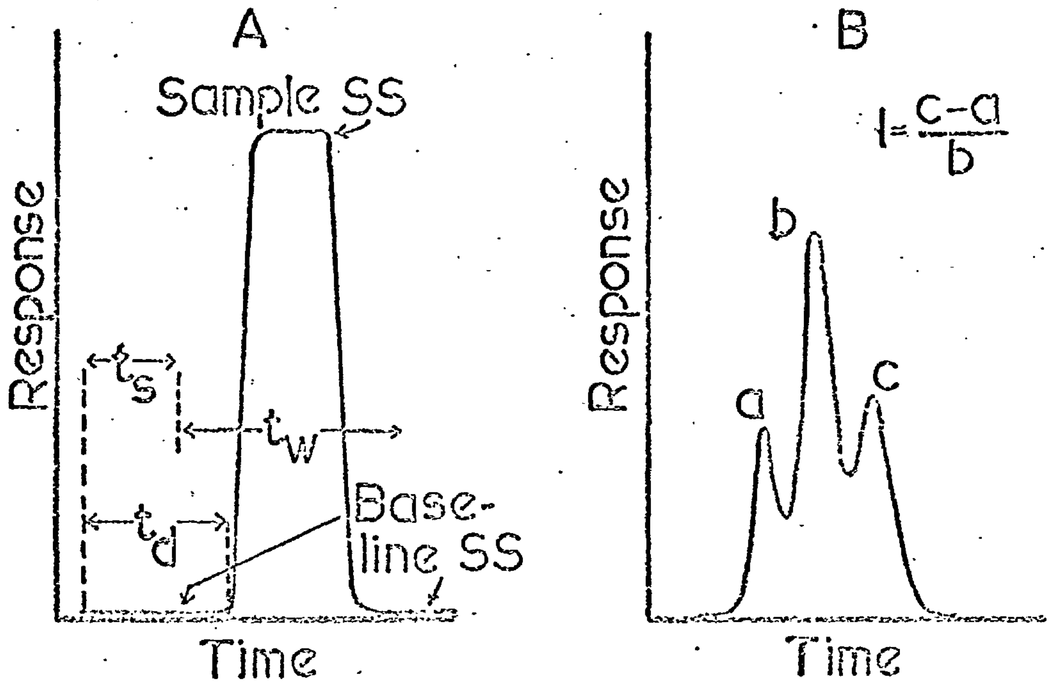


Figure 3. Definition of variables in continuous analysis systems

It shows the recorder tracing which plots sensor response when a sample is aspirated for a considerable length of time. While a blank (or wash) solution is being aspirated, a reading corresponding to the "baseline steady state" is traced on the recorder. The sampler probe is next placed in the sample at the beginning of the period, named "sampling time" (t_s).

The sensor does not observe this change for a period corresponding to the time required by the sample to traverse from the sampling point to the sensor. This is termed the "delay time" or "dead time" (t_d). At the end of t_d, the sensor response suddenly increases along a line called the "rise curve" and if the sampling time (t_s) is sufficiently prolonged, a stable reading will be recorded at the "sample steady state". At the end of t_s the sampler probe transfers from the sample to the blank (or wash) solution and this forms the beginning of the period "washing time" (t_w). After an interval corresponding to the delay time (t_d) the effect of this change will be observed by the sensor and the response decreases suddenly along the "fall curve" until, if t_w is long enough, the baseline steady state will again be reached. The sum of t_s and t_w is the "time between successive samples" (t_{bs}) which determines the "sample frequency" (f_s) expressed in samples per hour.

$$f_s = 3600/t_{bs}$$

when t_{bs} is expressed in seconds.

The time required for the sensor to transit between the steady states corresponding to consecutive samples is termed the "transition time" (t_t). It is contributed to mainly by sensor holdup and by those factors which cause spreading of the interface between consecutive samples. The sum $(t_d + t_t)$ is the "readout time" which represents the minimum time that is required after sample introduction to obtain the analytical result. When t_s is so short that the response does not reach sample steady state, that "percentage of the sample steady state concentration" (% SS) reached during the given t_s may be expressed as the apparent concentration obtained at the peak, divided by the concentration of the sample steady state times 100. "Interaction" is caused by spreading and subsequent mixing of the interface between consecutive samples. It can be measured[14] by running a sample of low concentration, a sample of high concentration, and then again the sample of low concentration (Figure 3B) under the conditions of t_s, t_w and t_{bs} chosen for actual operation. A concentration error due to interaction between the high sample and the second low sample can then be observed, and "percentage interaction" (% I) is given by the difference of the apparent concentrations of the low sample, divided by the high concentration which caused this difference, times 100. Interaction is decreased by having the sampler probe to be immersed in water whenever it is not in the sample.

The most significant discovery by Thiers and co-workers[15] was the observation that the transition between steady state conditions in continuous flow analysis follows kinetics which are approximately first order with respect to concentration. Analysis of the rise curves gives an expression,

$$dc/dt = k(C_{ss} - C_t)$$

where C_{ss} = final steady state concentration

C_t = apparent concentration at any given time t.

k = a proportionality constant

A typical first order kinetic relationship is obvious from the above expression and a plot of $-\log(C_{ss} - C_t)$ vs time gives a linear plot. It can therefore be said that during the transient state in continuous-flow analysis, the apparent concentration follows kinetics which are first order with respect to the difference between the apparent concentration and the steady state concentration towards which it is proceeding. In the case of the fall curves, the change follows kinetics which are first order with respect to the apparent concentration (C_t) itself since in this case the steady state concentration towards which the change is proceeding is zero. The slope of the straight line plots obtained gives the rates of the transition reaction and by analogy with the half-life concept in radioisotope usage, these slopes and rates were expressed in terms of "half-wash times" ($W_{\frac{1}{2}}$). A half-wash time is therefore defined as the time required to go from the apparent concentration represented by any point on the transition curve half-way (in concentration units) to the concentration at the steady state towards which the transition is heading.

A number of interrelationships of various parameters in continuous analysis can be achieved with the half-wash time principle. For quantitative calculation of this sort, one need only refer to a generalized chart on semilogarithmic paper of the concentrational distance from steady state remaining at any given time (expressed as a percentage of the original concentration distance) vs the time itself (expressed in units of half-wash time). Such a chart is reproduced in Figure 4.

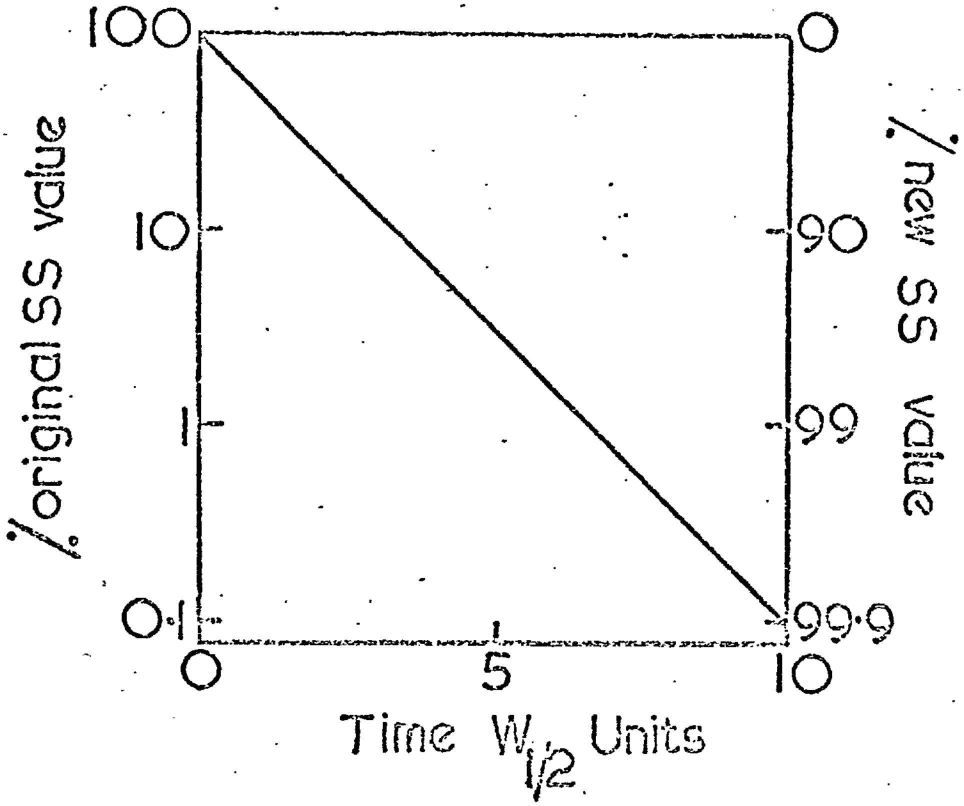


Figure 4. Generalized chart for calculation of interrelationships of various parameters in continuous analysis

If one wants to determine the percentage of steady state concentration reached at any given sampling time (t_s), one simply has to convert t_s into $W_{\frac{1}{2}}$ units by dividing by the latter and reading off on the chart the percentage steady state which corresponds to that number of half-wash times. It has already been said that interaction may be decreased by aspirating water or other wash liquid whenever the sample is not being aspirated. However, samples can be crowded together so closely by decreasing t_{bs} (for reasons of time-economy etc.), that the small but significant apparent concentration by which the signal has failed to reach baseline steady state can add itself to the peak of the subsequent sample. This proves to be the source of interaction.[14] The extent of such an interaction can be calculated again by the half-wash time principle. The value of t_{bs} is first converted again into $W_{\frac{1}{2}}$ units by division by the latter. The number obtained is the effective number of half-wash times between samples and obviously if this number were one, 50% of the concentration of any given sample would appear as part of the following sample; if it were two 25%, three, 12.5% and so on, as read off from the chart (Figure 4). Calculation of the decrease in precision to be expected theoretically as a result of variability of sampling time (t_s) can also be made with the help of the half-wash time principle and Figure 4. An improperly made sampler will give varying values of t_s from sample to sample either in random or systematic manner. t_s may also vary as a function of the height of the surface of the sample in its cup.

If t_s is sufficiently long for the sample steady state to be reached, then variations in t_s have no effect whatsoever on the concentration read. However, by reference to Figure 4 it can be seen that if t_s were only one $W_{\frac{1}{2}}$, where only 50% of the sample steady state is reached by the peak height, then a change of ± 0.1 $W_{\frac{1}{2}}$ in t_s would cause a ± 6% error in the concentration observed. One can therefore calculate, for any measured timing error, minimal values of t_s required to keep the effect of these timing errors on the precision within any chosen limits.

The above illustrations clarify the facts that the percentage of sample steady state reached and sample timing precision are affected by the value of t_s and not by t_{bs}, whereas the percentage interaction is affected only by t_{bs} and not by t_s and governing all these factors is the concept of half-wash time ($W_{\frac{1}{2}}$).

Returning to the continuous analytical system as a whole, again, the kinetic characteristic of the system should be such that the half-wash time ($W_{\frac{1}{2}}$) which governs the above-mentioned factors is kept to a minimum. This may be achieved by reducing holdup at the sensor to a minimum and also by controlling those factors that cause interfacial mixing as the stream passes through the equipment. The first requirement is met by careful cell design and the considerations involved are quite obvious; interfacial mixing is governed by a variety of factors and is much less easy to control. Size and geometry of the processing units, sensor unit, and connecting lines, all have an effect and so does the presence of stagnant regions or traps in which the sample may be held by absorption, adsorption, chemical reaction (e.g. precipitation) etc. Laminar flow has a parabolic velocity profile in tubes and therefore cause interfacial mixing.

A consideration of much less importance is the dead time or delay time (t_d). It affects the read-out time, but has no effect whatsoever on the sample frequency since at any instant, several samples can be passing consecutively through the processing train, just so long as each sample flows long enough past the sensor to permit it to reach the steady state or a reproducible fraction of the steady state corresponding to that sample. t_d may be minimized by reducing holdup in the processing units and flow system preceding the sensor.

Type of calibration

Any form of dependence of the response upon sample size may be used for ordinary analytical work, provided proper standards are available for calibration. The calibration may be either a linear plot that passes through the origin or not, or it may be non-linear. In continuous analysis, however, calibration with a single standard and direct readout of unknown samples is required so that a straight line calibration plot that passes through the origin is necessary. Plots that are linear but do not pass through the origin may still be used after automatic correction of the blank. However, when a non-linear dependence of response upon sample size exists, calibration with a single standard and direct readout is no longer possible. Calibration must be made with several standards and either a working curve must be used (which considerably cuts down the efficiency of the method) or an appropriate computation device must be employed to convert the response into concentration in which case it becomes doubtful that the excercise is worthwhile at all. In practice therefore, a linear dependence of response upon sample size is desirable for continuous analysis.

Sensitivity, accuracy and precision

In principle, only enough sample to fill the sensor would be needed for measurement, in continuous analysis; in practice, however, the sample has to flow long enough past the sensor to obtain a steady state response or a reproducible fraction of the steady state response. Only a fraction of the sample is therefore actually utilised for measurement and this waste of sample therefore corresponds to a loss of sensitivity. In order to reduce this wastefulness of sample and hence the decreased sensitivity, it is necessary to decrease t_s (time in sample) to a minimum, due considerations being given to the response time of the sensor (that is, the minimum time required for the sensor to detect any changes in sample concentration), the accuracy with which % SS (the percentage of steady state reading) may be reproduced (page 24) in any given time t_s and the timing of sampling that can introduce errors if t_s is small (page 26). It is in order to compensate for this waste of sample that continuous sensor units are designed for low hold-up, and the sensors themselves are chosen to be of a high sensitivity type so that a large volume of sample of very low concentration may be used for flushing past the sensor while still conserving the absolute amount of sought-for substance needed for measurement.

The accuracy and precision of continuous flow analytical methods are, in general, governed by two factors, namely, interaction between adjacent samples and drift.[14] The definition (page 21), source (page 25), and quantitative measurement of interaction from first principles (page 21) and from the half-wash principle (page 25.) have already been described. It has also been mentioned that interaction may be reduced by interposing water between samples. It is also obvious that the effect of interaction may be corrected by determining the percentage interaction which is then accounted for in the analytical result. Drift is a much more serious inherent problem in continuous analytical methods than in other analytical methods in general, since the precision of the former depends on the constancy of fractional completion of a reaction. The characteristic result of drift is that a given set of samples analyzed at one time will bear the same relationship to each other in their concentrations as when analyzed at a different time, but the two sets of answers will be significantly different. Evaluation of drift may be made by including known drift-control standards at frequent intervals throughout the operation of the instrument. The apparent change in concentration of these standards is a measure of instrumental drift. A calibration curve constructed at any one time must therefore always be altered to account for the drift existing at that particular time. This is done by arbitrarily dividing the sample into groups of samples nearest to each drift-control standard. The height of the peak for the drift-control standard for a given group is taken as the elevation of the calibration curve at that concentration at that time, and the calibration curve as originally constructed is shifted upwards or downward (parallel to the original curve) so as to pass through the correct value for the drift control standard in question.

A less obvious source of error in continuous analysis is the dependence of the analytical results on the depth of sample in the sample cups. Thiers and Oglesby[14] have recommended to use approximately similar volumes of samples in the sample cups for maximum precision and in amounts well in excess of the volume required When smaller amounts must be used, the volumes employed for standards and samples become critical and should be identical. The effect of sample depth on the analytical result will be more pronounced if the time of sampling (t_s) is small as has been discussed.

Recent developments in continuous analysis

An account of the recent developments in continuous analysis will be given here, but it shall, of necessity, be concerned only with those developments which involve the use or introduction of a principle new to continuous analysis either in instrumentation or in chemistry; other numerous works that concern with the improvements on existing principles shall however have to be omitted due to space limitations.

Kinetic methods of analysis

Kinetic method of analysis is one of the established analytical techniques where the concentration of a desired component is measured using principles of chemical kinetics. Analytical procedures based on the measurement of the rate of enzyme catalyzed reactions, for example, show great promise and are being developed rapidly. The concentration of a sought-for substance is found by measurement of its initial rate of reaction with other reagents that are all at controlled concentrations. Under such conditions, the sought-for concentration is proportional to the initial reaction rate. It is quite obvious that the use of the principle of continuous analysis where the sample is analysed in a flowing condition is ideally suited for kinetic methods of analysis. Suitable sensors may be placed at appropriate points along the solution path which detect changes in concentration as the sample moves forward, that is, with time. This is proportional to the reaction rate and hence to the concentration of the desired component in the sample. Automation becomes very convenient and direct readout of the answer is readily achieved. Instrumentation for continuous analysis by measurement of reaction rate has been designed in which the measurement system employed is either differential filter photometry[16] or differential amperometry.[17] In the latter case the instrument described measures continuously the rate of a chemical reaction, provided it is accompanied by a change in concentration of an electroactive species. A sample stream and a reagent stream containing all substances necessary for the reaction are pumped, each at a constant rate and are mixed for reaction to begin. The resultant reacting stream flows through an induction delay line, to permit any induction periods to pass if such exists. The stream then flows through an upstream tubular platinum electrode, an interelectrode delay line, a downstream tubular platinum electrode, and finally to waste. The interelectrode delay line is chosen long enough to produce sufficient electroactive material for accurate measurement. The difference in concentration of electroactive substance at the two electrodes is found by a differential amperometric measurement at a fixed applied potential. Since the pumping rate is constant and since the electrodes are at a fixed distance apart on the reacting stream, the measured differential current is proportional to the difference in concentration of the electroactive substance at the two electrodes, and is therefore also proportional to the reaction rate. Sensors and measuring devices used for such measurements should be of a high sensitivity type, so that reaction time required may be made short, and the reaction rate that is measured is then essentially an initial rate, which provides the best condition for linearity between the measured response and the sought-for concentration, which in turn permits direct readout of the result. Performance of the instrument was evaluated by analysing glucose by the well-known glucose oxidase method. In this method glucose is oxidised in the presence of air to gluconic acid and hydrogen peroxide, the reaction being catalyzed by the enzyme glucose oxidase. The hydrogen peroxide itself is not easily amenable for electrochemical detection, but in the presence of peroxidase, it reacts rapidly with ferrocyanide to produce ferricyanide which is amperometrically measured at the tubular platinum electrode. Linear calibration plots were obtained for concentrations of glucose from 0 to 100 ppm and the precision is high.

Isotope derivative method of analysis

The isotope derivative method is specifically for the determination of a substance in amounts too small for use of conventional methods and in the presence of substances too similar for quantitative separation before measurement. The principle of the method is that the sought-for substance is labelled with a radioactive reagent to form a radio-derivative. The interferences also become labelled. Then a known amount of inactive carrier derivative of the sought-for substance is added, and the mixture is processed to recover the purified carrier free of excess reagent and radio-derivatives of the interfering compounds. Measurement of the yield and specific activity of the purified carrier then permit calculation of the sought-for substance in the original sample, providing that the derivative-formation reaction has a reproducible yield and providing that the derivative does not decompose through the processing. This method, in spite of its great analytical potential, has not been widely accepted probably because the derivative forming reaction and purification steps usually involve much effort and time. This again is an ideal situation for introduction of continuous methodology. Thus Blaedel and Evenson[18] had presented a scheme for a continuous isotope derivative procedure for amino acid determination. The sample containing alanine and other amino acids is pumped at a constant rate to merge with a stream of radio-Co(II) of known concentration, also pumped at constant rate. The Co(II) is in excess over the amino acids. The radio-Co(III)-amino acid complexes are formed as the mixed stream passes through an electrolytic oxidizing column. The stream is next merged with another pumped stream of inactive carrier Co(III)-alanine of known concentration. Thereafter, the stream is processed to recover the Co(III)-alanine complex in radiochemically pure form. The chemical concentration as well as the radioactivity of the purified stream are both measured simultaneously, and the outputs of the two sensors are fed into a ratio recorder whose output is proportional to the concentration of alanine in the original sample. Direct readout of the alanine content should be possible after calibration of the equipment with a standard alanine sample.

Sequential multiple analysis

This development, in contrast to the former two, is on the instrumental side rather than on the chemical side of continuous analysis and as such only a brief account will be given. Sequential multiple analysis was first described by Skeggs[19] and his instrument was capable of performing eight tests simultaneously from a single serum sample and recording all eight tests sequentially on a single sheet of recorder paper. This system was later enlarged to twelve tests[12,13] by the Technicon (AutoAnalyzer) Corporation. The manner in which such a system works is a long story of ingenious manipulation of liquid streams, instrument design and electronics which shall not be described; the achievement of the system however is tremendous and deserves recognition. The sequential multiple analysis instrument (SMA 12/60, Technicon Corporation) is capable of analyzing for twelve components in a single two-millilitre of untreated sample at a rate of sixty samples per hour. Readout time is only nine minutes and the data, in the form of a serum chemistry profile, is recorded on precalibrated charts which are directly dispatchable to the user of the analytical service without any further computation or clerical steps. High accuracy is achieved by automatic blank correction and recording of results at steady-state levels.

Advantages and disadvantages of continuous analysis

The advantages of a continuous analysis system may be summarised as follows:

(a) Ease of automation.

Automation is relatively easy in continuous analysis, since the instrumental approach is such that a variation in signal is detected which can be conveniently amplified to actuate valves, compressors, pumps, heaters etc. in a chemical process system to regulate the conditions necessary for correct operation of the process.

(b) Reproducibility of "time".

Another great advantage associated with continuous analysis is the accurate reproducibility of "time" which allows the use of slow reactions and processes without the necessity to bring them to completion. Such reactions and processes are not adaptable to manual techniques where accurate reproduction of "time" is difficult, and therefore the reactions or processes involved must be made to go to completion which would take an unreasonably long time or may not even reach 100% completion at all. Hence many useful metastable processes such as hydrolysis, digestion, dialysis, solvent extraction etc. where 100% reaction rarely occurs within a reasonable period, are easily adaptable to continuous analysis.

(c) Sample handling.

All problems associated with the handling and transport of certain samples are eliminated in continuous analysis. Sampling of gas streams, handling of samples that can undergo change under the influence of air or sunlight, that are toxic, corrosive or otherwise dangerous or unpleasant are some of the problems that are easily overcome by continuous methodology.

(d) Speed of analysis.

The advantages of speed are quite obvious. Readout time in continuous analysis is usually in the order of a few minutes in contrast to several hours or even days by manual techniques. Considerable savings of money are therefore involved in large-scale production lines when a faulty product or process is detected by a rapid and dynamic analysis rather than a historic one. In the field of clinical analysis, also, such dynamic analyses allow diagnosis and treatment of cases on the basis of up-to-date analytical data rather than on analysis results of samples that existed in the patient some considerable length of time ago. The high frequency of analysis possible with continuous analysis also has many advantages. Frequent analysis allows production processes to operate closer to safety limits, and also has a tremendous impact in the field of preventive medicine.

(e) Minimising error.

The isolation from human intervention that characterises continuous analytical systems eliminates all sources of human errors. In principle, if a continuous analytical system can be made to indicate correctly for a standard, then it will indicate equally correctly for the sample.

The only disadvantage inherent in continuous analytical systems is the fact that only a fraction of the sample introduced into the system is actually utilised for measurement, the remainder being merely necessary to flow past the sensor long enough for the latter to reach a steady state or a reproducible fraction of the steady state. This waste of sample, therefore, corresponds to a loss of sensitivity.

Complexometry, polarography and continuous analysis

In order to extend the applicability of continuous analysis to much wider fields, an attempt has been made in this thesis to employ the classical analytical techniques of complexometry and polarography (voltammetry, in general), individually as well as in combination to continuous analysis systems. Such a course obviously holds many possibilities since the old and well-established method of complexometry is very versatile and still irreplaceable, and since the use of electrochemical sensors in continuous analysis, yet at an infant stage compared to other sensors such as optical sensors, offers all the advantages of electrochemical sensing, including the use of such latest developments in electroanalytical chemistry as cyclic and pulse techniques[20], anodic and cathodic stripping analysis[21] and so on, aimed at increasing sensitivity, selectivity etc.

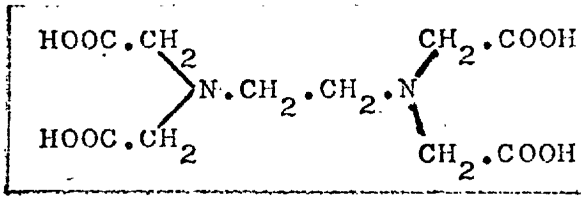
SECTION I

COMPLEXOMETRIC METHODS OF ANALYSIS

SECTION I

INTRODUCTION

Complexometry may be defined as those analytical procedures employing a group of compounds, named by Schwarzenbach as "complexones". These are aminopolycarbonylic acids, the most prominent member of which is ethylenediaminetetraacetic acid (EDTA)



the anion of which can act as a quinque - or sexi-dentate ligand by coordination to a metal ion through its two nitrogen atoms and three or four of the carbonylic acid groups, to form an octahedral complex which is greatly stabilised by the number of five-membered chelate ring it contains. This structure is also favourable to the preferred stereochemistry of most of the common metal ions. Even though certain aminopolycarboxylic acids (including EDTA) had been marketed since the 1930's, and metal complexes of some of these acids characterized in the early 1940's, it was only in 1945 that Schwarzenbach pointed out the analytical potentialities of these "complexones" by reporting the indirect determination of metal ions via the pH effects displayed on addition of an EDTA salt to the metal solution. Next he pointed out the use of metal sensitive indicators in analogy with acid-base indicators, which initiated the development of a whole series of metallochromic indicators and complexometric titrations.

The properties of EDTA that make it such a successful reagent had been traced by West[22] to:

(1) Formation of 1:1 complexes with almost all cations under suitable pH conditions, in contrast to ligands which form multiple complexes with metal ions so that the stoichciometry of their reaction is difficult to control for titrimetric purposes.

(2) EDTA also forms complexes of high stability so that sharp changes in pM values occur at end-points

(3) The complexes are so stable that the presence of many other complexing agents can be tolerated in solution without any adverse effects.

(4) Since EDTA is a tetrabasic acid possessing "strong" and "weak" protons, selectivity of reaction is possible by pH control, and

(5) EDTA complexes are soluble so that co-precipitation problems do not arise and EDTA is not a chromogenic reagent, although it does intensify pre-existing colour.

Complexometry, apparently, is very unselective in nature because of the high affinity of EDTA for most cations. However this is easily overcome by the use of "masking agents", this term has been defined by I.U.P.A.C. as "a substance preventing the reaction of one or more foreign substances in a determination by conversion into soluble complexes, different oxidation states or other unreactive forms". The analysis of very complex mixtures have been achieved by an ingenious use of these masking agents and pH control; separation techniques such as solvent extraction, ion-exchange etc. may also be used for further selectivity even though it is rarely necessary.

However problems arise when it comes to the determination of a group of elements with very similar properties, such as those of the same sub-group of the periodic table or the rare earths since, all the elements will react similarly to any masking agent used or pH control. Such a situation is perfectly exemplified by the alkaline earth elements especially magnesium and calcium which occur so closely in nature - either in the earth's crust, in natural waters or the human body. The following chapter deals with this particular problem, that is, of developing a method for determining calcium and magnesium in the presence of each other. The reasons for choosing this particular system are two-fold:

(a) The determination of calcium and magnesium in the presence of each other is basically an important analysis; the reasons for this are very obvious.

(b) The work will form the basis for subsequent studies since the same system (calcium/magnesium) shall be employed in Section II (Use of complexones in polarographic analysis) and Section III (Continuous analysis via complexometry using electrochemical sensors), in which a very clear demonstration of the principles outlined in each is possible with the calcium/magnesium system.

SECTION I

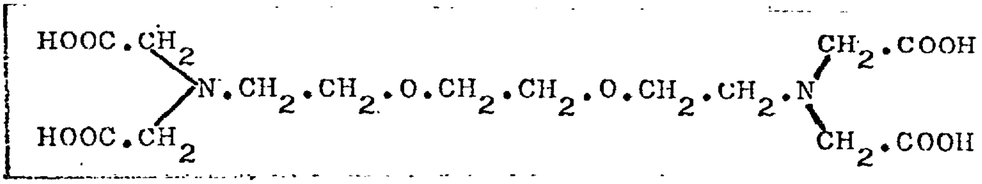
CHAPTER I

A COMPLEXOMETRIC METHOD FOR THE DETERMINATION OF CALCIUM AND MAGNESIUM IN THE PRESENCE OF EACH OTHER

1.1.1. Review of previous works

The determination of calcium and magnesium in the presence of each other had been a classic analytical problem since the beginning of chemistry, which appeared to have been solved by various methods, but only with serious limitations. Before the discovery of complexometric titrations, the commonest method involves separation of the calcium by precipitation as the oxalate with either a gravimetric or titrimetric follow-up. In addition to being time-consuming and tedious the accuracy of the method is seriously affected by problems of coprecipitation. The speed of analysis was greatly enhanced with the introduction of complexometric titrations where physical separation of calcium and magnesium was no longer necessary. The commonest procedure involves titration of the sample first for the sum of calcium and magnesium with EDTA in an ammonia buffer (pH 10) using Eriochrome Black T as indicator. Next, in a second aliquot of the sample, magnesium is precipitated as the hydroxide at a pH of approximately 13 and the calcium alone is then titrated with EDTA using murexide as indicator [23, 24]. The use of other indicators had also been suggested. The obvious disadvantage of the method is the coprecipitation of calcium with the magnesium hydroxide, especially at high Mg : Ca ratios. Various procedures had been proposed for eliminating this source of error. Thus a back titration procedure may be used in which EDTA is added in excess over the amount of calcium present and then precipitating the magnesium and back-titrating the excess of EDTA. Lott and Cheng[25] reported decreasing co-precipitation of calcium by the addition of polyvinyl alcohol. Burg and Conaghan reported the same effect with acetylacetone[26]. An additional problem caused by the hydroxide precipitation is the adsorption of the indicator. Belcher, Close and West[27] studied this in detail and here also the addition of polyvinyl alcohol or acetylacetone serves as an effective remedy[25, 26]. The precipitate is still considered a potential source of trouble, in spite of these improvements, and Tichomorova and Simackova[28] avoided this by titrating calcium at a high pH and still preventing the magnesium precipitation by complexing with tartrate. High results for calcium are however obtained[26,29], indicating co-titration of magnesium.

A turning point in the history of the titrimetric analysis of calcium and magnesium occured when Schwarzenbach[30] introduced a new complexone - ethylene glycol bis-(β-aminoethyl ether)-N,N^1-tetraacetic acid (EGTA),



$$\left[\begin{matrix}HOOC.CH_2\\ HOOC.CH_2\end{matrix}>N.CH_2.CH_2.O.CH_2.CH_2.O.CH_2.CH_2.N<\begin{matrix}CH_2.COOH\\ CH_2.COOH\end{matrix}\right.$$

which was reported to have a difference of 5.8 in the log $K_{stab.}$ of calcium and magnesium (log $K_{ca} = 11.0$, log $K_{Mg} = 5.2$) in contrast to only 2.0 with EDTA (log $K_{ca} = 10.7$, log $K_{Mg} = 8.7$). The first application of EGTA as a selective titrant for calcium in the presence of magnesium however, came only two years later, in a paper by Reilley[31] in 1957. The use of EGTA as a selective titrant was handicapped by the lack of a similarly selective indicator, however Reilley was able to get around this problem by the employment of potentiometric end-point detection. A series of papers then follow in quick succession which reported indirect visual end-point detection systems employing either zinc sensitised zincon[32,33] or PAN[34] indicators. Methods with photometric[29], fluorometric[35] and amperometric[36] end point detections have also been reported. The disadvantage in all these methods is that since the titration is carried out in the presence of unmasked magnesium, titration even with a fairly selective titrant is bound to give unreliable results especially at high magnesium concentrations. A different approach by Pribil[37] is to mask the calcium with a small measured excess of EGTA and titrate the magnesium with CDTA using Methylthymol Blue indicator. The calcium is determined by back-titrating the excess of EGTA with calcium using a fluorescent indicator, fluorexone. The end points are however, found to be poor.

1.1.2 Principle of the proposed method

The principle of the method involves an exchange reaction,

$$\mathrm{Ca} + \mathrm{MZ} + n\mathrm{A} \rightleftharpoons \mathrm{MA}_n + \mathrm{CaZ} \qquad (1)$$

(charges omitted for simplicity) where M represents a metal ion to be displaced, such as Zn or Cd, Z represents EGTA and A is an auxiliary complexing agent such as ammonia which suppresses the apparent stability of MZ and facilitates reaction towards the right. Solution conditions are such that participation in the above exchange reaction by magnesium is negligible; the latter is suitably masked and the liberated MA_n titrated complexometrically to give the concentration of calcium alone in the mixture. The concentration of magnesium can be obtained by difference after determining the total concentration of calcium and magnesium in a separate aliquot by the usual EDTA titration.

1.1.3. Theory

Nagakawa and Tanaka[38,39] derived the following relation for exchange reactions such as equation (1)

$$[Ca]_t = \left([MA_4] - \frac{[M]_t - [MA_4]}{[MA_4]} \cdot K_2 [A]^4 \right)\left(1 + \frac{[MA_4]}{[M_t] - [MA_4]} \frac{1}{K_1 [A]^4}\right) \qquad (2)$$

where K_1 is the equilibrium constant of the exchange reaction (1) and K_2 that of the following reaction,

$$MZ + 4A = MA_4 + Z$$

i.e. $$K_1 = \frac{[CaZ]\,[MA_4]}{[MZ]\,[Ca][A]^4} = \frac{K_{caZ}\,K_{MA4}}{K_{MZ}}$$

and $$K_2 = \frac{[MA_4]\,[Z]}{[MZ]\,[A]^4} = \frac{K_{MA}\,\alpha_{H(Z)}}{K_{My}}$$

All terms have their usual meaning in complexometry. In (2) the term $\frac{[M]_t - [MA_4]}{[MA_4]} \cdot K_2\,[A]^4$ may be neglected in comparison with $[MA_4]$ if $K_2\,[A]^4$ is sufficiently low.

Thus $$[Ca]_t = [MA_4]\,\left(1 + \frac{[MA_4]}{[M]_t - [MA_4]} - \frac{1}{K_1\,[A]^4}\right) \qquad (3)$$

The second term in parentheses of (3) may be neglected provided $K_1\,A^4$ is sufficiently high or $\frac{[MA_4]}{[M]_t - [MA_4]}$ is sufficiently low, and then

$$[Ca]_t = [MA_4] \qquad (4)$$

In the range where (4) holds, therefore, calcium can be successfully determined by titration of MA_4. A plot of $[MA_4]/[M]_t$ against $[Ca]_t/[M]_t$ for various values of $K_1[A]^4$ shows that the higher the value of $K_1[A]^4$ is, the wider is the range where (4) holds, i.e. $[Ca]_t$ is proportional to $[MA_4]$ to a value of $[Ca]_t/[M]_t$ more close to unity $K_1[A]^4$ should be more than 10^2 in order to apply (4) to a point $[Ca]_t/[M]_t = 0.5$.

The same considerations for the case of magnesium yields:

$$[Mg]_t = [MA_4]\left(1 + \frac{[MA_4]}{[M]_t - [MA_4]}\;\frac{1}{K_1^1\,[A]^4}\right)$$

where K_1^1 is the magnesium exchange reaction similar to (1). A plot of $[MA_4]/[M]_t$ against $[Mg]_t/[M]_t$ for various values of $K_1^1[A]^4$ shows that for sufficiently low values of $K_1^1[A]^4$ ($<10^{-3}$), the amount of MA_4 liberated by magnesium is very small and can be neglected. For a selective determination of calcium in the presence of magnesium, $K_1[A]^4$ should be sufficiently high and $K_1^1[A]^4$ should be sufficiently low. Since $K_1[A]^4/K_1^1[A]^4 = K_{CaZ}/K_{MgZ}$ a complexone should be chosen such that the difference in stabilities between its calcium and magnesium chelates is large. K_{CaEGTA}/K_{MgEGTA} ($10^{5.8}$) is much greater than K_{CaEDTA}/K_{MgEDTA} ($10^{2.6}$) and one can realise $K_1[A]^4$ higher than 10^2 and $K_1^1[A]^4$ less than 10^{-3} in an exchange equilibrium in which EGTA participates.

1.1.4 Preliminary experiments and studies

(a) Completeness and spontaneity of exchange reaction

The completeness and spontaneity of the exchange reaction,

$$Ca^{2+} + Zn\text{-}EGTA^{2-} + 4NH_3 \rightleftharpoons Ca\text{-}EGTA^{2-} + Zn(NH_3)_4^{2+}$$

towards the right hand side was tested by measuring the concentration of liberated $Zn(NH_3)_4^{2+}$, as determined by a complexometric titration with EGTA. The reaction was carried out in an ammonia buffer of pH9.6± 0.1 and concentrations of ammonia in the range 0.1 to 0.5 F; a 50% excess of Zn-EGTA is employed. Recovery of $Zn(NH_3)_4^{2+}$ is in all cases close to 100%. within the limits of experimental error, and the exchange reaction is instananeous, 100% recovery being attained at once without any further change on allowing to stand longer.

(b) Selection of titrants for $Zn(NH_3)_4^{2+}$ liberated by the exchange reaction

The titrant selected for $Zn(NH_3)_4^{2+}$ must satisfy certain requirements, for example, it must not react or interfere with any of the components of the exchange reaction except $Zn(NH_3)_4^{2+}$ itself. Of the three titrants studied, namely EDTA, CDTA and EGTA, EDTA and CDTA are entirely unsatisfactory presumably due to slow ligand exchange with the exchange reaction components such as the excess of Zn-EGTA or Ca-EGTA and very high results and diffuse end points are obtained. Titration with EGTA, however, gives excellent end points and accurate results.

(c) Selection of indicator

Only a few metallochromic indicators are available for the titration of zinc in ammoniacal solution at about pH 10; the most common are Eriochrome Black T, Pyrocatechol Violet and Zincon. Of these pyrocatechol violet is not very satisfactory since the colour change at the end point is from blue (metal-indicator colour) to violet (free indicator colour) which is not readily detectable. Zincon has been chosen in preference to Eriochrome Black T because of its higher selectivity. Zincon, the trivial name for O-[2-(2-hydroxy-5-sulfophenylazo)-benzylidene hydrazino]-benzoic acid, was first introduced by Rush and Yoe[40] as a zinc reagent for photometric analysis. Kinnunen and Merikant[41] showed that it is also an excellent indicator for the complexometric titration of zinc as it gives a sharp change from deep blue to orange-yellow. Zincon (the monosodium salt) has three acid hydrogens : a carboxylic hydrogen, a phenolic hydrogen and a secondary amine hydrogen and at pH10, the HIn^{3-} is the dominant species[33],

$$\begin{array}{ccccccc} H_3In^- & \rightleftharpoons & H_2In^{2-} & \rightleftharpoons & HIn^{3-} & \rightleftharpoons & In^{4-} \\ \text{Pink} & & \text{Yellow} & & \text{Orange-yellow} & & \text{Violet} \end{array}$$

(d) Masking of magnesium

The problem here is to find a masking agent for magnesium to mask it from being cotitrated even slightly with the zinc by EGTA. The reagent employed must not mask the zinc. The selection of a masking agent for magnesium is complicated by the fact that most of the conventional masking agents for magnesium also form insoluble zinc salts. It is a well-known fact that the alkaline earth metals can be masked by fluoride ions when zinc, cadmium, nickel or cobalt are to be titrated.[42] The stability constants for the fluorides of magnesium and zinc are $10^{5.19}$, $10^{1.26}$ respectively and the zinc salts are soluble. Fluoride ions therefore appear to be suitable.

However, the masking of magnesium by as much as twenty times excess of fluoride in an ammonia buffer medium of about pH 10 was found to be incomplete and free magnesium ions can be detected in the solution. It has been found that a 150 times excess of fluoride ions would mask the magnesium completely but such a high fluoride concentration is problematic as shall be obvious later. This difficulty was quite easily overcome, however, by carrying out the masking in a less polar medium by making the solution about 25% ethanolic since according to the Born equation, decreasing the dielectric constant, decreases the solvation energy and hence also the solubility. Some evidence of a slow interaction between the Ca-EGTA present in the solution and fluoride ions is found, but this is solved by optimising the concentration of fluoride as shall be described later. No such interaction with Zn-EGTA is however likely in view of the stability constants (Zn-EGTA = $10^{14.5}$, Zn-Fluoride complex = $10^{1.26}$) and this is confirmed experimentally. It is also established that the presence of up to 0.2M fluoride ions and up to 40% ethanol in the solution does not affect the titration of zinc with EGTA, at least in so far as, the sharpness of the end point colour change and accuracy are concerned.

1.1.5 Development of the method

Optimisation of conditions :

(a) Composition and concentration of buffer

Three ammonia buffers were studied, namely ammonia/ammonium chloride, ammonia/ammonium nitrate and ammonia/ammonium acetate. As already stated above a slow interaction between the calcium complexonate of EGTA and fluoride ions exist in the solution. The interaction is a nucleophilic substitution reaction and the ultimate reaction may be written as,

$$\text{Ca-EGTA} + n\text{F} \rightleftharpoons \text{CaF}_n + \text{EGTA}$$

omitting charges for simplicity. The rate of this reaction is found to be dependent on the concentration of the fluoride ions and therefore rules out the S_N1 mechanism. The substitution possibly follows a "concerted" mechanism in between the two extremes S_N1 and S_N2. The reaction is probably similar to the substitution of Ni-EDTA complex with cyanide ions in which a mechanism involving mixed nickel-EDTA-cyanide complexes had been shown to be very likely.43

The kinetics of the substitution will be considerably influenced by the presence of other complexing species in the solution. Of the anions of the three buffers studied, namely, chloride, nitrate and acetate, the acetate has the strongest complexing ability and can compete very favourably with the fluoride ions for the calcium. Steric factors also do not upset this consideration. The solution when swamped with acetate ions will therefore have the effect of forming a protective shield around the Ca-EGTA complex with acetate ions, thus hindering the approach of fluoride ions.

This prediction had actually been confirmed by experiments in which it was found that in chloride and nitrate buffers, the concentration of fluoride ions that can be tolerated is extremely small. For example in an ammonia/ammonium chloride medium when a solution containing 10 milligrams of calcium (0.25 millimole), after being allowed to undergo the exchange reaction with zinc-EGTA complexonate is titrated with EGTA in the presence of fluoride ions, it was found that only about 5 milligrams of fluoride (0.25 millimole) can be tolerated. At higher concentrations of fluoride, appreciable interaction between the calcium-EGTA complexonate and fluoride ions occur, as detected by an early and drawn-out end point as well as turbidity in the solution at high concentrations of calcium and fluoride.

The situation is much improved in the case of the ammonia/ammonium acetate buffer, where for a similar titration of 10 milligrams of calcium, up to about 100 milligrams of fluoride can be easily tolerated. The end points are sharp and accurate indicating that interaction between calcium-EGTA complexonate and fluoride ions is negligible. The ammonia/ammonium acetate buffer system was therefore chosen.

A compromise had to be made in optimising the concentration of buffer since for the exchange reaction a high ammonia concentration is preferred in order that $K'_{ZnEGTA} < K'_{CaEGTA}$, whereas for the titration a low ammonia concentration is preferred so that $K'_{ZnEGTA} \gg K'_{MgEGTA}$ where K' represents the apparent stability constants. Variation of the buffer concentration from about 0.1 to 0.4 F ammonia shows the optimum ammonia concentration for exchange and titration to be in the range 0.2 to 0.3 F, i.e. 3 to 5 ml. of <u>ca.</u> 3F NH_3/1F $NH_4.OOC.CH_3$ in 50 ml of solution.

(b) Concentration of fluoride.

Enough fluoride ions must be present in solution to ensure complete masking of magnesium, but at the same time they must not be present in such an excess as to cause interaction with the calcium-complexonate even in an acetate buffer medium. Consideration was given to the use of ions with high affinity for fluoride ions, to remove the excess of fluoride An element like boron may be used without itself introducing interference in the EGTA titration, however the idea was written off because of the competition that would occur for the fluoride ions by the magnesium ions on the one hand and fluoride attracting boron on the other with hindering or possible prevention of the original masking reaction. Equilibrium constants for the formation of BF_4^- from boric acid (H_3BO_3) and fluoride ion is $10^{20.0}$ and of MgF_2 is $10^{8.9}$ It appears best therefore to optimise the concentration of fluoride used. For this reason the tolerable limits of fluoride concentrations were determined for various amounts of magnesium and for various ratios of calcium to magnesium in the sample. The results are given in Figure 1.1.1. in which the tolerable excess of fluoride (assuming formation) of MgF_2 is plotted against the amount of magnesium present.

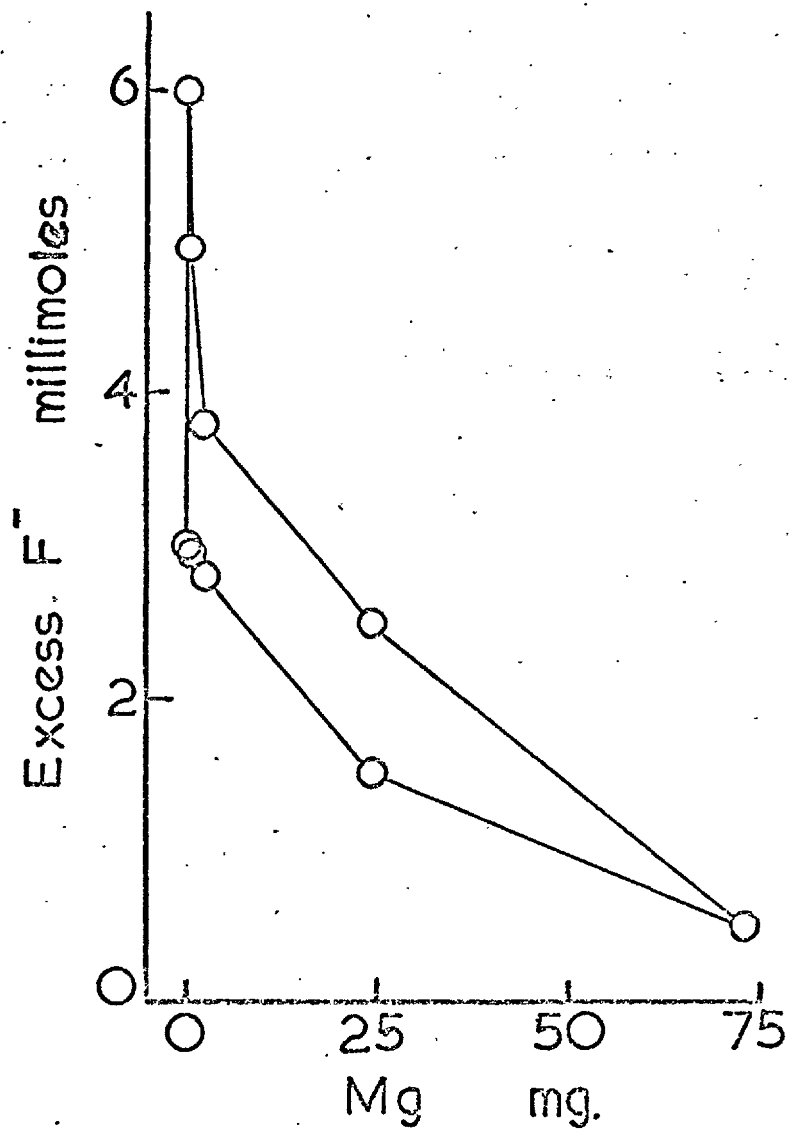


Figure 1.1.1 Permissible ranges of fluoride for masking magnesium. The two curves give the upper and lower limits of fluoride for a given amount of magnesium.

The amount of calcium titrated is in all cases 4 milligrams (0.1 millimole) so that the graph represents molar ratios of calcium to magnesium from 10:1 to 1:30. The two curves give the upper and lower limits of fluoride for a given amount of magnesium; accurate results are obtained in the area inside the curves, negative and positive errors being observed above and below the curves respectively. The range of concentration of fluoride that can be used without introduction of serious error, is thus quite wide at low magnesium concentrations, which gradually diminishes and disappears altogether at and beyond 75 milligrams of magnesium (1:30 molar ratio of Ca:Mg if 4 milligram of calcium is being titrated). Beyond this point therefore the concentration of fluoride to be used must be carefully controlled. However the ratio of calcium to magnesium that will be encountered in practice will rarely be as low as this and the choice of the appropriate fluoride concentration is a fairly simple matter.

(c) Concentration of Zn-EGTA

Concentrations of Zn-EGTA from 20% to 300% excess over the calcium present were tested and it was found that the exchange is already complete at 20% excess and no side effects such as slow interaction with other specied and drawn-out end points are found to be introduced by the use of a very large excess of the zinc complexonate. There is no limitation at all, therefore, to the amount of Zn-EGTA used so long as it is present in excess.

(d) pH of solution

The pH of the solution should be such that the most stable Ca-EGTA is formed; and no precipitation of zinc occurs. The dependence of the apparent stability of Zn-EGTA with pH is immaterial since in any case it will be strongly influenced by the auxiliary complexing agent, ammonia. A search in the literature[32] reveals that the apparent stability constant of Ca-EGTA complexonate increases linearly with pH from about pH 4 to 9.5 when it levels off and a limiting value of $10^{11.0}$ is attained. The optimum working pH range is found to be between 9.5 and 10, below which the stability of Ca-EGTA decreases rapidly and above which zinc hydroxide starts to precipitate in weak ammonia solutions.

(e) Concentration of ethanol

The concentration of ethanol employed has to be optimised since high ethanolic concentrations affect the colour system of the indicator. Ethanolic content of up to 40% has no effect but above this value, the colour of the free zincon indicator at pH ca. 10 in an ammonia/ammonium acetate buffer is pink instead of orange/yellow, which appears to be due to an effect on the acid dissociation of the indicator. The recognition of end-point colour change is thus effected since the change from blue to orange/yellow is a better contrast than the change to pink. The masking of magnesium by fluoride in amounts as recommended earlier is found to be efficient in 15 to 40% ethanolic solutions, and an ethanol concentration of 25% is in general recommended.

(f) Order of addition of reagents

This follows the logical sequence of allowing the exchange reaction to take place first in the ammonia/ammonium acetate buffer medium and only then the masking of magnesium by fluoride is carried out in a 25% ethanolic medium. The order can neither be reversed nor allowed to take place simultaneously such as by using a composite buffer containing all necessary reagents in one solution, since the calcium would then react with the fluoride first and kinetics of the exchange reaction will be jeopardized.

(g) Time of standing

The exchange reaction has been shown to be instantaneous and complete and to be independent of time. The masking of magnesium by fluoride ions in a 25% ethanolic medium is likewise spontaneous and time independent. However, a slow process of interaction between the calcium-EGTA complexonate and fluoride ions exists in the solution and can introduce errors if allowed to take place long enough. Thus it was found that accurate results (100% recovery of calcium) are obtainable up to a standing time after fluroide ion addition of five minutes; if allowed to stand for fifteen minutes only an 85% recovery is obtained and after twelve hours the recovery is 80%. The free EGTA complexone, produced by the interaction between the calcium-EGTA complexonate and fluoride ions, reacts with the zinc ammine liberated by the exchange reaction thus reducing the volume of EGTA titrant required. Hence the negative errors introduced with time. Titration of the solution should therefore start soon after (within two minutes) the addition of fluoride ions.

(h) Primary standards

Since the final step in the method is the titration of zinc with EGTA, the EGTA could have been standardised using a standard solution of zinc as the primary standard. However it is desirable that the sample and the standard go through the same reactions and steps in order that any errors may be compensated. A standard solution of calcium is therefore recommended as the primary standard. The standard calcium solution is allowed to undergo the same exchange reaction as the sample so that any error such as due to a slight difference from stoichimetric proportions of the zinc-EGTA complexonate are eliminated. The titration is also carried out in a 25% ethanolic solution so that identical solution conditions, ionic strength etc., are maintained and **an identical** colour change shall be obtained as with the sample.

1.1.6 Study of interferences

At a pH of 10 where the analyses are performed many metal ions are precipitated. These include ions such as lead, bismuth, antimony, titanium, zirconium, thorium, rare earths, berylium, niobium, tantalum, gallium, indium etc. Fluoride ions would mask a number of elements such as selenium, tin, vanadium and tungsten in addition to some of those mentioned above. Most common masking agents may be employed so long as they do not mask the zinc. A study on the tolerance of some possible interfering ions without the use of any masking agents was made and the results are shown in Table 1.

The most serious interference comes from the heavy metal ions, which are found to block the indicator as in the case of Eriochrome Black T even though it is less well-known. A rare hint of blockage of zincon indicator is given in a paper[41] on a back titration procedure for titration of these metals using zincon indicator even though these metal ions are known to give coloured complexes with zincon[40].

1.1.7 Experimental

(a) Reagent and solutions:

Ethyleneglycol bis (B-aminoethyl ether)-N,N,N'N'-tetra-acetic acid, 0.01M : Dissolve 7.6 grams of the acid in 10 ml of 4N sodium hydroxide and dilute to

Table 1 Summary of interference data.

Foreign ion	Interference, Remarks
Fe^{3+}	No interference up to 10 ppm
Pb^{2+}	- do -
Mn^{2+}	- do -
Al^{3+}	- do -
PO_4^{3-}	- do -
SO_4^{2-}	No interference yet up to 50,000 ppm.
Cu^{2+}	Blocks indicator above 0.5 ppm
Co^{2+}	Blocks indicator above 1.0 ppm
Ni^{2+}	Blocks indicator above 2.0 ppm.

2 litres. Warm if necessary. Standardize with standard calcium solution (see Procedure).

Calcium solutions (0.1M and 0.01M) : Dissolve 5.0045 grams of pure calcium carbonate in hydrochloric acid and dilute the solution to 500 ml (0.1M). Prepare the 0.01M solution by dilution. Use analytical grade reagents.

Magnesium solutions (0.1M and 0.01M) : Dissolve 12.325 grams of analytical reagent grade magnesium sulphate heptahydrate, in distilled water and dilute to 500 ml (0.1M). Prepare the 0.01M solution by dilution.

Ammonia/ammonium acetate buffer pH 10.3 : Dissolve 50.5 grams of ammonium acetate in 300 ml of water and add concentrated ammonia. Adjust the pH to exact value with ammonia and dilute to 500 ml. The composition of the buffer approximates 3.6F ammonia and 1.3 F ammonium acetate. Use analytical grade reagents.

Buffered Zn-EGTA complexonate 0.05 M : Prepare by mixing equivalent amounts (determined by titration using zincon as indicator) of 0.2 M zinc sulphate and 0.2 M EGTA and add an equal volume of buffer solution. The Zn-EGTA complex prepared without any buffer is unstable and the complex crystallizes out in a few hours. The solution is however, stabilised by the ammonia buffer.

Ammonium fluoride 1.0M : Dissolve 18.52 grams of ammonium fluoride (analytical grade) in distilled water and dilute to 500 ml.

<u>Zincon indicator solution</u> : Dissolve 0.013 gram of Zincon i.e. 0.[2-(2-hydroxy-5-sulphophenylazo)-benzylidene hydrazino]-benzoic acid in 0.5 ml of 4N sodium hydroxide, dilute to 10 ml, and store in a refrigerator. The solution is stable for approximately one week.

(b) <u>Procedure</u>

To 25-50 ml of the sample solution, containing 4-12 mg of calcium and o-15 mg of magnesium add 8 ml of the buffered zinc complex. Shake the solution and add 3.5 ml of 1M fluoride solution. (The volume of fluoride solution needed can also be found from Figure 1.1.1.). Add enough absolute alcohol to give a 25% v/v solution of it, and then 10 drops of indicator. About two minutes after the addition of the fluoride, titrate the solution with standard EGTA till the colour changes from deep blue to orange.

The concentration of magnesium may be obtained by difference after determining the total concentration of calcium and magnesium by the usual EDTA titration using Eriochrome Black T indicator.

(c) <u>Standardization of EGTA solution</u>

To 25.00 ml of 0.01M calcium solution, add 30 ml of water and 8 ml of the buffered zinc complex. Shake the solution and add ethanol (to give 25% v/v) followed by 10 drops of Zincon indicator. Titrate with EGTA from the blue to orange end-point.

1.1.8 Results and discussion

A representative selection of results obtained is shown in Table 2

Table 2

Calcium Taken (mg)	Calcium Found (mg)	Difference (mg)	Mg : Ca ratio
2.00	2.01	+0.01	1 : 1
4.01	4.10	+0.09	1 : 2
8.02	8.18	+0.16	1 : 4
12.02	12.17	+0.15	1 : 6
4.01	4.00	-0.01	1 :10
4.01	4.10	+0.09	1 : 2
4.01	4.03	+0.02	1 : 1
4.01	3.98	-0.03	2 : 1
4.01	3.94	-0.07	5 : 1
4.01	3.93	-0.08	10 : 1

The above results show that the presence of magnesium in different amounts and in various concentration ratios to calcium, do not have any significant effect on the method. No methodical errors can be detected. The accuracy of the results obtained are good and the precision is also high. In Table 2 the results given are the mean value of from 3 to 9 determinations on each sample; the standard deviation ranges from about 1 to 5 parts per thousand and the confidence interval at 95% confidence level amounts to plus or minus only a few hundreths of a milligram of the average values.

The proposed method is of general applicability for the analysis of calcium and magnesium in any sample, provided that before the method is applied the sample is suitably processed so as to separate substances present in matrix quantities such as iron in steel and suitable masking agents incorporated to mask other possible interfering ions present in lesser quantities. The method is especially suitable for the analysis of natural waters, since little or no pretreatment of the sample is necessary. Ions, other than calcium and magnesium, are present only in amounts which are well within the tolerance of the method as described in the study of interferences (Section1.1.6, Table 1).

Other alkaline earth metals (strontium and barium) if present, will be cotitrated with calcium, as with all other methods, since the ratio of the equilibrium constants of the calcium exchange reaction (K_{1Ca}) to the interfering exchange reaction (K_{1Sr} or K_{1Ba}) is not as favourable as with magnesium. Strontium and barium may be masked however with sulphate ions admittedly with the possible risk of loss of some calcium at high sulphate concentration.

An aspect of the outcome of the proposed method which had not been explored, is the possibility of removing the complex anions, Ca-EGTA^{2-} and the excess of Zn-EGTA^{2-} from the residual solution after the exchange reaction, by passing it through an anion exchange column, for example in the chloride form, so that the resulting solution only contains zinc, magnesium and foreign cations which did not undergo the exchange reaction, Analysis may now be completed with less complications since no consideration need longer be given to the presence of the complex anions. For example, an analysis for both may be achieved by a simple pH control, i.e. titrating zinc at pH _ca_. 7 and then titrating the magnesium in the same solution at pH _ca_. 10. Such a course would be impossible in the presence of complex anions since Ca-EGTA^{2-} is unstable at the low pH. A large number of other such possibilities exist. This approach would be advantageous in the analysis of complex samples where more manouvreability in manipulating solution conditions is desired; however it must be remembered that all this is achieved at the large expense of simplicity and speed of the method.

1.1.9 Conclusion

The proposed method offers a rapid, visual compleximetric procedure for the analysis of calcium in the presence of magnesium. All experimental parameters having been carefully evaluated and optimised, the recommended procedure is directly applicable as a practical analytical method for the analysis of natural waters and other aqueous samples, and with modifications to other samples. There are at present only two principally different visual compleximetric methods, apart from the proposed method, for the determination of calcium and magnesium, attempting to use the complexone EGTA in order to avoid titration at a high pH where magnesium hydroxide is precipitated. In one, the calcium is masked by a measured slight excess of EGTA, after which the magnesium is titrated with CDTA. Calcium is then determined by back-titrating the excess of EGTA[37]. Even though sound in principle the end points are found to be poor. The second type of methods are characterized by the use of zinc-sensitised indirect indicators[32,33]. The advantage of the proposed method over these methods is that in indirect indicator methods, a more critical, if not unpractical control of some parameters is necessary. For example, the shape of the titration curves are dependent upon magnesium concentration and therefore in order that the end-point detected is the equivalence point in all cases, titrations to different colour changes for different solutions have been recommended. The concentration of zinc ion added is also to be controlled so that colour change should occur at the equivalence point. The critical concentration of zinc ions is again dependent on the magnesium concentration.

Assurance at two separate points in the proposed method, for non-interference by magnesium - viz, in the exchange reaction where reaction conditions are controlled so that participation by magnesium is negligible and in the subsequent titration where the magnesium is siutably masked - is also an added advantage.

SECTION II

USE OF COMPLEXONES IN POLAROGRAPHIC ANALYSIS

SECTION II

INTRODUCTION

Polarography

Polarography, invented in 1922 by Heyrovsky,[44] is a controlled potential technique in which the electrolysis of an electroactive substance occurs at a dropping mercury electrode (DME), where the potential of the DME is constant or varied linearly with time; the electrolysis current is registered against the controlled potential of the DME (Current-potential curve). The DME has the advantage that contamination of the electrode surface by reaction products is avoided as the surface is constantly renewed; it also enables the attainment of high negative potentials due to the large overvoltage of hydrogen on mercury. The major disadvantage is the limited potential range on the positive side. To illustrate the principle of the method, suppose the potential applied to the DME in a polarographic cell containing an aqueous solution of IM potassium chloride is slowly scanned in the negative direction. At first, the cell current at 0V vs. a saturated calomel electrode (SCE) is zero, then rises gradually due to trace impurities and charging of the double-layer capacitance at the mercury-solution interface. This current is called the residual current (i_r). No charge transfer occurs until a potential of -1.8V is reached when the cathodic reduction of the potassium ions occur and a large current flows. If the above solution also contains some cadmium, the cell current rises sharply from that produced by the supporting electrolyte when the reduction potential of cadmium ions is reached,

$$Cd^{2+} + Hg + 2e \rightleftharpoons Cd(Hg)$$

As the current starts to rise, it is controlled by diffusion of the Cd^{2+} (or by the kinetics of the electron transfer reaction for irreversible processes) and eventually becomes limited by it. This limitation produces a current plateau which continues until the reduction potential of the supporting electrolyte is again reached. The net current produced by the diffusion of the electroactive species in the plateau region is called the diffusion current (i_d) and the ratio $i_d:i_r$ (signal : noise) determines the sensitivity of the technique. The potential of the DME where the diffusion current is half the limiting value is called the half-wave potential ($E_{\frac{1}{2}}$)

which is characteristic of the electroactive ion for a given supporting electrolyte. Other types of polarographic limiting currents exist but the most frequently encountered in analytical work is the diffusion current which is directly proportional to the concentration of the electroactive substance. A quantitative expression for this was derived by Ilkovic[45] from a consideration of Faraday's Laws of electrolysis and Fick's Laws of diffusion:

$$i_d = 607nCD^{\frac{1}{2}}m^{\frac{2}{3}}t^{\frac{1}{6}}$$

where n is the number of electrons transferred, C the concentration, D the diffusion coefficient, m the mercury flow rate and t the drop time. The Ilkovic equation assumes linear diffusion and curvature of the electrode was neglected; various corrections for spherical diffusion had been made but for most purposes the original Ilkovic equation was found to be adequate.

Polarography has found wide applications in the fields of analytical chemistry, and kinetic and structural studies of organic compounds and inorganic complexes.

Complexones in polarographic analysis

The use of complexones and complexometry had been dealt with in Section I. The principles of complexometry and polarography may be used to advantage in combination. The complexonates of metal ions and the complexones themselves give polarographic waves and the use of such polarographic data had been reviewed.[46] The various possibilities for the application of complexones in polarographic analysis may be classified into the following sub-sections.

(1) Utilisation of the chelation of complexones with metal ions to improve the resolution of polarographic waves.

This can be further subdivided into two types:

(a) Utilisation of the half-wave potential shift produced on chelation.

The formation of stable metal chelates generally results in a shift of the polarographic half-wave potentials of the corresponding metal ions to more negative values. The magnitude of this shift is related to the stability of the resulting chelate. It is this difference in the half-wave potential shifts that enables the interfering effect of one metal upon another to be overcome by producing sufficient separation between them. Thus for example Tl(I) and Pb(II) yield in acetate medium polarographic waves with practically identical half-wave potentials (-0.5V vs. SCE), but in the presence of EDTA the lead wave is shifted to -1.1V, whereas the thallium wave remains unaffected. Many such examples exist. Data on the $E_{\frac{1}{2}}$ shift also facilitate studies of the structure and stabilities of the complex ions.

(b) Utilisation of the wave shift and sometimes total suppression of the polarographic waves of metal complexonates due to adsorption phenomena at the DME.

It is a well-known fact that surface-active agents not only function as maximum suppressors but in some cases also influence the wave of the chelate.[47] This influence may manifest itself in an apparent shift of the half-wave potential, or a decrease in wave height. Of the various theories that have been advanced to explain the action of these surface active agents, the theory according to which an adsorbed film of the surface active agent inhibits electron transfer seems to be most generally applicable. Due to this inhibition the complexonate must cross an energy barrier before it can undergo the electrode reaction and hence the shift to more negative potentials for electrode reductions. Such wave shifts have been used to advantage for analytical purposes. In one instance, the complexone EDTA has been used in conjunction with common surface active agents in the determination of trace amounts of thallium in the presence of copper and other metals.[48] Reilley et al[47] have described an unusual effect of gelatin on the cathodic waves of certain metal complexonates. They showed that a certain concentration of surface active agent will decrease and even obliterate the wave height for the reduction of bismuth-EDTA complexonate (electrochemical masking) without causing appreciable distortion of the reduction wave of the free metal ions. This has been used to advantage in the sequential amperometric titration of bismuth and lead; the latter has a half-wave potential more negative than that of the bismuth-EDTA complexonate. The bismuth can be titrated at -0.25V to a sharp amperometric end point in the presence of a minimal concentration of gelatin to suppress maxima. The following titration of lead ion at -0.55V would not be as good since the diffusion current of lead is superimposed upon the large diffusion current of the bismuth-EDTA complexonate. However, by increasing the concentration of gelatin, the diffusion current due to bismuth-EDTA may be eliminated selectively and lead can be determined with greater sensitivity.

(2) Utilisation of substitution reactions of complexones.

Electrophilic substitution reactions such as,

$$MY + M^1 \rightleftharpoons M^1Y + M$$

where M represents a polarographically active metal ion, M^1 a polarographically inactive metal ion and Y a complexone, permit an indirect determination of metal ions that are polarographically inactive. The treatment of the equilibria of such substitution reactions had been dealt with in Section I, Chapter 1, and an application was made by Tanaka and Nakagawa[39] on the indirect polarographic determination of calcium in the presence of magnesium by an exchange reaction similar to equation (1), Section 1.1.2. These substitution reactions may also be used for the polarographic determination of the stability constants of the complexes.

(3) Utilisation of the anodic waves of complexones.

The anodic dissolution wave of mercury is shifted to a more negative potential if the solution contains a substance that forms simple or chelate complexes with mercury. For complexones of the EDTA type with four ionisable hydrogen atoms, the electrode reaction may be written as,

$$Hg^{\circ} + H_nY^{(4-n)-} \rightleftharpoons HgY^{2-} + nH^{+} + 2e$$

where n is an integer which may vary from zero to four, depending on the pH of the solution. The resulting wave is traditionally considered the anodic wave of the complexone. As a result of the formation of the mercury (II)-EDTA complexonate, the concentration of free EDTA decreases at the electrode surface, and more EDTA is transported to the electrode by diffusion. The corresponding current is therefore defined by the Ilkovic equation i.e. $i_d \propto C$. In the presence of metal ions that form stable chelates with EDTA, the diffusion current is decreased in proportion to the total concentration of metal present. In other words, a monitoring of the anodic current of the complexones provides a convenient way of following reactions in complexometry.

Of the possible uses of the complexones in polarographic analysis, just outlined, it is obvious that the last-mentioned is the most suitable for widespread and general application. The entire field of complexometry has been made available for polarographic analysis. The conventional complexometric principles of masking, pH control and selective titrants may be used to advantage in polarographic analysis. This approach also provides a means of automating analytical methods in complexometry by simply monitoring the on-stream anodic currents of the complexones. This aspect forms the basis of the work in Section III, but the present section is concerned with a fundamental study of the anodic polarographic behavious of the complexones, the use of the above-mentioned complexometric principles and their application to practical analysis.

SECTION II

CHAPTER 1.

POLAROGRAPHIC BEHAVIOUR OF COMPLEXONES

2.1.1. Introduction

It has been stated earlier that the anodic dissolution wave of mercury is shifted to a more negative potential, if the solution contains a substance capable of forming stable complexes with mercury. This may be considered to be analogous to the shift to more positive values of the cathodic discharge potential due to reduction of the supporting electrolyte at the DME, when electro-reducible metal ions are added to the solution. Polarograms derived in the latter process are called the cathodic polarographic waves of the metal ions, and by analogy the polarograms derived from the former process are termed the anodic polarographic waves of the complexing agents. The extent of the shift of the mercury dissolution potential is dependent upon the stability of the mercury complexes formed, and for this reason the complexones give well-defined anodic polarographic waves because of the very high stability of the mercury-complexonates formed.

A study of the polarographic behaviour of EDTA was first made by Matyska and Kössler[49] in 1951 and later by Gaffart, Michel and Duyckaerts.[50] The results of the two groups of workers disagree significantly, and a few years later, a revised paper by Matyska and coworkers[51] appeared in which a study of the polarographic behaviour of 1,2-diaminocyclohexane-N,N^1-tetraacetic acid (CDTA) was also included. The findings of these two groups of workers may be briefly summarised as follows:

EDTA and CDTA give well-defined one-step anodic polarographic waves corresponding to two-electron oxidations with the formation of Hg(II) chelates. The waves are diffusion controlled. There is disagreement, howevdr, in the conclusion towards reversibility of the electrode reaction. Matyska[51] found complete reversibility of the EDTA wave, whereas Michel[50] reported an appreciable difference between the half-wave potentials of the anodic EDTA wave and the cathodic Hg-EDTA wave. The CDTA wave was reported to be not fully reversible.[51] From a study of the dependence of $E_{\frac{1}{2}}$ with pH, stability constants of the Hg chelates had been determined and found to be $10^{22.15\pm0.05}$ by Michel et al[50] and $10^{21.6}$ by Matyska et al[51] for EDTA, and $10^{24\cdot3}$ for CDTA[51] in most of the pH range except in highly acidic and highly alkaline solutions where the hydrogen and hydroxo-complexes respectively would exist in appreciable quantities.

In the present chapter a fundamental study of the polarographic behaviour of the complexone EGTA was made. Polarographic data of the complexone, hitherto unreported, will be described. Stability constants of the mercury-EGTA chelate at different pH had also been determined.

Polarographic Behaviour of EGTA

2.1.2. General polarographic behaviour

EGTA gives a one-step polarographic anodic oxidation wave corresponding to the formation of mercury complexes. From a study of pH dependence of the waves, it is shown that the only dominating species produced is that corresponding to the formula HgZ^{2-} in the pH range 5•4 to 11•9, as shall be described in Sec. 2.1.6. The waves are found to be diffusion-controlled from a study of the dependence of limiting current on mercury reservoir height (Figure 2.1.1.),

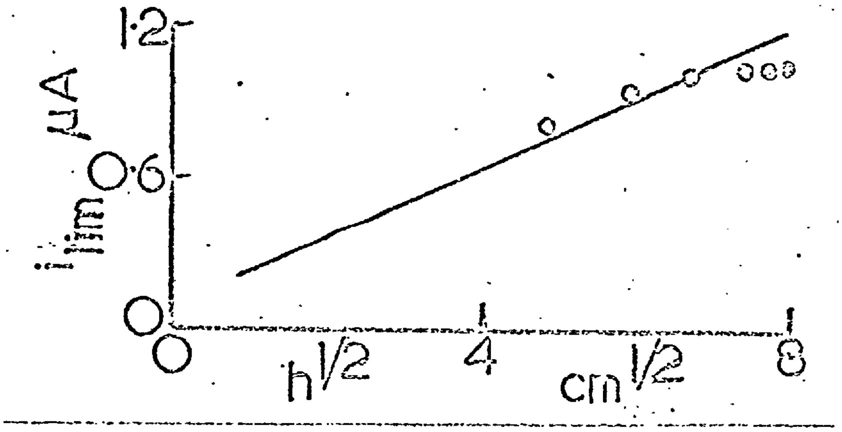


Figure 2.1.1. Dependence of i_{lim} on $h^{\frac{1}{2}}_{corr}$ for a solution of 1.82×10^{-4}M EGTA in sodium tetraborate buffer pH 8.36 and 0.5M KNO_3.

The shape of the i_{lim} vs $h^{\frac{1}{2}}_{corr}$ plot also seems to indicate a slight kinetic influence on the wave; further evidence for this is given later. A linear dependence of the diffusion current on concentration was also found and are described in the analytical applications in later chapters.

2.1.3. Estimation of n

The number of electrons (n) involved per molecule of the complexone in the electrode reaction was determined from diffusion current data by the relation,

$$n_u = \frac{I_u}{I_s} ns$$

where I denotes the diffusion current constant, and the subscripts u and s denote the unknown and standard respectively. The above relation applies only if the diffusion coefficients of the unknown and standard are comparable. For this reason EDTA was chosen as the reference depolariser since the structure, and charge carried by the molecule which determines diffusion are similar to EGTA. The type of reaction which occurs at the electrode is also similar in that mercury-complexes are formed in both cases. From the value of I for EDTA of 2·86 as determined by Michel et al[50] and the author's value of I for EGTA of 2·90 (Table 2.1.1.), and since n = 2 is established for EDTA,[49-51] the value of n for EGTA is found to be 2; hence the anodic wave observed for EGTA at the DME is due to a two-electron oxidation to form a Hg(II)-EGTA chelate.

2.1.4. Reversibility of the electrode reaction

The fully reversible nature of the electrode reaction was established from the fundamental criterion of reversibility, namely, the cathodic wave obtained with the oxidised form (Hg(II)-EGTA) and the anodic wave of the reduced form (EGTA) have the same half-wave potential. (Figure 2.1.2.)

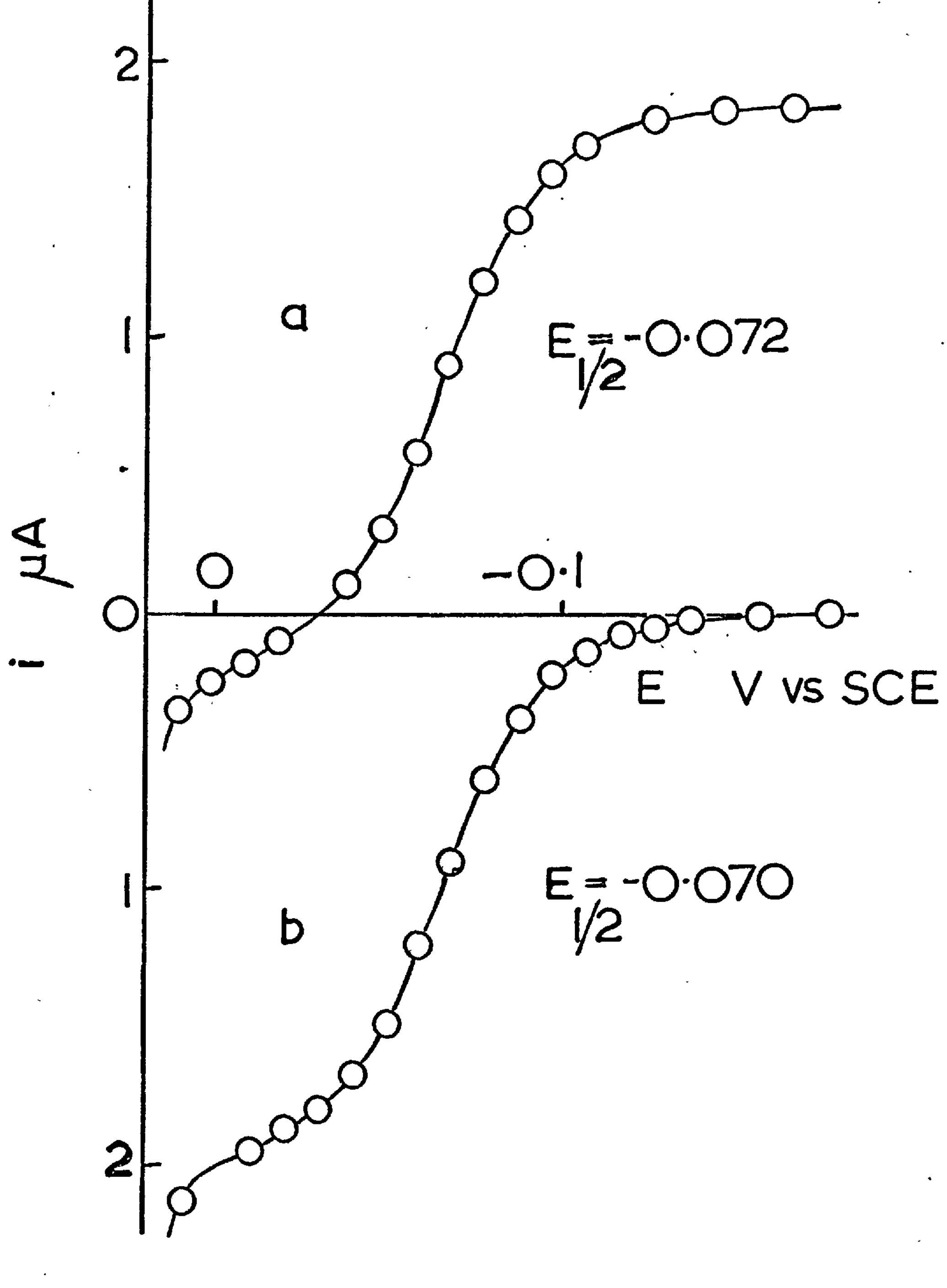


Figure 2.1.2 Manually plotted polarograms of 4.55×10^{-4}M (a) Hg-EGTA (b) EGTA in ammonia/ammonium nitrate buffer pH 9.3.

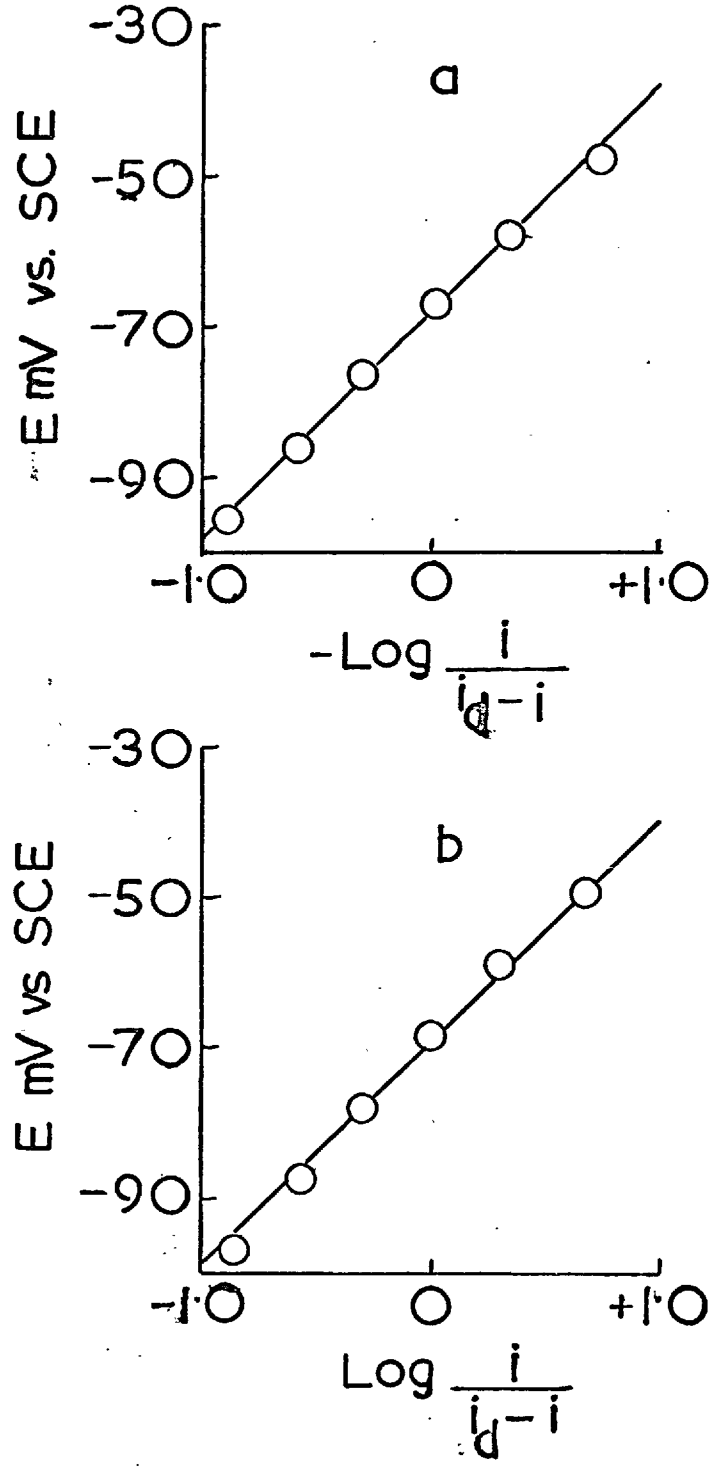


Figure 2.1.3 Logarithmic analysis of the rising portion of (a) cathodic wave of Hg-EGTA and (b) anodic wave of EGTA. Data from Fig. 2.1.2. Slopes= 29 mV/log unit.

Further evidence was provided by a logarithmic analysis of the rising portion of both the cathodic and anodic waves, as shown in Figure 2.1.3. Linear plots were obtained over the range of values of the log term from -1·0 to +1·0 (corresponding to current values ranging from about 9 to 90% of the diffusion current), having the near theoretical slopes of 0·030V per unit log-term for a reversible two-electron reaction.

2.1.5. Current-potential relationship

Since EGTA undergoes the same type of anodic oxidation reaction at the DME as EDTA, namely, the formation of Hg(II) chelates, the current-potential relationship will be expected to be similar to that for EDTA.[49-51]

At a potential positive enough to oxidise mercury, oxidation of the mercury to mercury (II) occurs,

$$Hg \rightleftharpoons Hg^{2+} + 2e \qquad (1)$$

In the presence of EGTA (H_4Z), the Hg^{2+} and EGTA react to form the complex HgZ^{2-}, in a solution which is neither too acidic nor too alkaline,

$$Hg^{2+} + Z^{4-} \rightleftharpoons HgZ^{2-} \qquad (2)$$

and hence the anodic oxidation potential of mercury is shifted to less positive values. The decrease in concentration of Z^{4-} at the electrode surface is counteracted by dissociation of the species $H_4Z, H_3Z^-, H_2Z^{2-}, HZ^{3-}$ and diffusion towards the electrode surface. The Nernst relationship for the reversible redox system (1) gives

$$E = E_o + \frac{RT}{2F} \ln \left[Hg^{2+}\right]_o \qquad (3)$$

where E is the potential of the DME, E_o the standard potential of the Hg/Hg^{2+} couple, and the other terms have their usual meaning. The subscript nought denotes concentration at the electrode surface. The stability constant of the complex HgZ^{2-} is defined by,

$$K_{HgZ} = \frac{\left[HgZ^{2-}\right]}{\left[Hg^{2+}\right]\left[Z^{4-}\right]} \qquad (4)$$

The value of $\left[Z^{4-}\right]$ may be easily computed from a knowledge of the dissociation constants (k_1, k_2, k_3, k_4) of EGTA. Thus if C represents the total concentration of the free complexone, then

$$C = \left[H_4Z\right] + \left[H_3Z^-\right] + \left[H_2Z^{2-}\right] + \left[HZ^{3-}\right] + \left[Z^{4-}\right] \qquad (5)$$

and $C = P\left[Z^{4-}\right]$

where

$$P = \frac{[H^+]^4 + k_1[H^+]^3 + k_1k_2[H^+]^2 + k_1k_2k_3[H^+] + k_1k_2k_3k_4}{k_1k_2k_3k_4} \qquad (6)$$

A combination of equations (3), (4) and (6) gives

$$E = E_o + \frac{RT}{2F} \ln \frac{[HgZ^{2-}]_o P}{K_{HgZ}\ C_o} \qquad (7)$$

Since the limiting current is diffusion-controlled, the following relation exists,

$$i = a\ (C - C_o) \qquad (8)$$

where i is the current, a is a constant and C and C_o are the bulk and surface concentrations of the free complexone. The limiting current at $C_o = 0$ is given by

$$i_d = a\ C \qquad (9)$$

and a combination of (8) and (9) gives,

$$(i_d - i) = a\ C_o \qquad (10)$$

The sum of the concentrations of the total free complexone and the HgZ^{2-} will be a constant and may be written as,

$$[HgZ^{2-}] + C = \text{constant} \qquad (11)$$

Substitution in equation (8) gives,

$$i = a\,([HgZ^{2-}]_o - [HgZ^{2-}]) \qquad (12)$$

Since the concentration of HgZ^{2-} is negligible in the bulk of the solution, equation (12) reduces to

$$i = a\left[HgZ^{2-}\right]_o \qquad (13)$$

A solution of equations (7), (10) and (13) give the current-potential relation,

$$E = E_o + \frac{RT}{2F}\ln\frac{I}{K_{HgZ}}\frac{i}{i_d - i} \qquad (14)$$

and for $i = \frac{1}{2}i_d$,

$$E_{\frac{1}{2}} = E_o + \frac{2{\cdot}303\ RT}{2F}(\log P - \log K_{HgZ}) \qquad (15)$$

This equation predicts that the half-wave potential is independent of concentration but is however dependent on pH, since P is a function of pH (equation 6). It also permits a calculation of the stability constant K_{HgZ} from pH and potential data. If species other than HgZ^{2-} are present in dominating proportions, for example, hydrogen-complexes at low pH and hydroxo-complexes at high pH, it will be evident by a failure of equation (16). These theoretical predictions are confirmed in the following sections.

2.1.6. Dependence on pH

Table 2.1.1. summarises the results obtained on a study of the dependence of the EGTA waves on pH from pH 2·3 to 13·0. Constant ionic-strength buffers, maintained at an ionic-strength of 0·5, were used and potentials were measured using a three-electrode system as described in the experimental section.

Table 2.1.1. Summary of data on the study of the dependence of the EGTA anodic wave on pH.

1	2	3	4	5	6	7
pH	Log P	$E_{\frac{1}{2}}$ mv vs SCE	$E_{\frac{1}{2}}-E'_o$ mv vs SCE	LogK_{HgZ}	id uA/mM	I
2.30	-	n.w.	-	-	n.w.	-
3.34	-	n.w.	-	-	n.w.	-
4.35	-	n.w.	-	-	n.w.	-
5·43	7·45	+141·0	-464·1	23·46	4·47	2·69
6·02	6·27	+119·5	-485·6	23·00	4·59	2·76
6·35	5·61	+99·2	-505·9	23·06	4·76	2·86
7·00	4·32	+52·3	-552·8	23·37	4·76	2·86
7·45	3·43	+21·9	-583·2	23·54	4·88	2·93
8·09	2·20	-8·1	-613·2	23·34	5·24	3·15
8·36	1·72	-23·0	-628·1	23·39	5·26	3·16
8·90	0·90	-51·2	-656·3	23·53	5·00	3·00
9·28	0·49	-62·3	-667·4	23·50	4·95	2·97
9·80	0·18	-68·7	-673·8	23·42	4·76	2·86
10·05	0·11	-70·9	-676·0	23·42	4·78	2·87
10·61	0·03	-77·4	-682·5	23·57	4·28	2·57
11·10	0·01	-77·1	-682·2	23·54	4·52	2·71
11·85	0·00	-77·5	-682·6	23·54	-	-
12·45	-	n.w.	-	-	n.w.	-
12·95	-	n.w.	-	-	n.w.	-

Key to table:

(1) Values of Log P in column 2 are computed from equation (6) using the dissociation constants for EGTA, $k_1 = 10^{-2}$, $k_2 = 10^{-2\cdot68}$, $k_3 = 10^{-8\cdot85}$ and $k_4 = 10^{-9\cdot46}$.

(2) The value of E_o^1, the formal potential of the Hg/Hg^{2+} couple, for computation in columns 4 and 5, is obtained by the relation

$$E_o^1 = E_o + 0\cdot029 \log f_{Hg^{2+}}$$

where $f_{Hg^{2+}}$ is the activity coefficient of Hg^{2+} and which is equal to 0·5 at an ionic strength of 0·5 and for a charge of two.[52] Taking the value of E_o for the Hg/Hg^{2+} couple as +0·854 V vs. Normal Hydrogen Electrode (NHE), the value of E_o^1 is found to be +0·8451 V vs. NHE or +0·6051 V vs. SCE.

(3) Values of Log K_{HgZ} are computed from equation (15).

(4) The measured capillary characteristics of the DME are as follows:

$$m = 1\cdot468 \text{ mg/sec}, \quad t = 4\cdot61 \text{ sec and } m^{\frac{2}{3}}t^{\frac{1}{6}} = 1\cdot665$$

and the values of the diffusion current constants (I) in column (7) are computed using these values.

(5) n.w. denotes no wave.

Discussion of results

The small variation of the limiting currents with pH, is presumably due to the slight kinetic nature of the wave as indicated earlier. Figure 2.1.5 shows this variation together with the composition of the solution in terms of the percentages of various dissociated forms of EGTA at different pH values.

The stability constant of the mercury-EGTA chelate HgZ^{2-} had so far been determined only potentiometrically and the values of $10^{23.20}$ at 20°C and $\mu = 0.1$ obtained by Schwarzenbach[53], $10^{23.8}$ at 25°C and $\mu = 0.1$ obtained by Reilley[31,54] and $10^{23.12}$ at 20°C and $\mu = 0.1$ obtained by Mackey et al[55] all using potentiometric mercury electrodes agree well with the author's average value over the pH range 5.43 to 11.85 of $10^{23.47}$ at 23°C and $\mu = 0.5$ obtained polarographically.

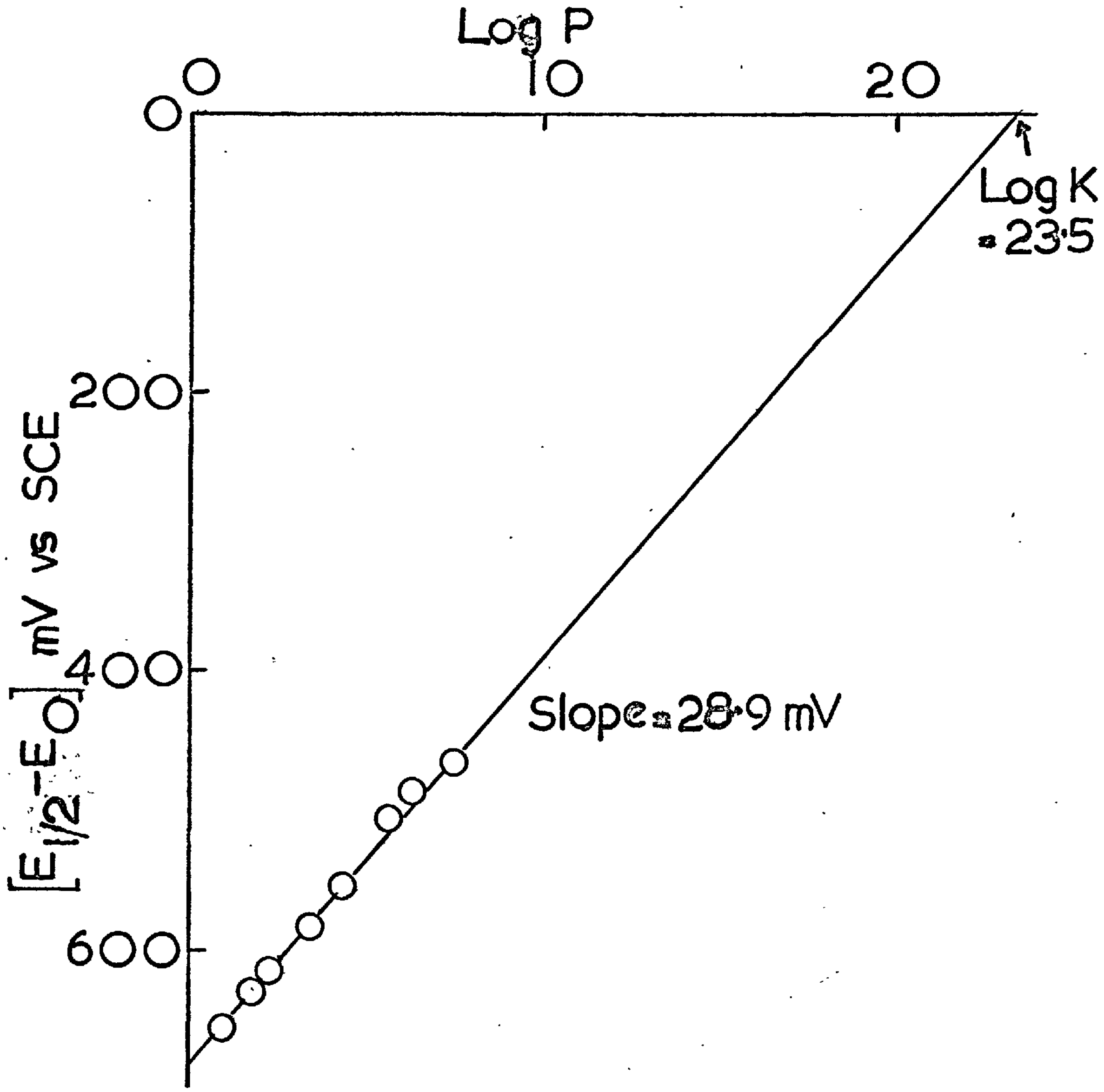


Figure 2.1.4 $(E_{\frac{1}{2}}-E_o^{'})$ vs Log P. Data from Table 2.1.1.

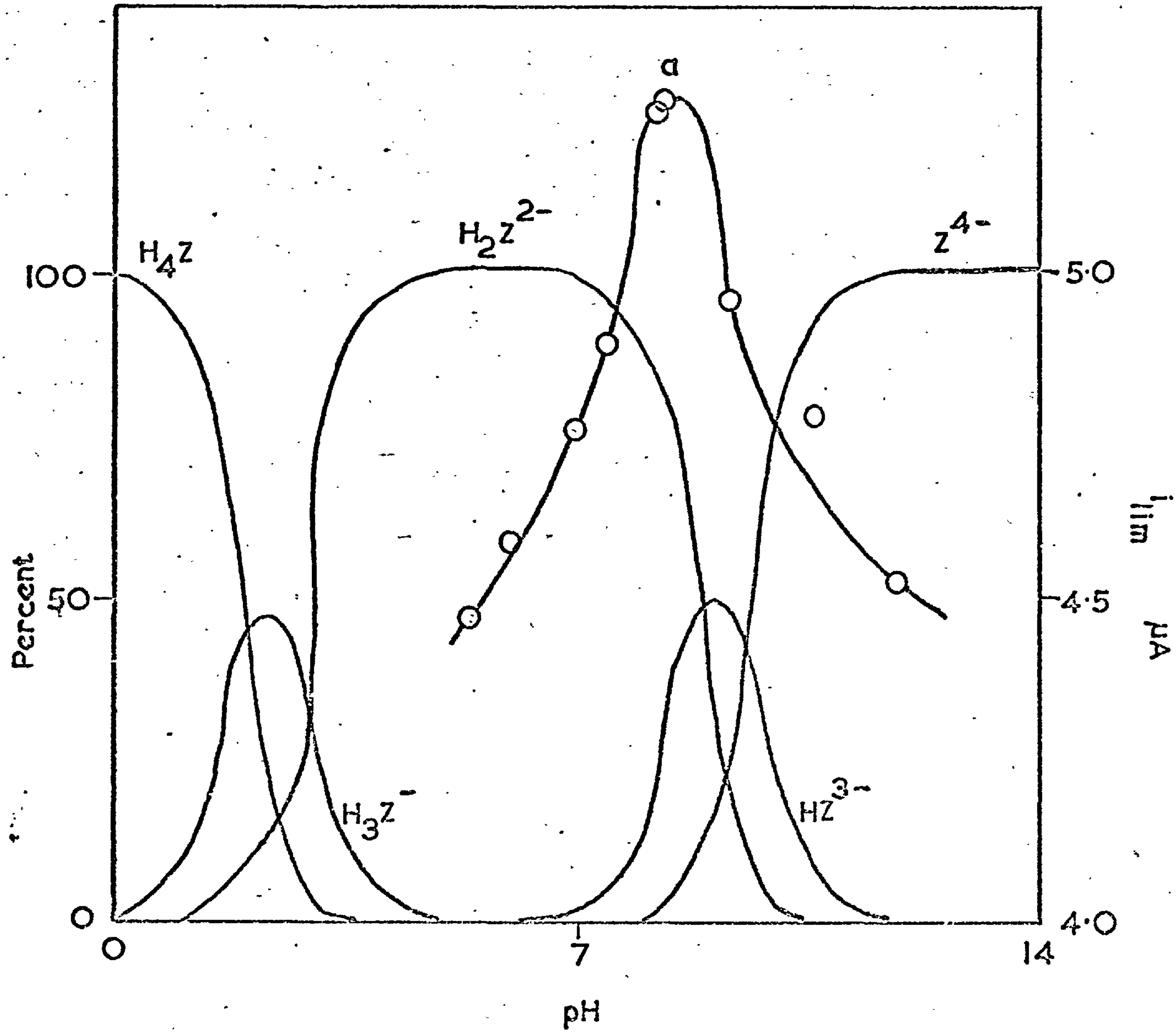


Figure 2.1.5 Dependence of limiting current on pH (curve a) shown together with the composition of the solution in terms of the percentages of the various dissociated forms of EGTA (curves as indicated) at different pH values. Data from Table 2.1.1.

A plot of $(E_{\frac{1}{2}} - E'_{o})$ against log P (Figure 2.1.4) gives a straight line having a slope of 28·9 mv/unit of log P and an intercept on the log P axis of 23·5 which is in perfect agreement with equation (15) which predicts a slope of 29 mv/unit of log P at 23°C and an intercept of 23·5 which is the average value of log K_{HgZ} over the pH range 5·43 to 11.85. This experimental confirmation of the validity of equation (15) also shows that within the range of pH studied, the only complex existing in considerable proportion is that corresponding to the formula HgZ^{2-} as was assumed in the derivation of equation (15). The presence of other mercury complex species, in appreciable proportions, particularly hydrogen complexes at low pH (high log P) and hydroxo complexes at high pH (low log P) would have been obvious by a deviation of the experimental points from the straight line at high and low log P values. The facts that this is not the case and that no polarographic anodic waves of EGTA are obtainable below pH 5·4 and above pH 11·85 show that complex species of the type $HgHZ^{-}$ and $Hg(OH)Z^{3-}$ are not formed by the electrode process in contrast to EDTA.[49]

2.1.7 Experimental

Constant ionic-strength buffers

The pH dependence studies in section2.1.6 are made using buffers maintained at a constant ionic-strength of 0.5 (KNO_3). Constant ionic-strength McIlvaine buffers as recommended by Elving[56] for polarographic work are used in the pH range 2.4 to 7.4, and alkaline buffers due to Bates and Bower[57] are used in the pH range 8.0 to 13.0. The compositions are given in Table 2.1.2.

The preparation of EGTA has been described previously (Section I, Chapter 1). The EGTA solution is standardized for the purpose of calculating diffusion current constants by titrating against a standard zinc solution at pH 9 (ammonia buffer) using zincon as indicator.

Table 2.1.2. Composition and pH values of buffers maintained at an ionic strength of 0·5.

pH	Na_2HPO_4 M	Citric acid M	Ionic-strength of buffer system, M	M, KNO_3 to produce ionic-strength of 0·5
2·4	0·012	0·094	0·025	0·475
3·4	0·057	0·071	0·112	0·388
4.4	0·088	0·056	0·190	0·310
5·4	0·112	0·044	0·302	0·198
6.0	0·126	0.037	0·344	0.156
6·4	0.139	0·031	0·371	0.129
7.0	0.165	0·018	0.427	0.073
7.4	0·182	0·009	0·488	0·012
	$Na_2B_4O_7$ M	HCl M	Ionic-strength of buffer system, M	M, KNO_3 to produce ionic-strength of 0·5
8.0	0.0125	0.0205	-	0·5
8·4	0·0125	0·0166	-	0·5
9·0	0·0125	0.0046	-	0·5
	$Na_2B_4O_7$ M	NaOH M	Ionic-strength of buffer system, M	M, KNO_3 to produce ionic-strength of 0·5
9·4	0·0125	0·0060	-	0·5
10.0	0.0125	0.0183	-	0·5
10·4	0·0125	0·0221	-	0·5
	Na_2HPO_4 M	NaOH M	Ionic-strength of buffer system, M	M, KNO_3 to produce ionic-strength of 0·5
11·0	0·025	0·0041	-	0·5
11·4	0·025	0.0091	-	0·5
12·0	0.025	0·0269	-	0·5
	-	NaOH M	Ionic-strength of buffer system, M	M, KNO_3 to produce ionic-strength of 0·5
12·4	-	0·0324	0·0324	0·4676
13·0	-	0·1320	0·1320	0·3680

Apparatus

A System Heyrovsky polarograph type LP 55A (Czechoslovakia) was used. Photographic recording provided with the polarograph, however, was not utilised; instead a Servoscribe recorder (Smiths Industries Ltd.) was attached for recording polarograms. A 5 ml capacity Kalousek cell with a saturated calomel reference electrode was employed. Polarograms are recorded using the conventional two-electrode system. Capillary characteristics has been given earlier (Table 2.1.1).

For accurate measurements of the potential of the DME, such as for constructing manual polarograms for log-plots etc., and for measuring half-wave potentials, a three-electrode system was employed. The potential of the DME was measured under zero current conditions using an auxiliary reference SCE using a Hewlett-Packard Digital Voltmeter type 3439 A.

pH measurements were made with a Pye pH meter (W.G. Pye and Co., Ltd., Cambridge).

2.1.8. Conclusion

The complexone EGTA undergoes a reversible two-electron oxidation at the DME giving rise to a one-step, diffusion-controlled anodic polarographic wave. Experimental results obtained on a study of the variation with pH of the half-wave potentials of the waves fit in perfectly with theory and it has also been shown that within the pH range where anodic polarographic waves for EGTA are obtainable (i.e. pH 5·43 to 11·85), the only complex formed predominantly by the electrode reaction is that corresponding to the formula HgZ^{2-}. The stability constant of HgZ^{2-} has also been determined polarographically and found to be $10^{23\cdot47}$ ($t = 23^{\circ}C$, $u = 0\cdot5$). It has also been shown indirectly that hydrogen and hydroxo complexes of the type $HgHZ^{-}$ and $Hg(OH)Z^{3-}$ are not formed by the electrode reaction in contrast to EDTA which form these complexes at low and high pH respectively.

The diffusion current constant for the anodic waves of EGTA has been determined.

SECTION II

CHAPTER 2

ANALYTICAL APPLICATIONS OF D.C. POLAROGRAPHY OF COMPLEXONES; AN INDIRECT D.C. POLAROGRAPHIC METHOD AND AN AMPEROMETRIC TITRIMETRIC METHOD FOR THE DETERMINATION OF CALCIUM AND MAGNESIUM IN THE PRESENCE OF EACH OTHER.

2.2.1. Introduction

An introduction to the problem of the analysis of calcium and magnesium in the presence of each other including a literature survey of the complexometric titrimetric methods employing visual, as well as instrumental end-point detection techniques had been given in Section I, Chapter 1. The direct polarographic determination of the alkaline-earth metal ions in aqueous solution had been shown to be seriously interfered by maxima, hydrolytic reactions, catalytic hydrogen waves etc.; studies in non-aqueous solvent systems such as N,N-dimethylacetamide indicate some possible analytical applications.[58] However, for the most general application it is obvious that indirect polarographic methods must be resorted to. Thus indirect polarographic methods of the displacement type in which the alkaline earth metal ions undergo an electrophilic substitution reaction with the complexonates of polarographically active metals, have been reported.[39,59] The liberated electroactive metal ion is a measure of the alkaline earth metal concentration. In only one instance, the anodic currents due to electro-oxidation of the free complexones at a stationary mercury electrode was used to detect the end-point of the complexometric titrations of the alkaline earth metals with the complexones.[60]

In the present chapter the anodic polarographic waves of the complexones have been employed in the development of an indirect d.c. polarographic method and an amperometric method for the analysis of calcium and magnesium in the presence of each other. The methods provide a clear illustration of the combination of complexometric and polarographic principles to advantage.

2.2.2. Principles of the methods

The proportional decrease in the anodic current of a known concentration of complexone with added metal ion forms the basis of the polarographic method. By the use of selective complexones, namely, EGTA for reacting with calcium, an analysis for the latter in the presence of magnesium is achieved. Magnesium concentration is obtained by difference after a similar determination of the sum of calcium and magnesium using EDTA. Similarly in the amperometric method, calcium is determined by a selective titration with EGTA and magnesium by difference after titration for the sum with EDTA. The end-point in both cases is marked by the appearance of an anodic current due to the presence of the complexones.

The above principles, however, are not easily realised in practice since the stability of the magnesium-EGTA chelate ($K = 10^{5\cdot2}$) is not sufficiently low to be free of interference especially in methods where an excess of the complexone has to be present. The search for a suitable masking agent, conventional or otherwise, for masking the magnesium _in the presence of_ calcium, therefore forms a relatively large portion of the work in this chapter.

2.2.3. Optimum conditions

(a) Solution pH

The pH at which the analysis is to be performed must be such that the reaction of the metal ions with the complexones is spontaneous and complete; the metal complexonates must not dissociate appreciably as to give rise to kinetic currents and no precipitation of the metal ions must occur.

In order to choose a pH that will satisfy the above requirements, a plot of the logarithm of the conditional stability constants of the various chelates concerned against pH was constructed, and shown in Figure 2.2.1. The conditional stability constant (K^1_{MY}) was calculated using the equation[61]

$$K^1_{MY} = \frac{K_{MY}}{\alpha_M \alpha_Y} \qquad (1)$$

where K_{MY} is the absolute stability constant, and α_M and α_Y are the so-called alpha-coefficients or "side-reaction" coefficients. Since in this particular case, the alkaline earth metals do not form hydrogen or hydroxo-complexes, α_M may be neglected whilst α_Y, a pH dependent function, is calculated from the equation[61]

$$\alpha_{Y(H)} = 1 + \sum_{j=1}^{4} [H^+]^j K_{HjY} \qquad (2)$$

using the values according to Schwarzenbach[53] of $\log K_1 = 10{\cdot}26$, $\log K_2 = 16{\cdot}42$, $\log K_3 = 19{\cdot}09$, $\log K_4 = 21{\cdot}09$ for EDTA and $\log K_1 = 9{\cdot}43$, $\log K_2 = 18{\cdot}28$, $\log K_3 = 20{\cdot}96$ and $\log K_4 = 22{\cdot}96$ for EGTA.

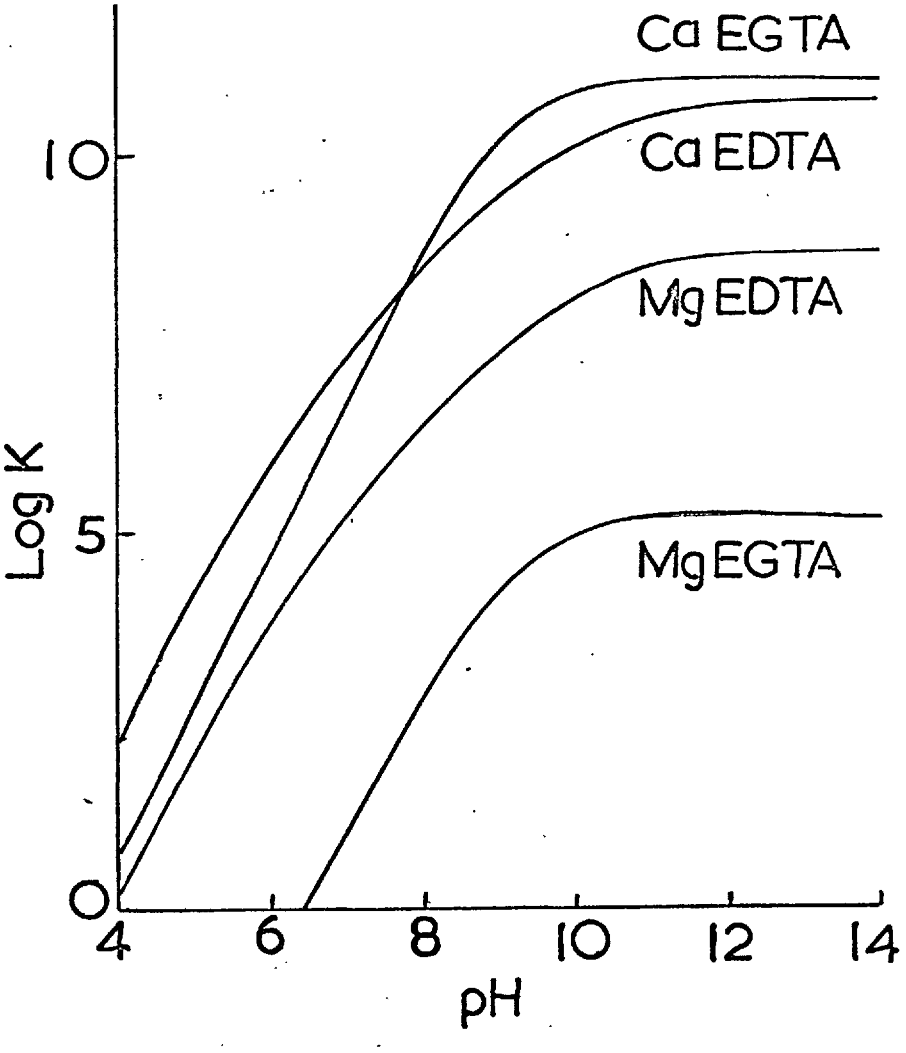


Figure 2.2.1 Variation of the conditional stability constants of the calcium and magnesium chelates of EDTA and EGTA with pH.

Figure 2.2.1 shows that the conditional stability constants of the chelates increase with increasing pH and attains a limiting value at and above pH 10. A solution pH of around 10, therefore seems appropriate since below pH 10 the stability decreases rapidly and kinetic currents will be observed at sufficiently low pH as was reported by Michel[62] at pH 6.4; at pH values higher than 10, magnesium would start to precipitate. That kinetic currents due to dissociation of the metal-chelates in the vicinity of the DME are absent at pH ca 10 had been confirmed by experiments in which anodic currents of the solutions of complexones containing an excess of the alkaline earth metal ions were measured and found to be non-existent.

(b) Buffer composition and concentration.

The choice of buffer composition is only limited by the fact that anion components of the buffers that strongly complex with mercury shift the anodic dissolution potential of mercury towards less positive potentials. Consequently the anodic waves of the complexones will be less well-defined. Thus it was found that both EDTA and EGTA do not give any anodic wave at all in buffers containing acetate ions which strongly complex with the mercury. Ammonia/ammonium nitrate buffer has therefore been used throughout this work because of the non-complexing property of the nitrate ion. Even in this case a very high concentration of ammonia is to be avoided for the same reasons. The optimisation of the concentration of buffer is complicated by the fact that it has an important effect on the suppression of maxima observed in the anodic waves of the complexones as discussed in section (c). Triethanolamine buffer may be used interchangeably with the ammonia buffer and has the advantage of masking certain interfering ions, but has the disadvantage of low conductivity.

(c) Polarographic maxima.

Both the EDTA and EGTA anodic waves had been found to exhibit polarographic maxima. The maxima always occur on the rising portion of the polarographic waves, are rather acute and fall abruptly and discontinuously from the maximum current to the limiting value. No maxima occur, however, on the anodic waves of both the complexones when the concentration of the depolariser is less than 5×10^{-4}M at a drop time of around 4 seconds; neither are maxima observed in concentrated solutions of supporting electrolyte and the dependence of the height of the maximum with concentration of buffer, as shown in Figure 2.2.2 agrees well qualitatively with the theory by Popova and Kryukova[63] that for maxima of the first kind there exists an empirical relation for the optimum conductivity k_{opt} of the electrolyte at which the maximum attains its highest value:

$$k_{opt} = 1{\cdot}5\ c$$

and with the conductivity increasing above the optimal value, the maximum current decreases:

$$i_{max} = Kk^{-b}$$

where c is the depolariser concentration in gram equivalents per litre, k is expressed in $ohm^{-1}cm^{-1}$, K is a constant and b varies between 0•6 and 0•7. All the above observations lead to the conclusion that the observed maxima are of the first kind.

The dependence of maxima on drop-time and mercury flow-rate cannot yet be expressed by simple relationships and experiments had been performed in order to facilitate choice of optimum conditions and the results are shown in Figure 2.2.3 and Figure 2.2.4.

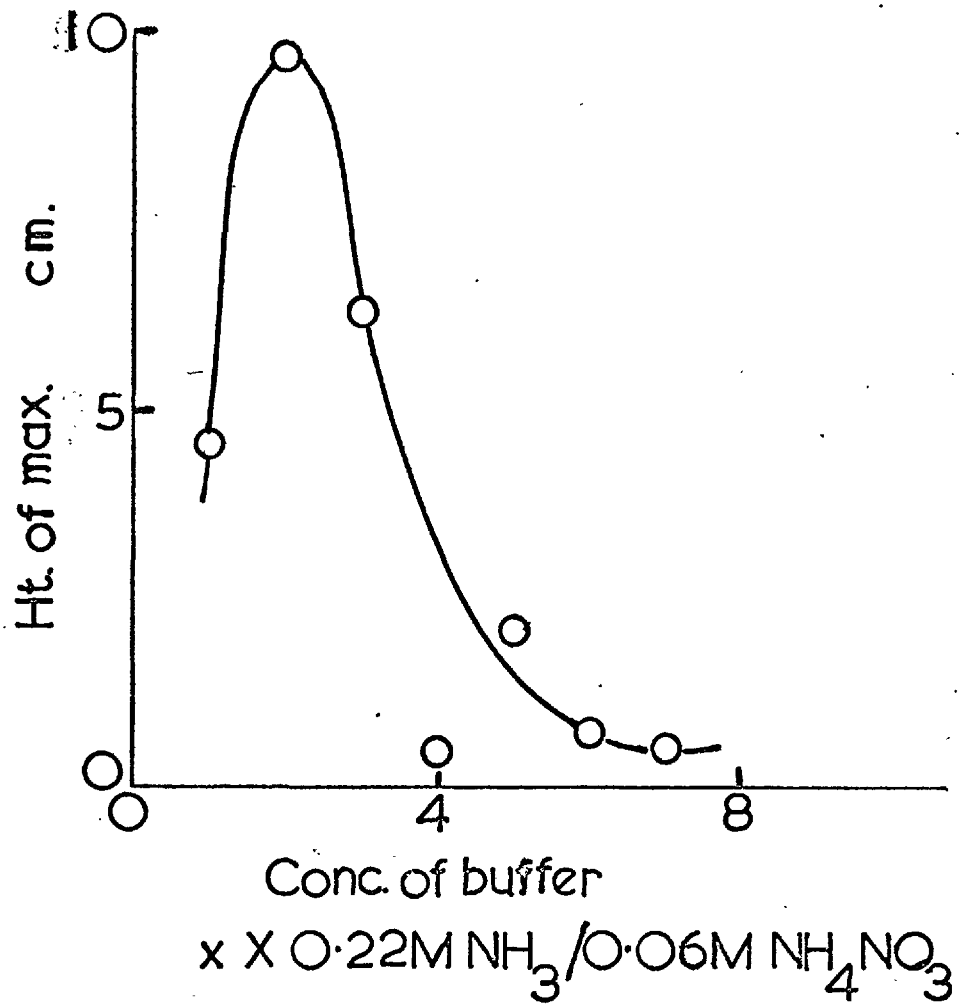


Figure 2.2.2 Variation of the height of the maximum on the anodic wave of 1.92 mM EDTA with electrolyte concentration.

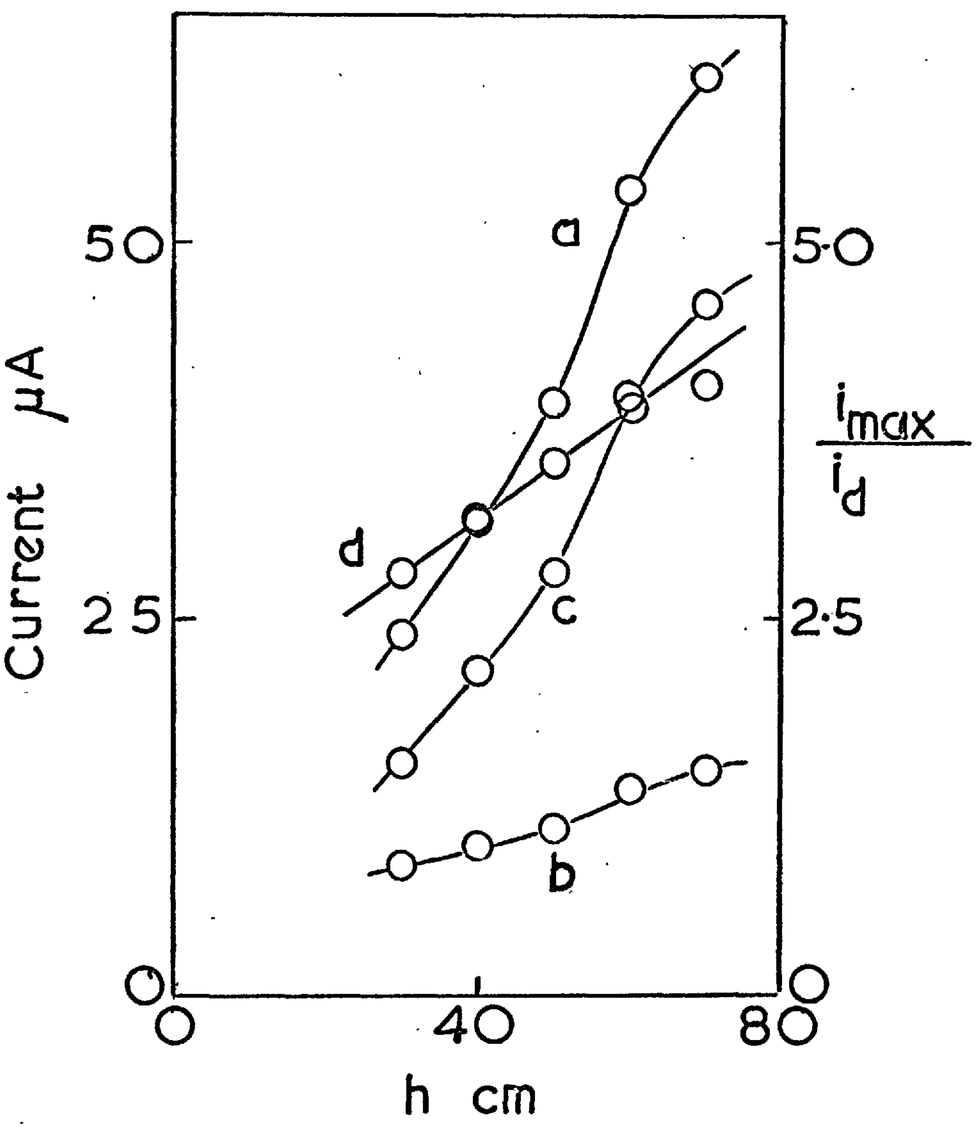


Figure 2.2.3 Effect of height of mercury head on the maximum of the anodic wave of 1.92 mM EDTA in 0.22M ammonia/0.06M ammonium nitrate.
(a) i_{max} (b) i_d (c) $i_{max}-i_d$ (d) i_{max}/i_d

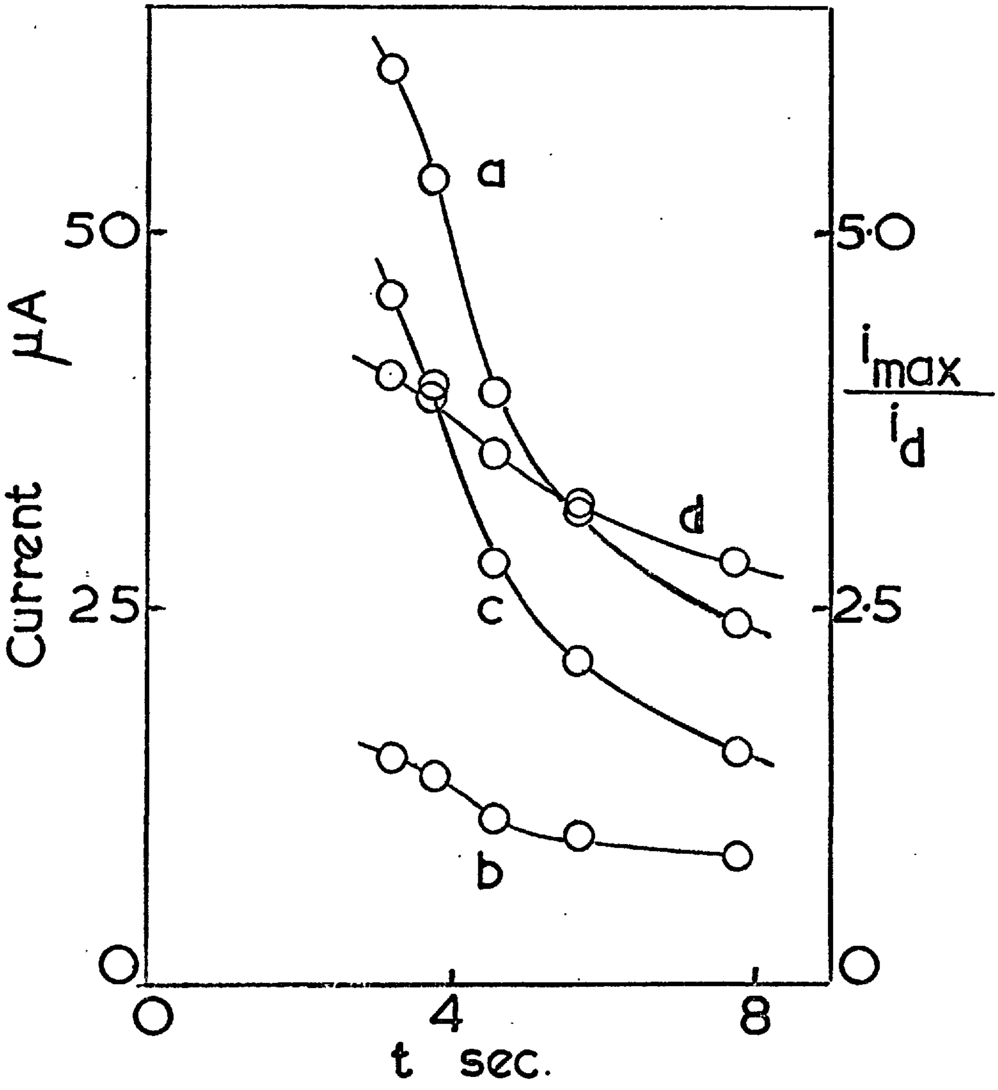


Figure 2.2.4 Effect of drop-time on the maximum of the anodic wave of 1.92 mM EDTA in 0.22M ammonia/ 0.06M ammonium nitrate.
(a) i_{max} (b) i_d (c) $i_{max}-i_d$ (d) i_{max}/i_d

The figures show a considerable influence of the height of the mercury head and the drop time on the maxima; the maxima decreasing with decreasing height of the mercury head or increasing drop time. A long drop-time together with the use of an appropriate surface active agent could therefore be used for suppression of the observed maxima; a drop-time of around 4-5 seconds (in the region of rapidly decreasing maximum in Figure 2.2.4) is considered optimum. Even though longer drop-times would still lessen the maxima, uncertainties in the diffusion phenomena at too high drop-times advise against this. The use of high concentrations of buffer to suppress maxima (Figure 2.2.2) is also inadvisable for the obvious reason that high concentration of impurities may accompany the high concentration of buffer.

The examination of the effect of different types of maximum suppressors on the observed maxima show that the maxima can be suppressed to varying degrees of perfection or complexity by all types of suppressors studied as summarieed in Table 2.2.1.

Table 2.2.1. Effect of different maximum suppressors on the anodic waves of EDTA and EGTA.

Substance	Type	Effective* Concentration %	Maximum Suppression Remarks
Triton X-100	Neutral	0·001-0·002	Yes. Complications at rising portion above 0·002%
Methyl red	Zwitterion	>0·004	Yes.
Cetavalon	Cationic	<u>ca</u>0·01	Yes. Can be used in a wide range of concentration without complication.
Sodium lauryl-sulphate	Anionic	<u>ca</u>0·002	Yes. Potential shift at high concentration to positive values.
Iodide	Anionic	<u>ca</u>0·001	- do -

* Information here has been largely generalized. Applies generally to concentration of complexones in the vicinity of 2 x 10^{-3}M, at a drop-time of 4 seconds.

In conclusion to this section it may be generalized and said that maxima may be avoided when concentrations of complexones less than or equal to 5 x 10^{-4}M are employed. If higher concentrations are to be used, the observed maxima may be suppressed with the maximum suppressors as described. Optimum drop-time is around 4 seconds and the optimum concentration of buffer has been found from

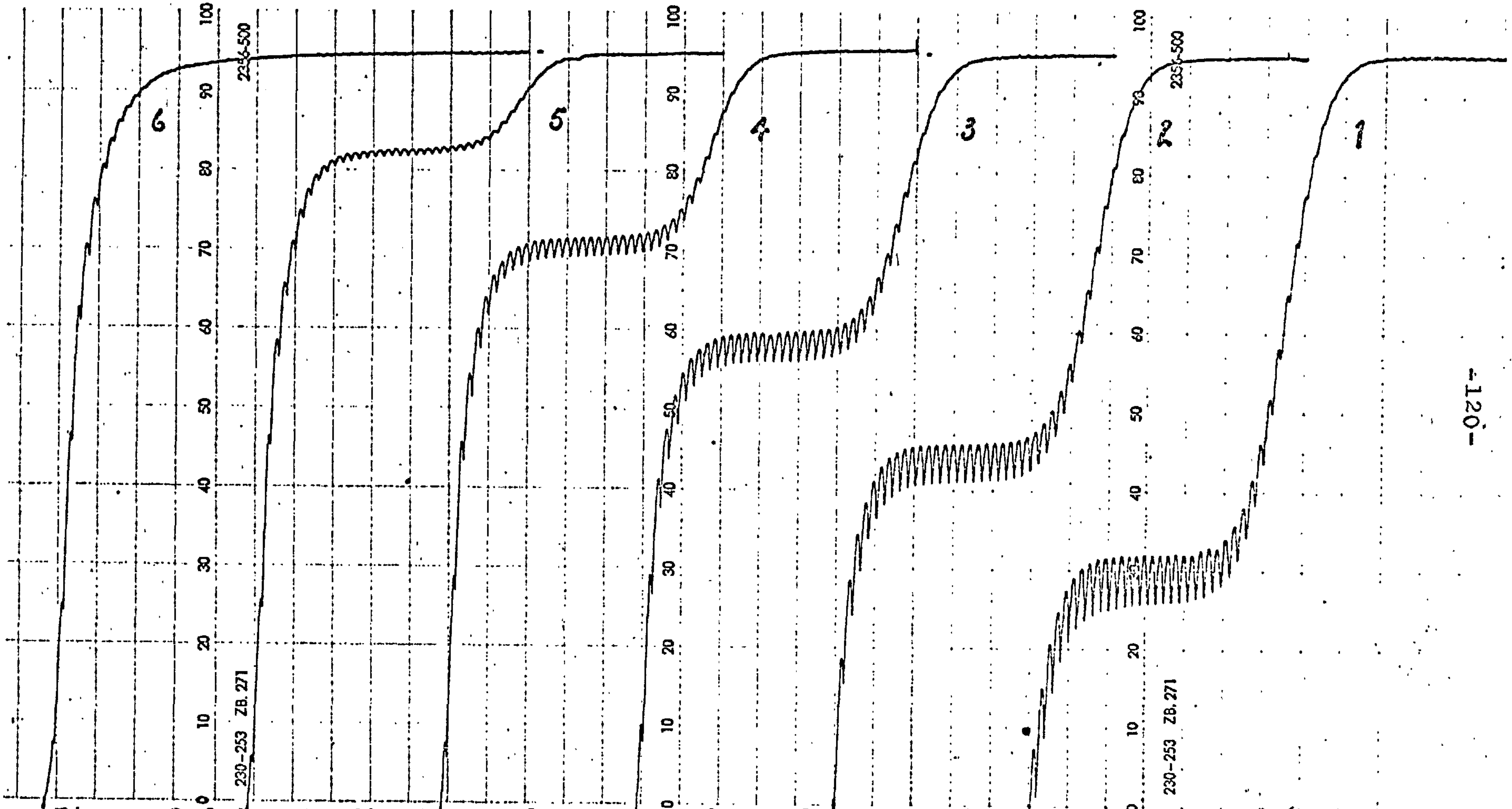


Figure 2.2.5 Anodic d.c. polarograms of *ca.* 1 mM EDTA in 0.22M ammonia/0.06M ammonium nitrate, 0.002% Triton X-100 containing (1) 0 (2) 0.2 (3) 0.4 (4) 0.6 (5) 0.8 (6) 1.0 mM (Ca+Mg) in a 1:1 ratio. 33 mV/column, all curves start at -0.3V vs SCE, sens.=1/10 (9.3 uA f.s.d.).

considerations of buffering capacity, shape and definition of polarographic waves, and maxima to be <u>ca</u> 0·22M NH_3/0·06M NH_4NO_3.

(d) Masking agents for magnesium in the presence of calcium.

Linear calibration plots for EDTA and EGTA were obtained within the normal polarographic working range of 0 to 2 x 10^{-3}M. Calibration for the analysis of the sum of calcium and magnesium by measuring the decrease in the height of the anodic waves of EDTA also presents no problem, as can be seen in Figure 2.2.5 and Figure 2.2.6.

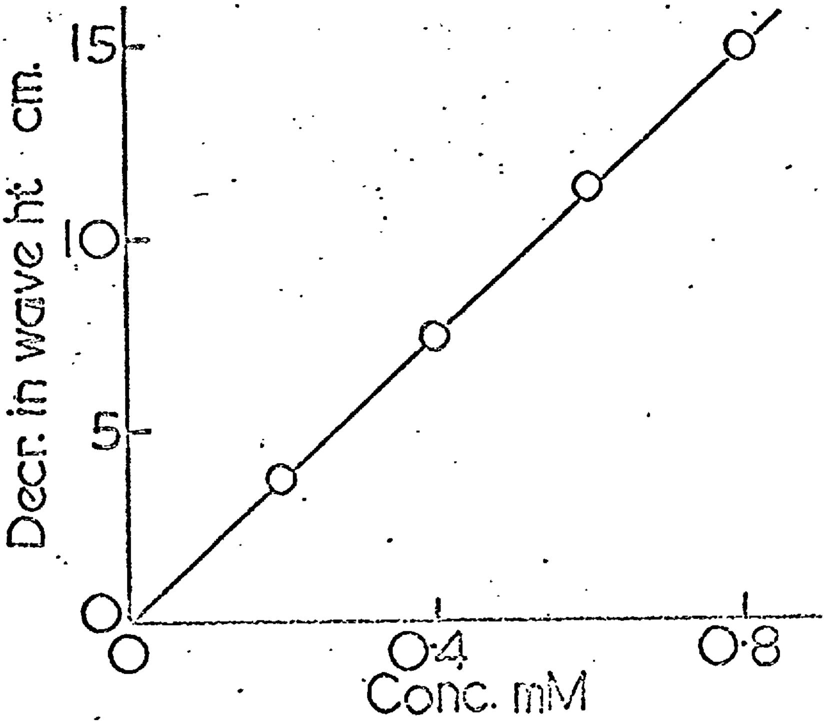


Figure 2.2.6. Calibration curve for the analysis of (Ca + Mg). The blank solution is <u>ca</u> 10^{-3}M EDTA in 0·2M NH_3/0·05M NH_4NO_3.

However, considerable errors are involved in the calibration plots for analysis of calcium alone, in the presence of magnesium, by measuring the decrease in anodic currents of EGTA as can be seen by the large deviation of the experimental curve (b) from the theoretical curve (a) in Figure 2.2.9. This is caused by reaction between magnesium and the excess of EGTA present, in spite of the low stability-constant of Mg-EGTA ($10^{5\cdot2}$). It therefore becomes essential to mask the magnesium, in order to remove this interference.

However, for very obvious reasons it is quite improbable yet to find a ligand that would react exclusively with magnesium in the presence of calcium. But when the calcium is to be subsequently reacted with EGTA after the masking of magnesium, as in this particular case, the problem becomes simpler. Ligands can be found which combine with magnesium forming complexes of stability greater than $10^{5\cdot2}$ (the stability-constant of Mg-EGTA). These ligands will certainly also react with the calcium; however, a further specification that the ligands should form complexes with calcium having stability constants less than $10^{11\cdot0}$ (the stability-constant of Ca-EGTA), can be added so that, even though both calcium and magnesium are masked by the added ligand, subsequent addition of EGTA to the masked solution will cause the calcium-ligand complex to be demasked and titrated whilst the magnesium-ligand complex remains intact. Table 2.2.2 gives a list of some ligands that meet the above-mentioned requirements obtained from a survey of the literature.[64,65]

Consideration was given to the following three points in deciding on the suitability of a substance as a masking agent for the present purpose:

(i) Masking efficiency

(ii) Non-interference in polarographic measurements, and

(iii) Commercial availability.

According to the theory of masking just outlined, log K_{Mg} and log K_{Ca} values in Table 2.2.2 provide an approximate measure of masking efficiency; high values of (log K_{Mg} - 5·2) and (11·0 - log K_{Ca}) indicating high masking efficiency and vice versa. Point (ii) is less easy to predict since interference in polarographic measurements can take different forms. Interference can occur in the form of polarographic waves of the substances used for masking, either before or after the waves of the complexones, the former affecting sensitivity and

Table 2.2.2. Some possible masking agents for magnesium in the presence of calcium, for subsequent titration with EGTA.

No.	Ligand	Log K_{Ca}	Log K_{Mg}
1.	Disodium-1,2-dihydroxybenzene-3,5-disulphonate (Tiron)	5·80	6·86
2.	Tripolyphosphoric acid	4·95	5·80
3.	Nitrilotriacetic acid (NTA)	6·41	5·41
4.	Aminomethylphosphonic acid-N,N-diacetic acid	7·18	6·28
5.	B-Aminoethylphosphonic acid diacetic acid	5·44	6·33
6.	N,N^1-Ethylenebis-N,N^1-(2-O-hydroxyphenyl) glycine	7·20	8·00
7.	N,N-Dihydroxyethylethylene diamine diacetic acid (HEDDA)	4·80	5·70
8.	Trimethylene diamine tetraacetic acid	7·12	6·02
9.	N,N,N^1,N^1-tetrakis-(phosphonomethyl)-1,2-cyclohexane diamine (CDTMP)	5·50	6·40
10.	trans-Piperazine-2,3-dicarboxylic acid	<1	5·80
11.	N-2-Phosphoethyl imino diacetic acid	5·44	6·33
12.	N-(2-Hydroxy-5-nitrobenzyl)-imino diacetic acid	6·44	6·85
13.	N-(2-Hydroxybenzyl) imino diacetic acid	6·74	7·28
14.	4-(p-Arsonophenylazo)-3-hydroxynaphthalene-2,7-disulphonic acid	5·50	5·90
15.	Eriochrome Black A	5·25	7·20
16.	Eriochrome Black B	5·70	7·40
17.	Eriochrome Black T	5·40	7·00
18.	Eriochrome Black R	5·58	7·64

The stability constants quoted are for an ionic strength of 0·1 and temperature of 20-25°C.

the latter affecting the definition of the shape of waves of the complexones. Interference can also occur by the adsorption of the substances used for masking, on the surface of the DME, such as is possible with large dye stuff molecules like the Eriochrome Black compounds (Nos. 15-18, Table 2.2.2).

The validity of the theory was established experimentally and the results are shown in Figure 2.2.7 and Figure 2.2.8.

The diffusion current due to a certain concentration of EGTA is decreased on addition of magnesium and this decrease is a measure of interference. Figure 2.2.7 shows the efficiency of masking of three potential masking agents studied, as measured by the efficiency of attainment of the diffusion current to the original value with increasing concentration of masking agent. Figure 2.2.8 shows that due to the second specification in the selection of masking agents, namely, that log K_{Ca} should be less than 11·0, the presence of the masking agents does not prevent or hinder reaction between the calcium and EGTA. Any such prevention or hindrance would be indicated by an inclination of the experimental lines towards the broken horizontal line in Figure 2.2.8, which is

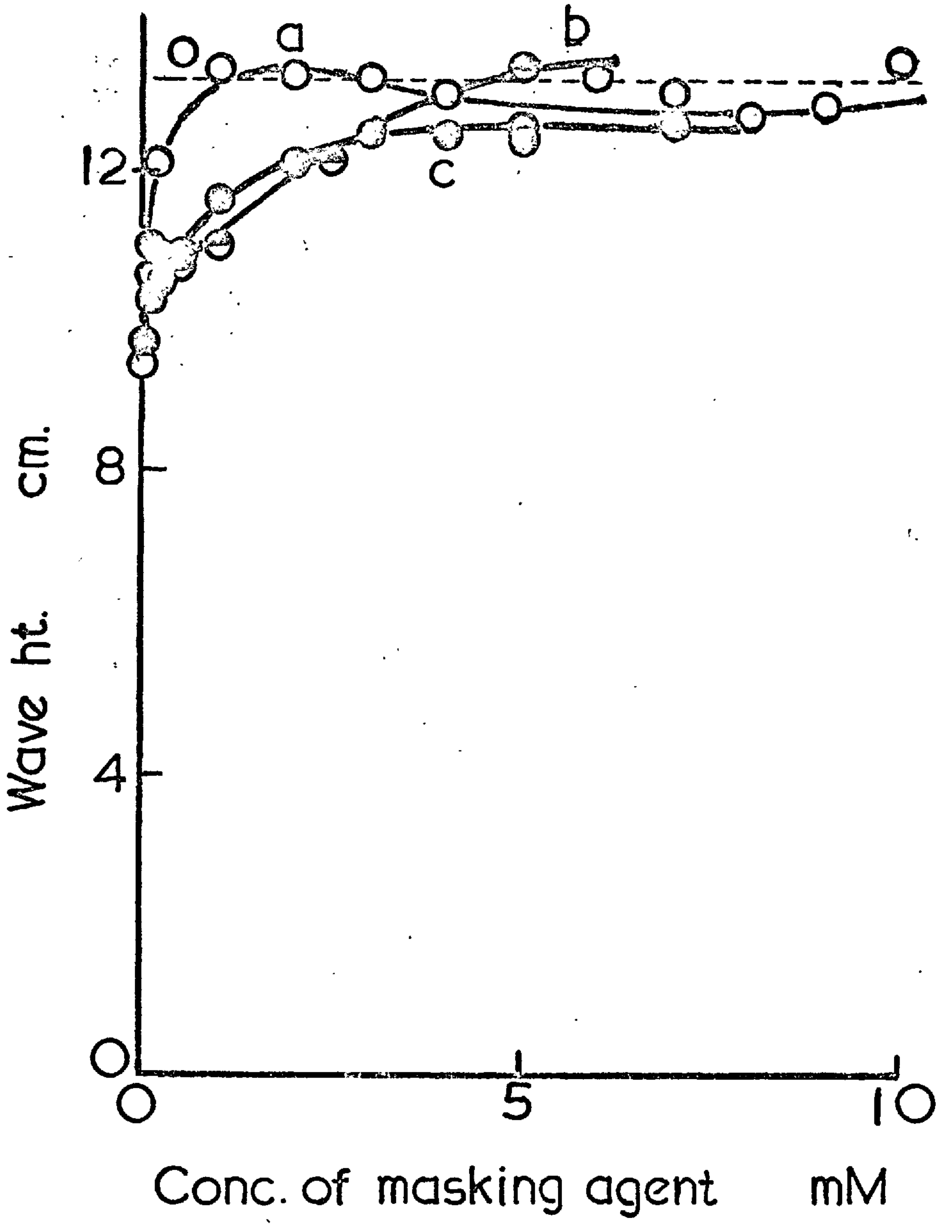


Figure 2.2.7 Masking of magnesium against reaction with EGTA. Wave heights of 0.6 mM EGTA in 0.15M ammonia/0.04M ammonium nitrate in the presence of 0.25 mM magnesium, plotted against concentration of different masking agents (a) sodium tripolyphosphate (b) Eriochrome Black T (c) tiron. Broken line represents wave height in the absence of magnesium.

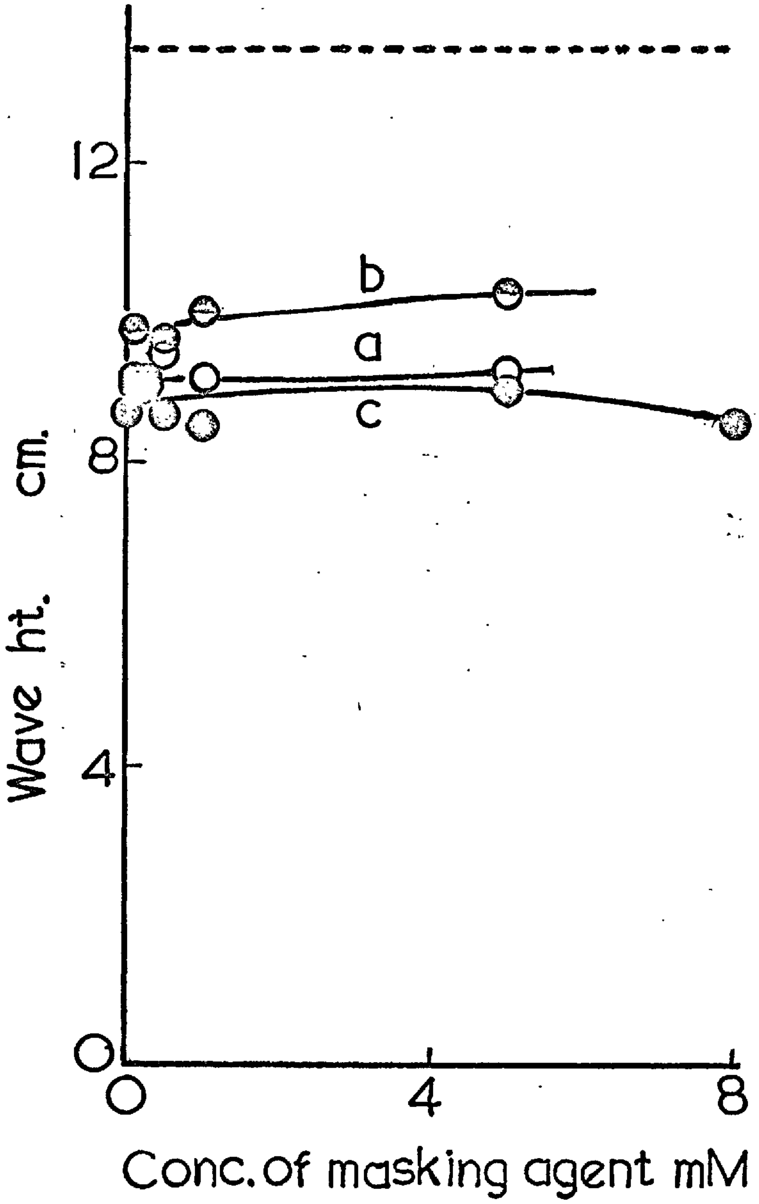


Figure 2.2.8 Non-interference of masking agents for magnesium on reaction between calcium and EGTA. Wave heights of 0.6 mM EGTA in 0.15M ammonia/ 0.04M ammonium nitrate in the presence of different masking agents; (a) sodium tripolyphosphate (b) Eriochrome Black T (c) tiron. Solution also contains 0.2 mM calcium. Broken line represents wave height in the absence of calcium.

found not to be the case. A study of Figure 2.2.7 shows the sodium tripolyphosphate to have the highest masking efficiency. Substances like nitrilotriacetic acid (NTA) in Table 2.2.2 were found to be unsuitable since the polarographic wave for NTA which immediately follows the EGTA wave badly influences the latter. Sodium tripolyphosphate was thus chosen as the most suitable masking agent for magnesium, on grounds of masking efficiency, non-interference in polarographic measurements and commercial availability. The overall effect of the use of sodium tripolyphosphate as a masking agent is shown in Figure 2.2.9.

The diffusion current vs. concentration of calcium plot is highly displaced from the theoretical curve in the absence of any masking agent, whereas in the presence of sodium tripolyphosphate the experimental and theoretical curves almost coincide.

2.2.4. Results and discussion

(a) Indirect polarographic method.

The determination of the total concentration of alkaline earth metals by indirect d.c. polarography of EDTA is quite straightforward and presents no problems as was stated earlier and shown in Figure 2.2.6. A similar determination for calcium

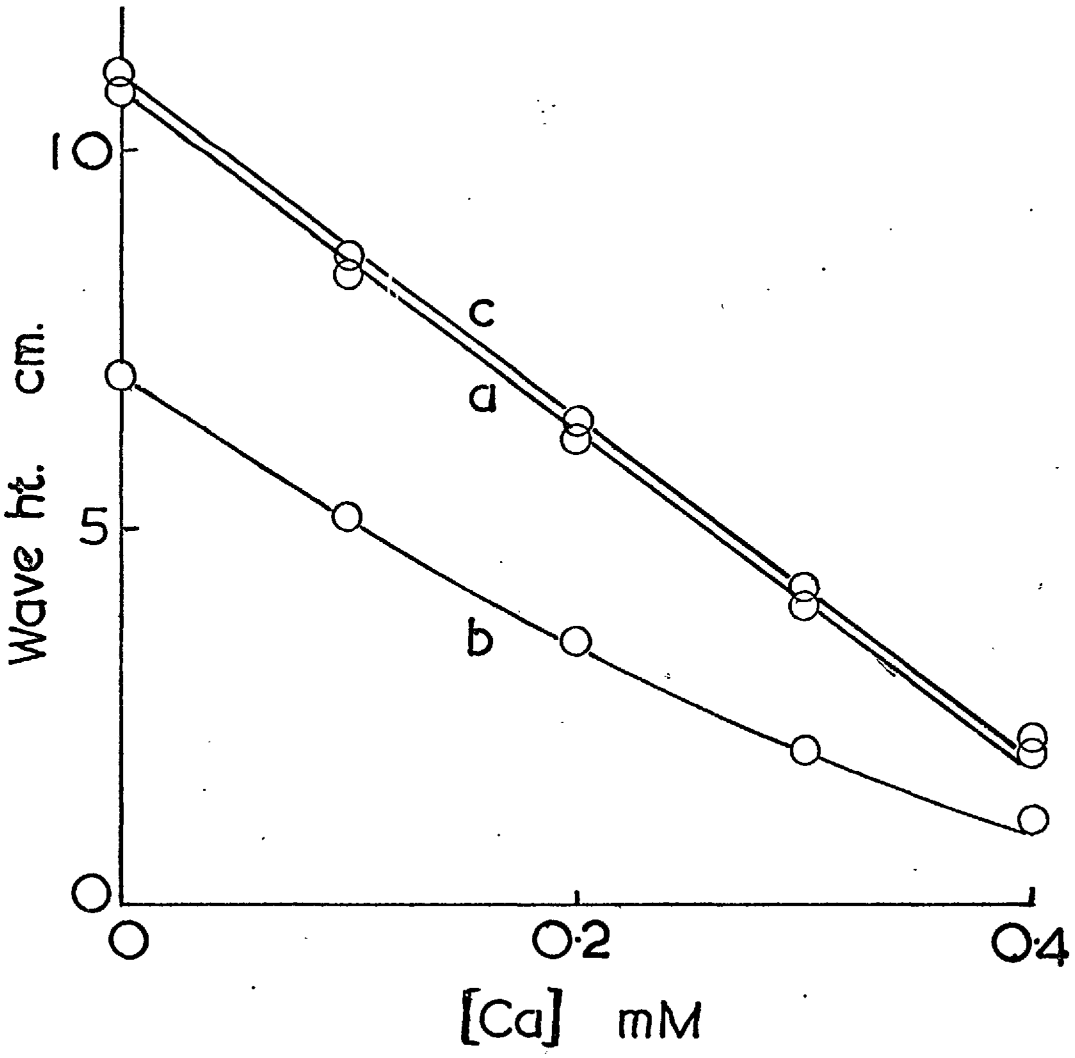


Figure 2.2.9 Overall effect of the use of masking agent. Wave heights of 0.6 mM EGTA in 0.15M ammonia/0.04M ammonium nitrate plotted against calcium concentration. Solutions also contain 0.25 mM magnesium; (a) theoretical (b) without any masking agent (c) in the presence of 2 mM sodium tripolyphosphate.

alone in the mixture using EGTA was interfered by the presence of magnesium which had been shown to be solved by using sodium tripolyphosphate as a masking agent. An evaluation of this second procedure is given in Figure 2.2.10 in which plots of $(i_{blank} - i_d)$ vs. concentration of calcium in the presence of different concentrations of magnesium are shown to be almost coincident with each other and with the plot for the case where no magnesium is present. Figure 2.2.11 shows a set of polarograms for one of the cases of Figure 2.2.10.

Table 2.2.3 gives some representative results on the analysis of synthetic samples treated as unknowns.

Table 2.2.3. Some representative results on the analysis of synthetic samples treated as unknowns, by the indirect d.c. polarographic method.

-	Ca + Mg	
	Present (mM)	Found (mM)
-	0·50	0·49
-	0·50	0·49
-	0·50	0·50
-	0·80	0·79
-	0·80	0·79
-	0·80	0·79
Magnesium present (mM)	Calcium	
	Present (mM)	Found (mM)
0·50	0·50	0·48
0·50	0·50	0·50
0·50	0·50	0·50
1·00	0·50	0·50
1·00	0·50	0·51
1·00	0·50	0·50
2·00	0·50	0·51
2·00	0·50	0·50
2·00	0·50	0·52

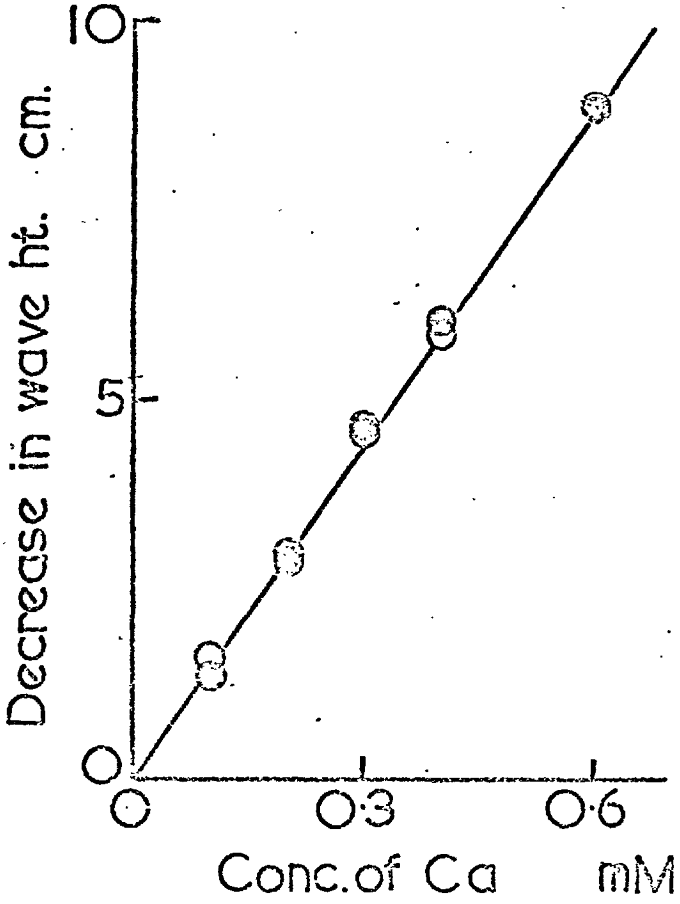


Figure 2.2.10 Evaluation of the method. Decrease in wave heights of EGTA plotted against calcium concentration. Solutions also contain (a) 0 mM (straight line) (b) 1 mM (unshaded circles) and (c) 2 mM (shaded circles) of magnesium. 2.5 mM sodium tripolyphosphate used as masking agent.

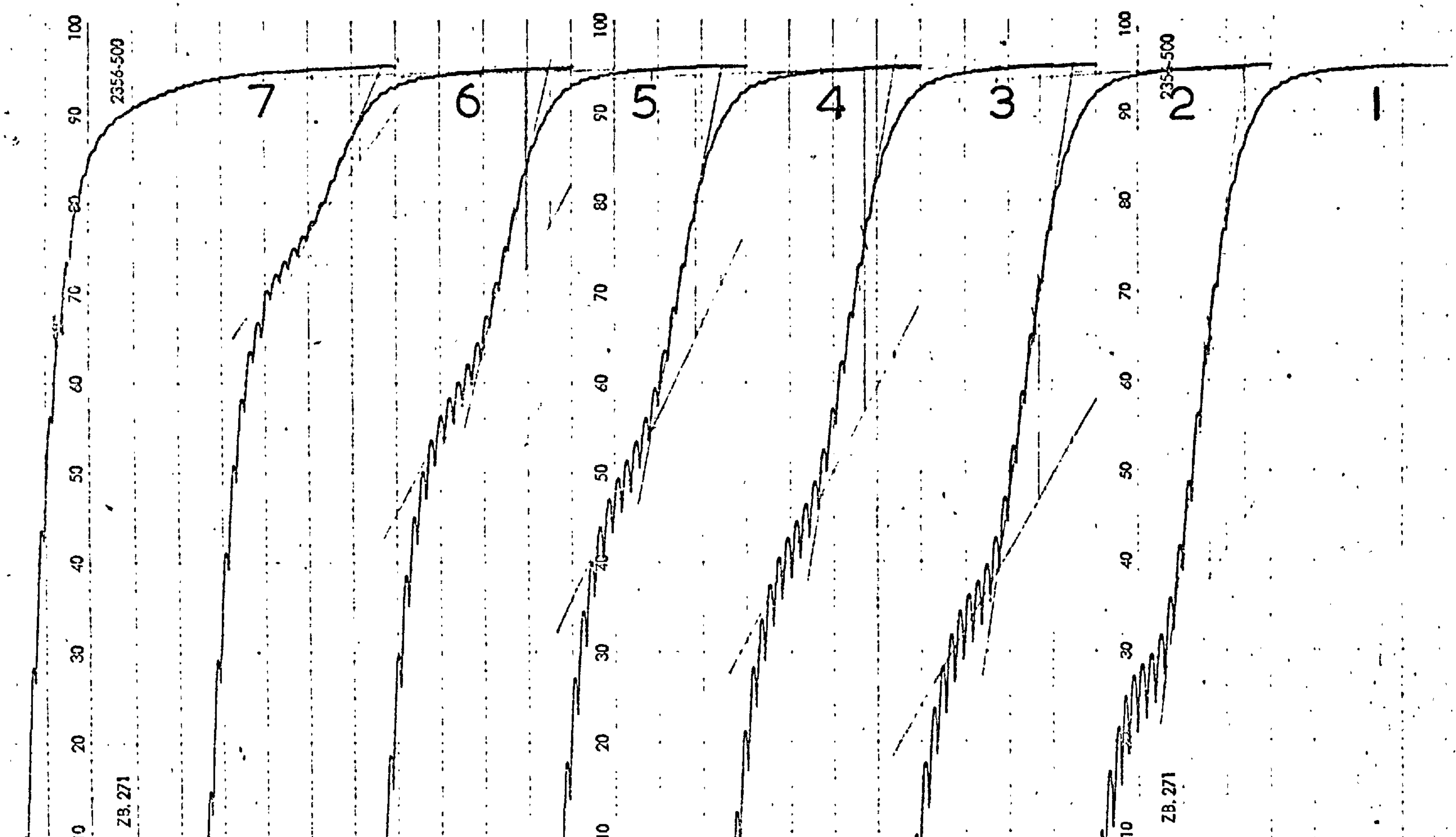


Figure 2.2.11 Anodic d.c. polarograms of *ca.* 0.8 mM EGTA in 0.22M ammonia/ 0.06M ammonium nitrate containing (1) 0 (2) 0.1 (3) 0.2 (4) 0.3 (5) 0.4 (6) 0.6 (7) 0.8 mM calcium. Solutions also contain 1 mM magnesium & 2.5 mM sod. tripolyphosphate. 33 mV/column, all curves start at -0.25V vs SCE, sens.=1/7 (6.5 uA f.s.d.).

The concentration of the complexones used in both cases in the above table was 1·0 x 10^{-3}M and in all the analyses above the decrease in diffusion current due to addition of the metal ions amounts to at least 50% of the original diffusion current. Larger errors accompany a lesser decrease in diffusion current due to uncertainties in the measurement of wave height and should be avoided.

(b) Amperometric titrimetric method.

For analysis of calcium alone in samples containing not very high concentrations of magnesium, the use of masking agents for magnesium was not found to be necessary in the titrimetric method, since any reaction between magnesium and EGTA after the calcium equivalence point merely decreases the slope of the portion of the titration curve after the equivalence point without appreciably affecting the position of the end-point on the volume axis. Results obtained without the use of any masking agent are thus shown in Table 2.2.4.

Table 2.2.4. Some representative results on the determination of calcium in the presence of magnesium by the amperometric titrimetric method.

Mg:Ca Molar ratio	Calcium	
	Present (mg)	Found (mg)
3:1	0·080	0·079
3:1	0·080	0·078
1:1	0·401	0·401
1:1	0·401	0·408
1:1	0·401	0·403
1:2	0·080	0·079
1:2	0·080	0·080

At high concentrations of magnesium exceeding a 3:1 ratio of magnesium to calcium, the increasing broadness of the curvatures at the end-point accompanying the decreasing slope of that portion of the titration curve after the equivalence point causes a large negative error to be incurred, if the magnesium is allowed to react with the excess of EGTA, unmasked. This may be seen in the titration curve for a magnesium to calcium ratio of 5:1 in Figure 2.2.12 as compared with those of 0:1 to 3:1.

Another disadvantage for not masking the magnesium is that a successive titration first for calcium with EGTA and then for magnesium in the same solution with EDTA cannot be performed. The added EDTA in

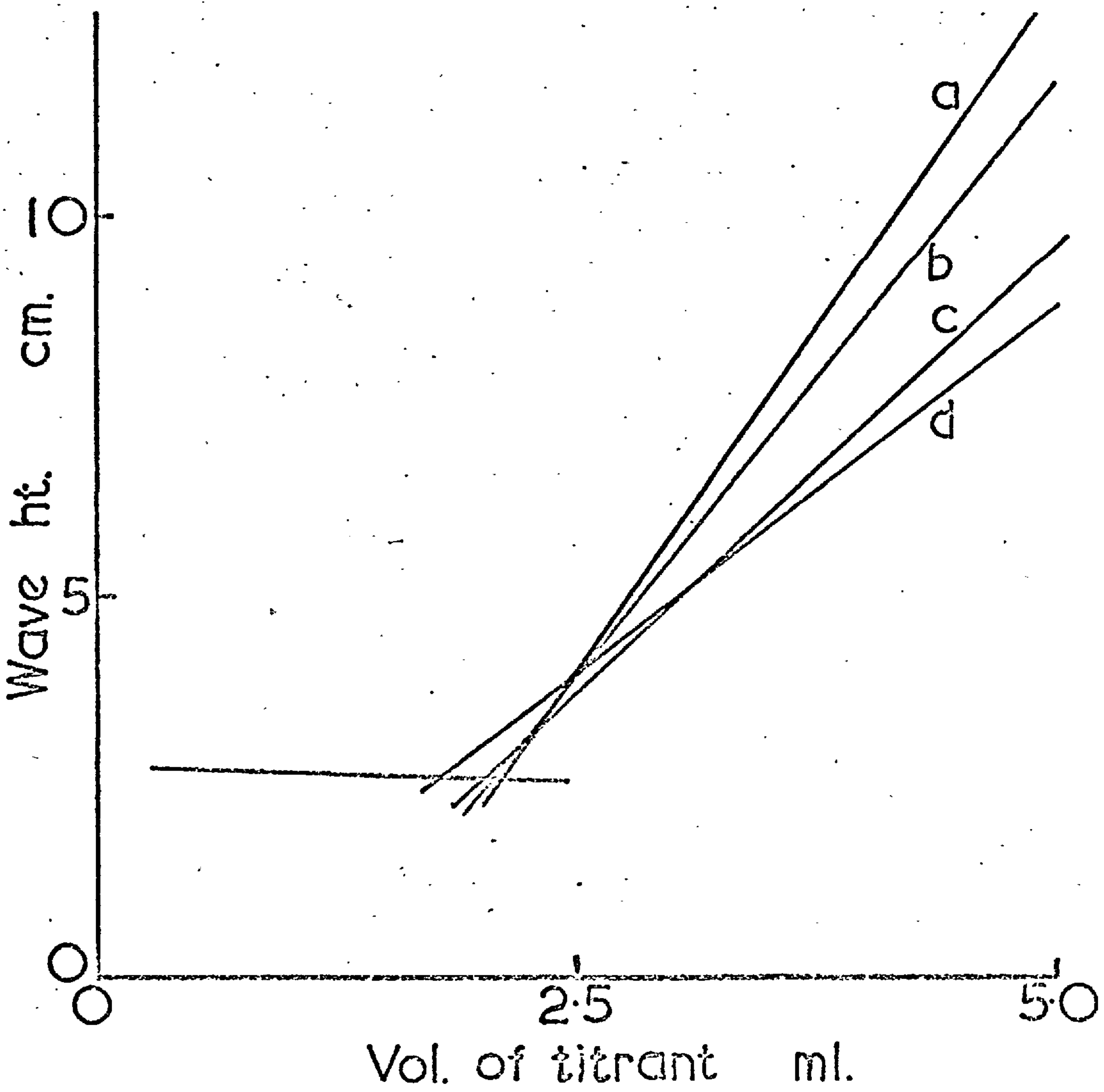


Figure 2.2.12 Amperometric titration curves of solutions containing increasing ratios of magnesium:calcium ; (a) 0:0.002 (b) 0.001:0.002 (c) 0.006:0.002 (d) 0.010:0.002. Applied potential= 0V vs SCE.

the second titration undergoes a nucleophilic chelate exchange reaction with the magnesium-EGTA, liberating free EGTA. The initial portion before the equivalence point of the second titration curve is therefore not horizontal since EGTA also gives an anodic current at the applied potential for EDTA. The diffusion current therefore continues to rise with a relatively large slope with the consequence that the second end-point is obscure.

In cases where large concentrations of magnesium are involved or where analyses for both calcium and magnesium in the same aliquot of sample is required, therefore, sodium tripolyphosphate or other masking agents as described in section 2.2.3 (d) must be employed.

In both methods ions which precipitate at or above pH <u>ca</u> 10 do not interfere as discussed in Section I, Chapter 1. Those masking agents which give anodic waves at potentials close to those of the complexones cannot be used but otherwise most conventional masking agents as well as those listed in Table 2.2.2 may be used. It is extremely difficult to quote an absolute sensitivity figure for the methods without involving instrumental sensitivity parameters. However, some idea on the sensitivity of the polarographic method may be obtained by an empirical calculation if one assumes that 0·1 microampere is a reasonable lower limit of measuring diffusion currents:

$$C = \frac{i}{Im^{\frac{2}{3}}t^{\frac{1}{6}}} = \frac{0 \cdot 1}{3 \times 2} = \underline{ca}\ 2 \times 10^{-5} M$$

since the diffusion current constant for both complexones is approximately 3 and a value of 2 for $m^{\frac{2}{3}}t^{\frac{1}{6}}$ roughly corresponds to the normal capillary characteristics of $t = \underline{ca}\ 4$ sec. and $m = \underline{ca}\ 2$ mg/sec. This sensitivity figure applies to a normal d.c. polarographic procedure and will increase if, for routine analysis modified techniques such as fast linear sweep cathode ray polarography or oscillopolarography etc. are employed to which the method should be equally applicable.

Preliminary investigations showed that the methods are applicable without any modification to the analysis of calcium and magnesium in sugar samples (crude syrup before crystallization). Direct analysis on blood serum however caused problems due to the high surface active properties of the samples and the latter must therefore be treated before analysis.

Mixed titrants

Preliminary investigations on an interesting use of a bi-component titrant consisting of two complexones in admixture had been made. Even though initiated purely out of curiosity, it had been found that such usage could simplify analytical procedures as will be shown, again, for the analysis of calcium and magnesium using EGTA and EDTA. A special advantage in this particular case is that no masking of magnesium is necessary as will be shown later. Figure 2.2.13 shows the polarograms of a 1:1 mixture of EDTA and EGTA with increasing concentration of magnesium. The diffusion current due to EDTA (first wave) decreases whereas that due to EGTA (second wave) remains constant, as expected from the stability constants $10^{8.7}$ and $10^{5.2}$ for magnesium-EDTA and magnesium-EGTA chelates respectively. A similar increase in the concentration of calcium decreases both waves simultaneously, corresponding to the stability constants $10^{10.7}$ and $10^{11.0}$ for calcium-EDTA and calcium-EGTA chelates respectively. Thus if one has two calibration curves, namely, total diffusion current of EDTA and EGTA vs. metal ion concentration (calcium or magnesium or their

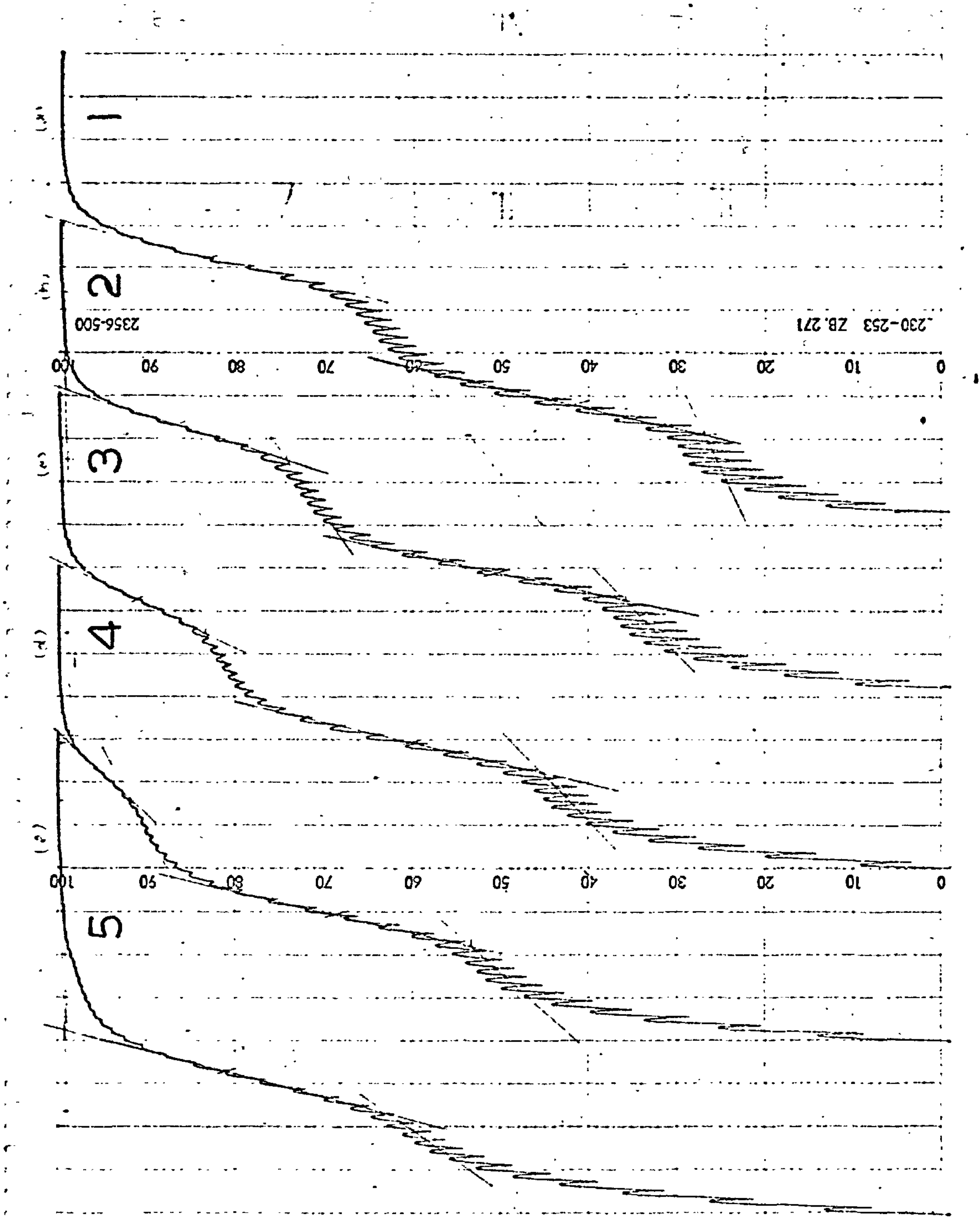


Figure 2.2.13 Anodic d.c. polarograms of the mixed titrant (EDTA+EGTA, 0.5 mM with respect to each) in 0.3M ammonia/0.1M ammonium nitrate containing (1) 0.1 (2) 0.2 (3) 0.3 (4) 0.4 (5) 0.5 mM magnesium. The first wave is EDTA and the second one EGTA. 33 mV/column, all curves start at -0.3V vs SCE, sens.=1/7 (6.5 uA f.s.d.)

mixture) and diffusion current of EGTA alone vs. calcium concentration it is obvious that for the determination of both calcium and magnesium in a mixture, all that one needs is a single polarogram of a solution of the mixed titrant containing the sample (calcium + magnesium). Concentrations of calcium alone and of total calcium and magnesium are then read off from the calibration curves.

An added advantage is that since an excess of EDTA is always present, no problem of the interfering reaction between magnesium and EGTA exists and therefore no question of masking of magnesium arises.

2.2.5. Experimental

The apparatus used have been described earlier (Section II, Chapter 1). The DME had the following capillary characteristics: m = 1·965 mg/sec (at OV) and t = 4·1 sec (at OV).

The preparation and standardization of EGTA as well as solutions of calcium and magnesium have been described earlier. EDTA was prepared from its disodium salt and similarly standardized. An approximately 0·1M stock solution of the masking agent (sodium tripolyphosphate) was prepared. The ammonia buffer used approximates the composition $3{\cdot}6M\ NH_3/1{\cdot}0M\ NH_4NO_3$ (pH 9·8).

Procedures

(a) Indirect d.c. polarographic method.

(i) Determination of total calcium and magnesium.

Prepare in a volumetric flask a solution containing $1{\cdot}0 \times 10^{-3}M$ EDTA in $0{\cdot}22M\ NH_3/0{\cdot}06M\ NH_4NO_3$ and 0·002% Triton X-100, and also containing the sample which when made up to volume will have a concentration of calcium and magnesium enough to decrease the anodic diffusion current of EDTA by at least 50%. Record a polarogram, measure the anodic diffusion current and read off concentration of total calcium and magnesium from a calibration curve constructed under similar conditions using standard solutions of calcium or magnesium. The concentration of EDTA may be varied to suit the concentration of sought-for substances present in the samples.

(ii) Determination of calcium alone in the presence of magnesium.

Prepare in a volumetric flask, a solution containing $1{\cdot}0 \times 10^{-3}M$ EGTA in $0{\cdot}22M\ NH_3/0{\cdot}06\,M\ NH_4NO_3$, 0·001% Triton X-100 and $2{\cdot}5$ to $10{\cdot}0 \times 10^{-3}M$ sodium tripolyphosphate and also containing the sample which when made up to volume will have a concentration of calcium enough to decrease the anodic diffusion current of EGTA by at least 50%. Record a polarogram, measure the anodic diffusion current and read off concentration of calcium from a calibration curve constructed under similar conditions using standard solutions of calcium alone in the absence of any magnesium. The concentration of EGTA may be varied to suit the concentration of sought-for substance present in the sample.

(b) Amperometric titrimetric method.

(i) Determination of calcium alone in the presence of magnesium.

Titrate a solution of the sample in ammonia buffer (ca $0 \cdot 22\,M\,NH_3/0 \cdot 06\,M\,NH_4NO_3$) and 0·001% Triton X-100 against a standard solution of EGTA. Detect the end-point amperometrically by applying to the DME a potential of OV vs. SCE which lies on the limiting current plateau of the EGTA anodic wave. The anodic current increases linearly from zero beyond the end-point. The concentration of the EGTA titrant has to be adjusted to suit the amount of sought-for substance present so that a large increase in volume is not incurred during the titration.

(ii) Determination of total calcium and magnesium.

The procedure is the same as above but using EDTA as the titrant. The applied potential 0V vs. SCE lies also on the limiting current plateau of the EDTA anodic wave.

If the sample contains a high concentration of magnesium, incorporate a suitable concentration of masking agent (2·5 to 10 x 10^{-3}M sodium tripolyphosphate) in procedure (i). The same applies if after procedure (i) a successive titration in the same solution for magnesium with EDTA is to be performed. (Explanations have been given in section 2.2.4).

2.2.6. Conclusion

The advantageous combination of complexometric principles (such as the use of selective titrants, masking agents etc.) with the technique of d.c. polarography has been demonstrated by the development of practical analytical methods for the important determination of calcium and magnesium in the presence of each other.

The concept of mixed titrants is introduced and an interesting use of the same is also described.

SECTION II

CHAPTER 3

ANALYTICAL APPLICATIONS OF A.C. POLAROGRAPHY OF COMPLEXONES.

2.3.1. Introduction

Alternating current (a.c.) polarography of complexones has until now evaded investigation. In the present chapter will be described a study on the a.c. polarography of EDTA and related compounds with a view to analytical applications, especially in trace analysis, even though one may remember that a.c. polarography is basically not a trace technique; it is supposedly less sensitive than its d.c. counterpart. Potentials of the technique for trace analysis will be demonstrated by the analysis of calcium in the presence of magnesium using a method similar to that developed earlier (Section II, Chapter 2).

A.C. polarography consists essentially of superimposing a small alternating voltage on a linearly increasing d.c. potential applied to a DME, and plotting the alternating current produced against the d.c. potential. At potentials corresponding to the foot of a d.c. polarographic wave, the alternating current is entirely due to charging and discharging of the double layer and is therefore relatively small. The same processes are responsible for the alternating current at potentials corresponding to the plateau of the d.c. wave since the electroactive species is consumed as fast as it reaches the electrode surface at any time during the alternating voltage cycle. At potentials corresponding to the rising portion of the d.c. wave, however, the effect of a superposed sinusoidal alternating voltage is to produce periodic concentration changes of the oxidized and reduced forms at the interface. These concentration changes are accompanied by periodic diffusion processes and the flow of alternating current (faraidic alternating current). The greatest variations of the composition of the layer of solution around the drop occur at the half-wave potential and a maximum alternating current is thus observed at that potential. The "a.c. polarographic wave" thus obtained is peak shaped. The maximum alternating current obtained is a function of the reversibility of the electrode reaction. Very little or no concentration changes of the oxidized and reduced forms can occur if the half-reaction is irreversible and hence the alternating current at the peak is much smaller than for reversible reactions. This aspect of the technique permits a study of the rates of fast electron-transfer processes. A linear relationship between the peak height (alternating current at summit potential, Δi_s) and concentration under favourable conditions also permits analytical applications.

The most important advantages of the technique in analytical work over its d.c. counterpart are the possibility of working in the presence of air, low temperature coefficient, better resolution of closely-spaced waves and non-interference by huge waves preceding the one under investigation. A comprehensive treatment of the technique is given in a monograph by Breyer and Bauer.[66]

2.3.2. General a.c. polarographic behaviour of EDTA and related compounds.

Figure 2.3.1 shows the a.c. polarographic waves of EDTA and two related compounds, EGTA and CDTA. The experimental observations of

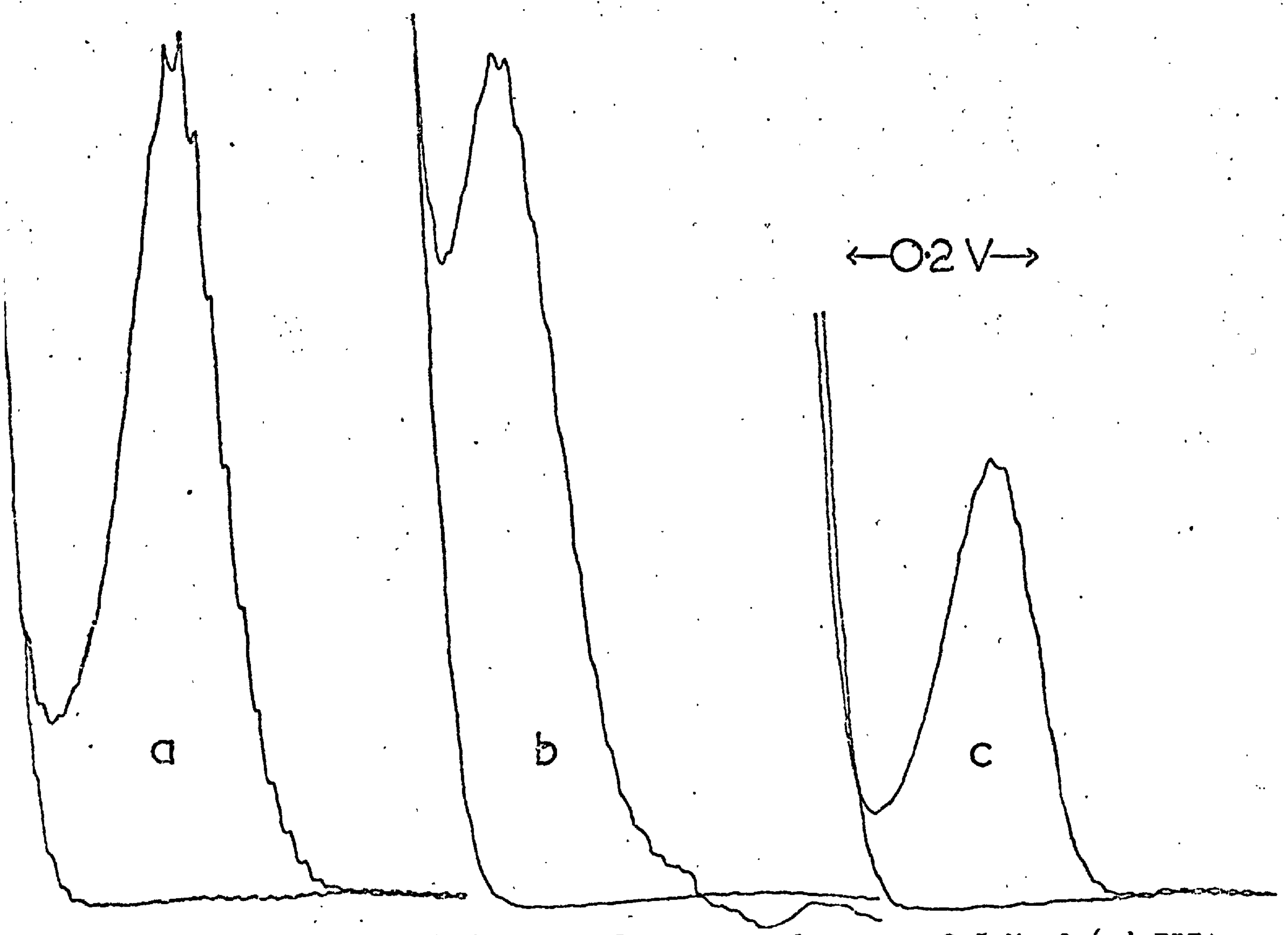


Figure 2.3.1 A.C. polarograms of some complexones. 0.5mM of (a) EDTA (b) EGTA (c) CDTA in ammonia buffer (0.3M ammonia/ 0.1M ammonium nitrate). All polarograms start at -0.4V vs SCE.

the a.c. waves do not agree with the theoretical treatment assuming simple electrode reactions. For example, whereas the simple electrode reaction theory predicts that the summit potentials (E_s) in the a.c. waves be equal to their corresponding d.c. half-wave potentials ($E_{\frac{1}{2}}$) and that the width of the a.c. waves at half-height be equal to 90.5/n where n = 2 in this particular case, none of these come true in the experimental results given in Table 2.3.1.

Table 2.3.1. Some a.c. polarographic data of complexones (from Figure 2.3.1)

Complexone	$E_{\frac{1}{2}}$ (d.c.)	E_s (a.c.)	$\Delta E_{s/2}$	Relative* a.c. peak height
EDTA	-0.083	-0.093	80	3.3
EGTA	-0.010	-0.003	-	4.7
CDTA	-0.110	-0.100	117	2.3

* Relative to d.c. wave height i.e. a.c. peak height/ d.c. wave height.

The experimental observations on the dependence of the a.c. peak heights (Δi_s) on concentration of depolariser, shown in Figure 2.3.2, also contradicts with the simple electrode reaction theory that they be linear.

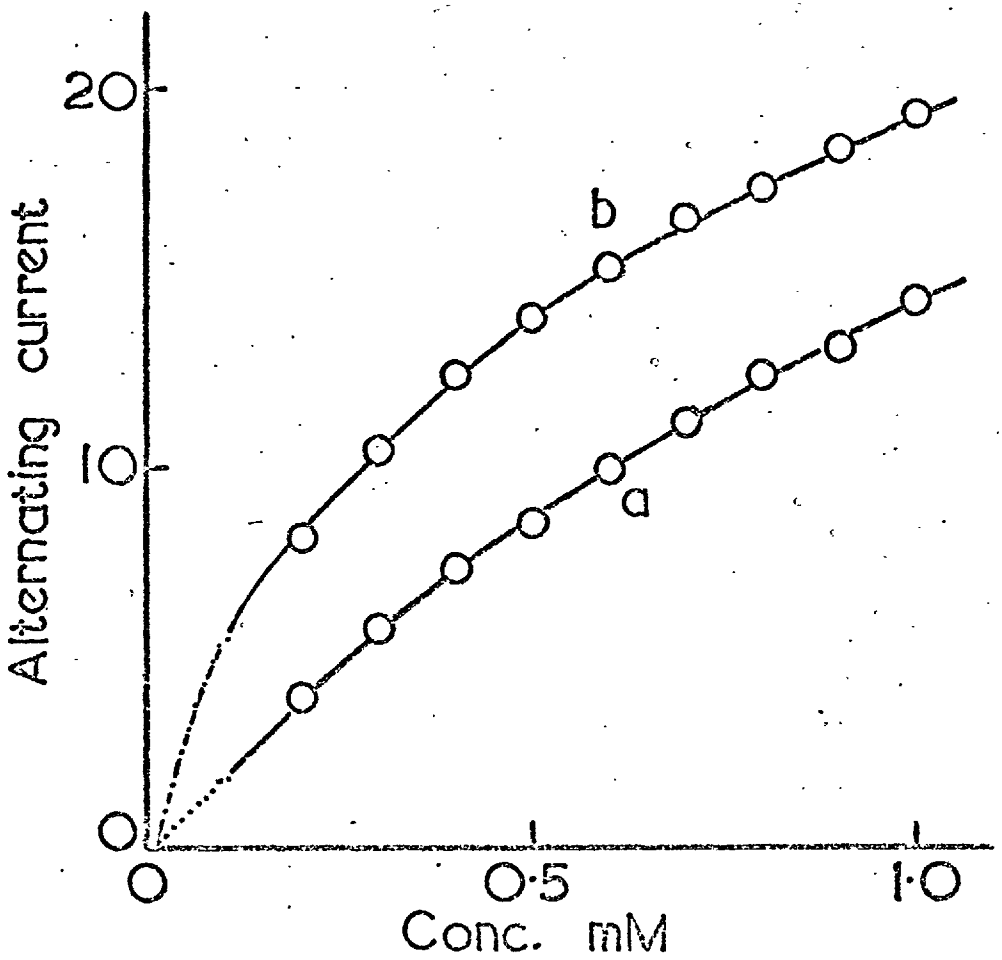


Figure 2.3.2 Dependence of alternating current at summit potential on concentration of depolariser in the a.c. polarography of some complexones: (a) EDTA (sens.=1/50) (b) EGTA (sens.=1/30) in 0.3M ammonia/0.1M ammonium nitrate.

The experimental observations are found to be best interpreted by means of a theory which takes into account the adsorption of the depolariser.[66] The difference between $E_{\frac{1}{2}}$ and E_s is given by this theory as,

$$E_{\frac{1}{2}} - E_s = \frac{RT}{nF} \ln \frac{b_o}{b_R} + \frac{RT}{nF} \ln d^{\frac{1}{2}} \qquad (1)$$

where b_R and b_o are the Langmuir adsorption coefficients and d is the ratio of the diffusion coefficients of the reduced and oxidized species. The shape of the concentration dependence curve is also satisfactorily explained by the equation

$$\triangle i_s = \frac{KaBC}{1 + 2BC} \qquad (2)$$

where K is a factor that includes a number of parameters such as the number of electrons transferred per molecule, the amplitude and frequency of the alternating voltage, etc., <u>a</u> is a parameter that depends on the size of the depolariser molecules in the adsorbed state and

$$B = \frac{b_o b_R}{b_o + b_R} \qquad (3)$$

Equation (2) predicts a non-linear relationship between peak height and concentration. However, at low concentrations ($1 \gg 2BC$), the peak height is a linear function of concentration, i.e.

$$\Delta i_s = KaBC \text{ for } 1 \gg 2BC \qquad (4)$$

which can be clearly seen in Figure 2.3.2 for both complexones; the slope of the straight line (= KaB) will be larger than that for an unadsorbed species (= K) i.e. higher sensitivity, since in the case of adsorption there will be a surface excess of the depolarizer. At higher concentrations the peak height becomes increasingly independent of concentration as in Figure 2.3.2 and ultimately at very high concentrations ($2BC \gg 1$), the peak height reaches a limiting value equal to $\frac{1}{2}Ka$. Calculations of the adsorption coefficients b_O and b_R should in principle be possible since from the shape of the curves in Figure 2.3.2 one can easily obtain the value of B (using equation (2)) and the ratio b_O/b_R may be computed from the separation between $E_{\frac{1}{2}}$ and E_s (equation (1)). The summit potential for EDTA is found to be entirely independent of concentration (2×10^{-5} to 10^{-3} M) whereas that for EGTA varies slightly between -0.007 to +0.013 V vs. SCE in the concentration range 6×10^{-5} to 10^{-3} M.

The last column in Table 2.3.1 gives a rough measure of the reversibility of the electrode reactions. Thus a very small value for CDTA indicates that the electrode reaction is not fully reversible which is in agreement with d.c. polarographic data in which a difference of about 60 mV was found between the half-wave potentials of the anodic wave of CDTA and cathodic wave of Hg-CDTA.[51] The large value for EGTA indicates a fully reversible nature of the electrode reaction supporting the d.c. polarographic evidence described in Section II, Chapter 1. An intermediate value for EDTA seems to indicate an intermediate degree of reversibility between EGTA and CDTA perhaps corresponding to a disagreement in the d.c. polarographic studies where Matyska et al[51] reported full reversibility of the EDTA wave whereas Michel et al[50] reported a difference of about 25 mV between the half-wave potentials of the anodic and cathodic steps. Even though these qualitative observations on electron transfer rates are desirable in a more quantitative form, the relatively simple equipment used (see Experimental) do not permit such a study to be made.

2.3.3. Analytical applications

Although the general shape of the concentration dependence curves are curved, it has been shown by theory (equation (4)) and by experiment (Figure 2.3.2) that at low concentrations the dependence is linear. This can be seen more clearly in Figure 2.3.3 which shows the usefulness of the a.c. polarographic technique for analytical purposes in the range 10^{-5} to 2×10^{-4} M for EDTA and 2×10^{-5} to 10^{-4} M for EGTA. Even though due to its higher adsorption the EGTA gives a higher a.c. polarographic response than EDTA (significance of B in equation (4)), the concentration range where a linear relationship exists is less for EGTA than for EDTA. This is also easily explained by means of equation (4) since B being large for EGTA the concentration (C) must be kept low enough so that the condition $1 \gg 2BC$ is maintained. It must be borne in mind however that the strength of adsorption and hence the sensitivity and range of analytical utility depends markedly on such experimental parameters as the nature of the supporting electrolyte and pH of the solution etc. No generalizations can be made and separate studies must be made experimentally for each particular case.

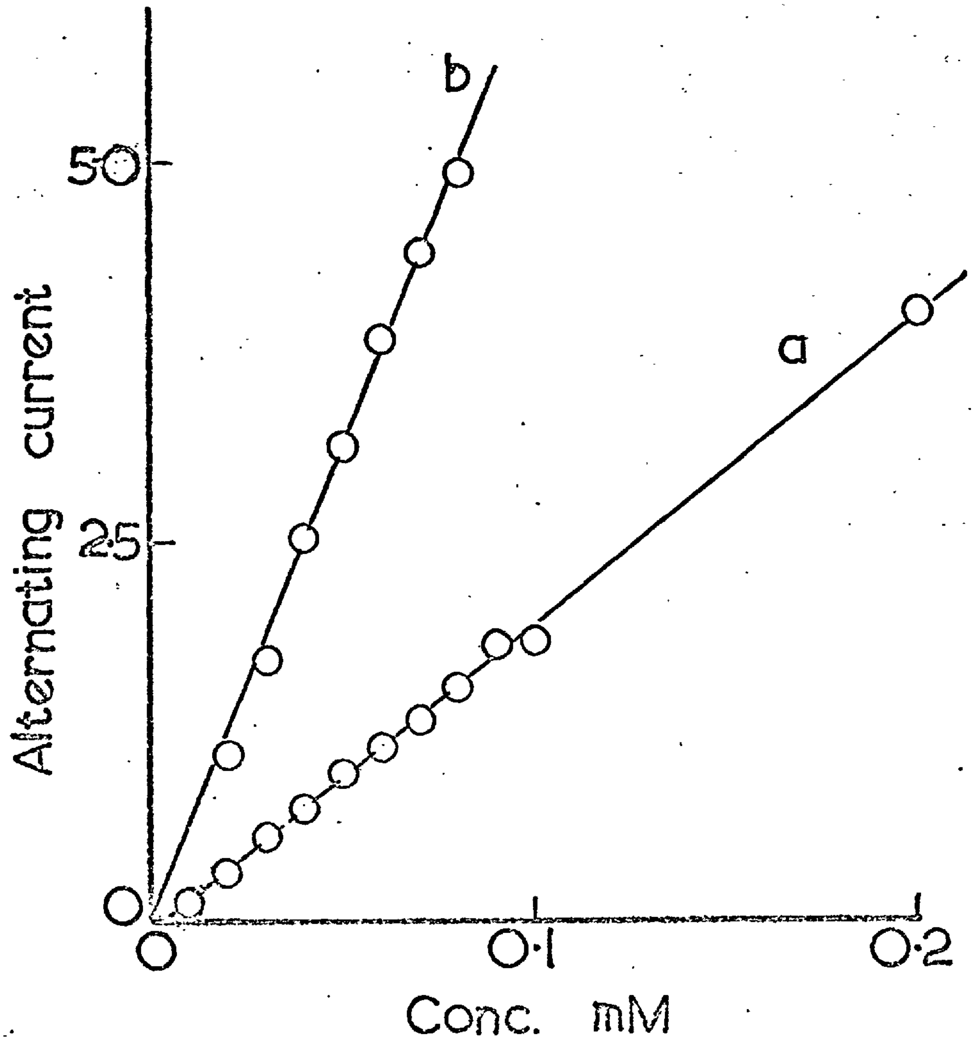


Figure 2.3.3 Dependence of alternating current at summit potential on concentration of depolariser in the a.c. polarography of some complexones: (a) EDTA (sens.=1/50) (b) EGTA (sens.=1/30) in 0.3M ammonia/0.1M ammonium nitrate.

The summit potentials are totally unaffected and peak heights are only diminished by an insignificant amount when a.c. polarographic measurements of the complexones are made in the presence of air. The technique is therefore suitable with large electrodes used for higher sensitivity, where the utility is limited to a large degree by interference due to slight traces of oxygen.

A practical application of the a.c. polarography of complexones for trace analysis is illustrated by the analysis of from 0•2 to 1•0 ppm calcium in the presence of magnesium by an indirect method similar to that developed in Section I, Chapter 2. An evaluation of the method is given in Figure 2.3.4 where the closeness of the experimental points (shaded circles) to the calibration curve illustrates the accuracy of the method.

2.3.4. Experimental

Preparation of solutions had been described earlier.

The equipment used was a Cambridge General Purpose Polarograph in conjunction with a Cambridge "Univector" A.C. Polarograph Unit (Cambridge

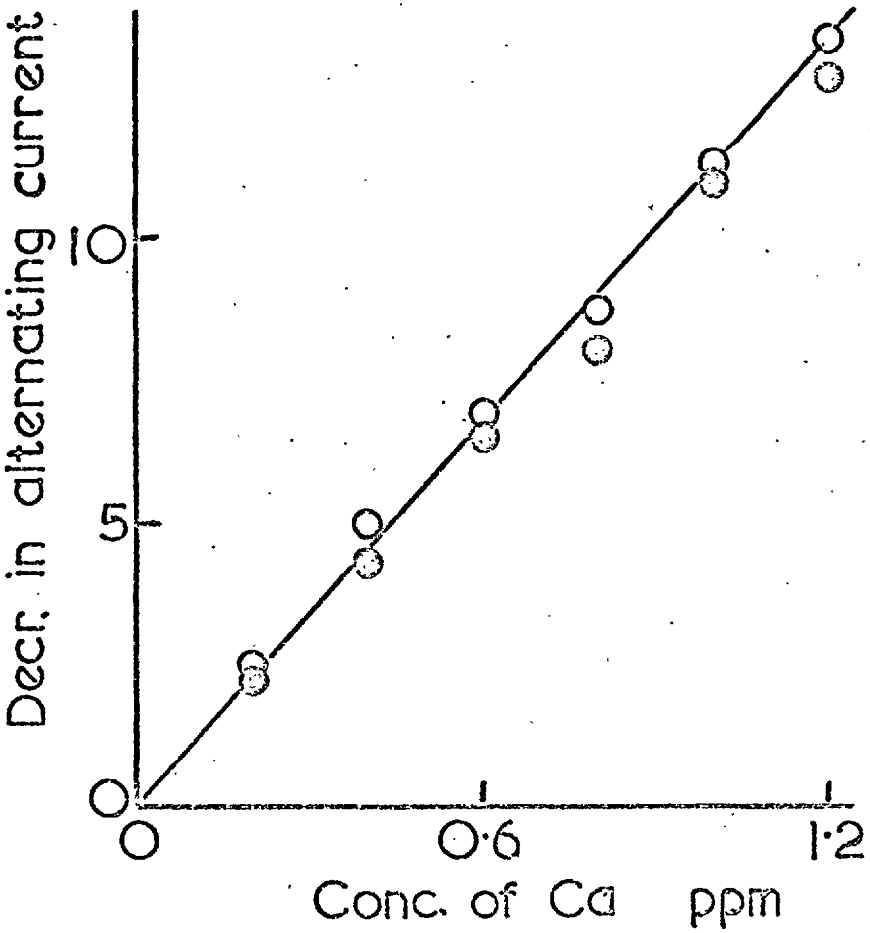


Figure 2.3.4 Dependence of decrease in a,c. on concentration. Unshaded and shaded circles represent concentrations of calcium in the absence and presence (0.1 mM) of magnesium respectively. Reagent solution consists of 0.05 mM EGTA in 0.3M ammonia/ 0.1M ammonium nitrate and 0.2 mM sodium tripolyphosphate.

Instruments Co. Ltd.); a Servoscribe recorder (Smiths Industries Ltd.) was used for recording polarograms. The Univector A.C. Polarograph Unit contains a phase-sensitive base-current suppression circuit and is therefore quite sensitive for analytical work. The amplitude and frequency of the alternating voltage however cannot be readily varied and is therefore not suitable for rate studies. The amplitude is ca 25 mV and frequency ca 35 c.p.s.

The DME had the following capillary characteristics under open circuit conditions:

$m = 1{\cdot}965$ mg/sec. and $t = 4{\cdot}1$ sec.

5 ml aliquots of the solutions are used for polarographic measurements in a Kalousek cell.

2.3.5. Conclusion

Attempts had been made to explain the observed a.c. polarographic behaviour of the complexones using existing theory. The most satisfactory interpretation of experimental data was achieved by means of the theory which takes into account the adsorption of depolariser. Qualitative a.c. polarographic data had been shown to support the d.c. polarographic evidence on the question of the reversibility of the anodic oxidation of the three complexones EDTA, EGTA and CDTA. Analytical potentials of the a.c. polarography of complexones had been given and demonstrated by a trace analysis of calcium in the presence of magnesium.

SECTION III

CONTINUOUS ANALYSIS VIA COMPLEXOMETRY USING ELECTROCHEMICAL SENSORS

SECTION III

INTRODUCTION

The principle of "continuous analysis" has been introduced extensively at the beginning of the thesis. This final section deals with the application of electrochemical sensors for continuous analysis using complexometric principles. It must be admitted here that even though a rather broad term "electrochemical sensors" has been used, only voltammetric sensors are the real concern of the thesis; the generalised term being used simply because the electrodes and electrode configurations that will be described would also be applicable to other types of electrochemical phenomena other than voltammetry, such as potentiometry or coulometry.

The idea of using voltammetric electrodes for continuous analytical sensing was conceived quite early in the century and a report on the first polarographic analyser[67] appeared in the literature as early as 1933, followed in 1947 by a paper by Müller[68] on a study of the platinum micro "by-pass" electrode where he pointed out the analytical possibilities. Later publications on the subject dealt with the design of polarographic flow-through cells[69-72] and the use of special electrodes such as a shielded DME[73] to ensure non-empirical diffusion conditions, a rapidly dropping DME[74] to desensitise against agitation or sample flow, a wide-bore DME[75,76] to extend the period of reproducibility of results and mercury pool electrodes[77,78] for higher sensitivity. Experimental set-ups for adaptation of the technique of continuous polarographic sensing to ion-exchange separations,[79-82] gas analysis by gas-chromatography[83] had been described, and continuous polarographic analysers for specific purposes such as for dissolved oxygen,[74-76,84,85] oxygen in blood _in vivo_,[86] oxygen in gases,[77,87] uranium,[88,89] α-amino acids,[90] sulphur dioxide,[91] Ti^{3+} and Fe^{3+},[92] carbon monoxide,[92] cyanide,[73,93] mercury[77] and hydrogen sulphide,[83] had been developed.

Whilst reports on the performance of the individual analysers had been encouraging, it is obvious that a major draw-back in the development of the technique of continuous polarographic analysis as a whole, is the inflexibility of the analysers developed. The lack of a common underlying "principle" in the latter inhibits growth of the range of applications. For example, in the analysers given above, although measurement of well-defined limiting currents at appropriate applied potentials provide a simple answer for electroactive species, it is much less so with substances like α-amino acids[90] and carbon monoxide[92] where the chemistry involved is complex, and even more important is the lack of a foundation for wide application. In the carbon monoxide analyser, for instance, an equivalent amount of iodine set free by passing the gas containing carbon monoxide over heated iodine pentoxide is continuously oxidised to iodate by a dilute alkaline solution of hypobromite, excess hypobromite being continuously reduced by sodium sulphite. The cathodic current due to iodate is proportional to the concentration of carbon monoxide. The method, though ingenious, simply lacks a foundation for general application.

In this last section of the thesis, the "principle" of complexometry is introduced to the technique of continuous polarographic analysis. This provides an underlying principle with which wide applications are possible and in theory virtually all methods of complexometric analysis are made available to be put on a continuous basis with polarographic sensing. This technique of "complexometric continuous-polarographic-analysis" as it might logically be termed, is equally useful for electroactive as well as electroinactive substances since it only involves measurement of anodic waves of complexones, and is also equally applicable to metals as well as non-metals in view of the large number of indirect complexometric methods for non-metals. Selectivity is also improved since the selectivity provided by complexometry by the use of selective complexones, masking agents and choice of pH supplements that already provided by polarography. In the first chapter of this section the design and development of a continuous polarographic analyser for the <u>simultaneous</u> determination of calcium and magnesium, using complexometric principles are described and in the second and last chapter an investigation on the use of some continuous electrochemical sensors other than the DME is described, again with an emphasis on the use of complexometric principles.

SECTION III

CHAPTER 1

APPLICATION OF COMPLEXOMETRIC PRINCIPLES IN CONTINUOUS POLAROGRAPHIC ANALYSIS. A CONTINUOUS POLAROGRAPHIC ANALYSER FOR SIMULTANEOUS ANALYSIS OF CALCIUM AND MAGNESIUM.

3.1.1. Introduction

Continuous automated methods for the analysis of calcium and magnesium have been surprisingly rare in spite of the obviously large demand for such analytical methods. Colorimetric (absorptiometric) sensing is employed in all cases. In an AutoAnalyzer recommended method for calcium,[94] for example, the absorbance of a coloured calcium complex is measured compensating for any interference by magnesium by empirical additions of magnesium to the standard. Most magnesium methods are based upon the development of a coloured complex between magnesium hydroxide and a "lake" forming reagent.

The development of a continuous polarographic analyser for the simultaneous analysis of calcium and magnesium is described in the present chapter; together with it the design and characteristics of a general purpose polarographic flow-through cell is presented and polarographic behaviour unique to measurements in a flowing stream are discussed.

3.1.2. Polarographic flow-through cell.

A cross-sectional diagram of the cell is shown in Figure 3.1.1. It is constructed from a block of clear perspex (3" x 2" x 1") so that the functioning of the DME, the process of nitrogen gas removal, solution passage, etc., can be easily observed. The axial 3 mm hole for the solution stream is first drilled on a lathe and then the passages for the DME (4 mm), nitrogen exit (4 mm) and mercury collection pool (4 mm) are drilled to a depth at which the drill point just enters the axial hole. The outside ends of the holes are enlarged to a diameter of 7 mm to a depth of approximately 1 cm so that tight-fitting polythene tubing (or other suitable material) sleeves can be push-fitted into the holes. The drilled holes are then examined to ensure freedom from any irregularities; the axial hole for the solution stream especially should be smooth and free from rough spots or traps which might hold gas bubbles or mercury drops. They would also cause turbulence in the solution stream.

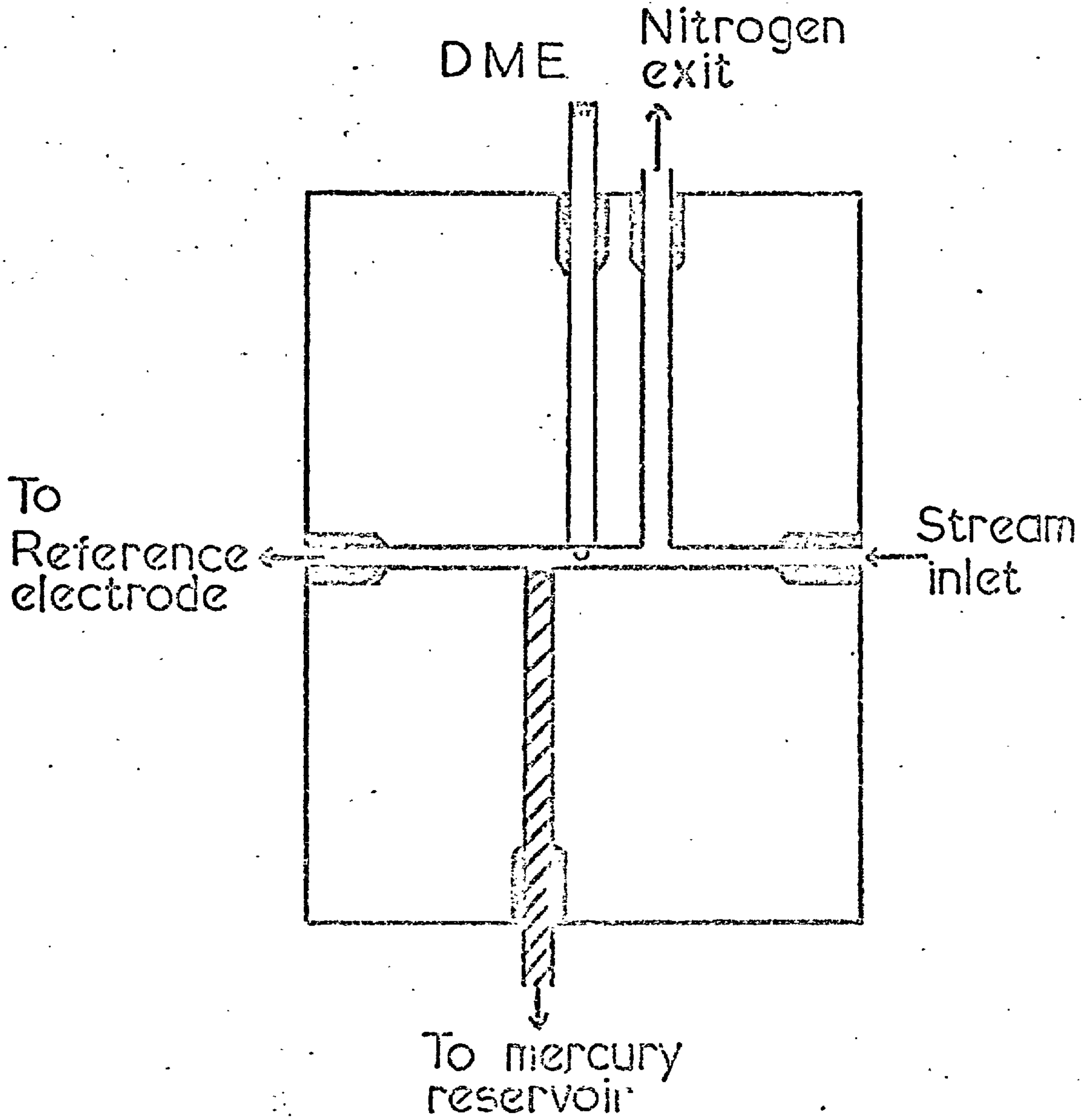


Figure 3.1.1 Polarographic flow-through cell.

The solution stream flows from right to left in in the cell, as shown in the diagram, so that bubbles of gas (nitrogen) are removed before the solution reaches the DME; the cell is slightly tilted downwards (a few degrees from the horizontal) so that mercury drops roll down the slope to be collected in the mercury pool downstream. The mercury pool is connected via polythene tubing to a huge mercury reservoir the height of which is adjusted so that the level of the mercury pool is a few millimetres below the solution stream. The mercury pool level remains practically constant for long periods of time because of the large surface area of the levelling reservoir.

Four important factors have been given the utmost consideration in the design of the cell, namely, low sample hold-up, absence of stagnant regions, elimination of inter-sample mixing and finally the avoidance of turbulence. The first three ensure a fast response of the cell to changes in sample composition and the last factor prevents the introduction of uncertain diffusion characteristics.

Sample hold-up is absolutely nil in the cell described since the sample solution is merely pumped past the electrode without any "holding" of the sample. The DME is positioned so that the mercury drops fall from a position level with the top wall of the axial hole; any positioning above this level would introduce a stagnant region. The mercury collection pool, for the same reason, has not been placed directly beneath the DME since a stagnant region would have appeared in the small volume between the bottom wall of the axial hole and the mercury meniscus. Turbulence around the electrode, caused by the vibrating mercury meniscus (due to the pulsating action of the peristaltic pump, etc.) is, at the same time, eliminated.

The nitrogen gas bubbles, used as a segmenting gas for efficient mixing and pumping and as a deoxygenating gas, also serves to separate consecutive samples. It is therefore desirable that the removal of nitrogen bubbles from the solution stream be done as close to the DME as possible in order to avoid lateral inter-sample mixing. Exit of gas however is found to cause considerable turbulence around the electrode and a compromising optimum distance of about 6 mm between the DME and nitrogen exit have been obtained by repeated experimentation at different gas flow rates.

The process of nitrog~~r~~en gas removal is accomplished by pumping the solution out of the cell at a rate smaller than that at the cell entrance. The nitrogen gas, together with some solution, is thereby efficiently removed.

Calomel reference electrodes are normally used and are connected to the solution stream via an inverted T-piece at the exit end of the cell. A potassium nitrate-saturated agar plug contact or for long term purposes a fritted glass contact may be used in the vertical arm of the T-piece. The apparent resistance of the cell, measured with a Phillips Conductivity Bridge (PR 9500/01), using a SCE and a potassium nitrate-saturated agar plug contact is <u>ca</u> 4K in a 0·1M perchloric acid electrolyte solution in static condition.

Figure 3.1.2 shows the cell response to abrupt changes in sample concentration. Calculation from data similar to Figure 3.1.2 showed that on changing the solution from 0·22M NH_3/0·06M NH_yNO_3 containing no EDTA to one containing 5 x 10^{-4} M EDTA, the current rose to 95% and 100% of the steady state value in one and two minutes respectively. On changing in

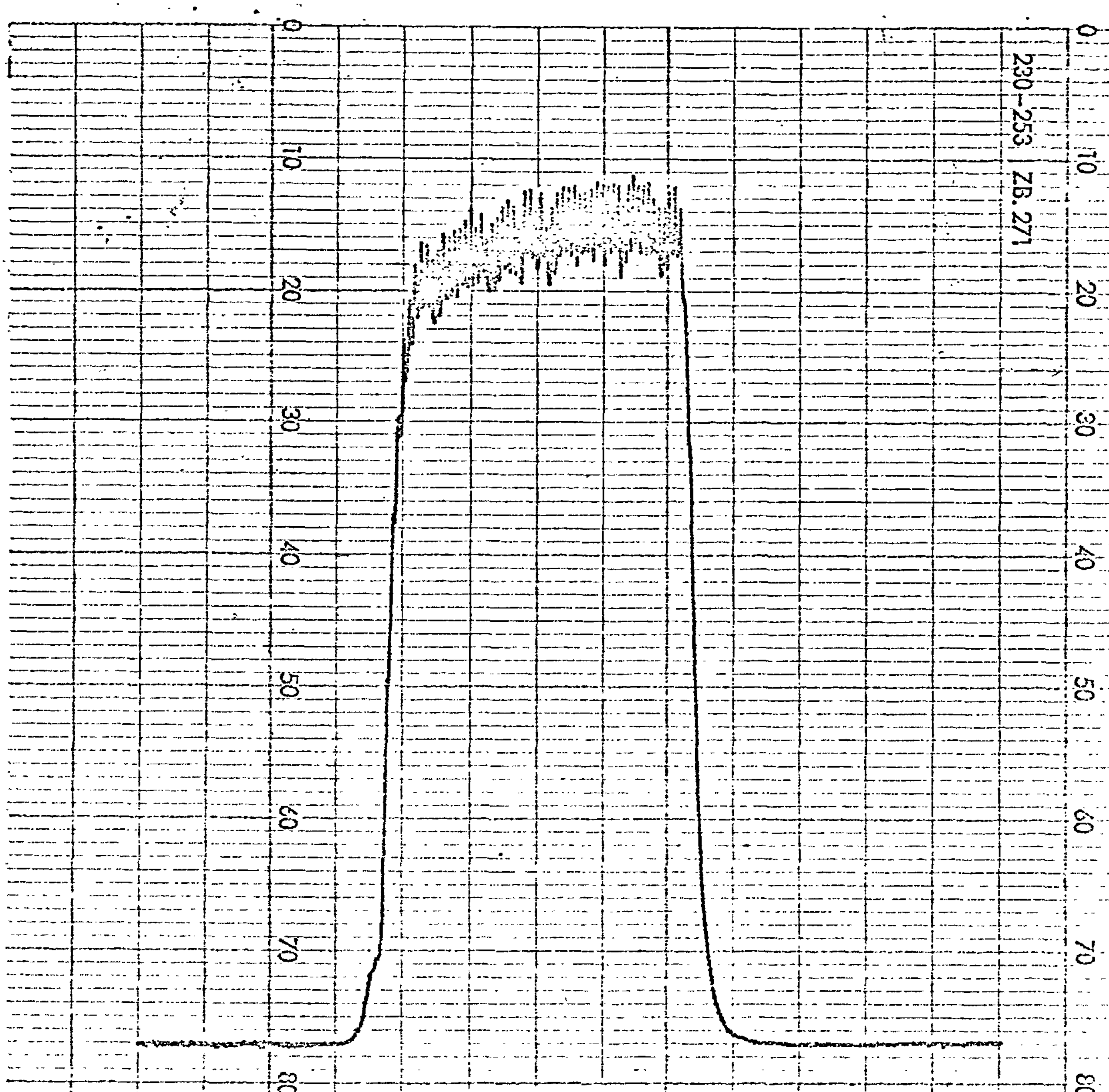


Figure 3.1.2 Cell response to changes in sample composition from 0.22M ammonia/0.06M ammonium nitrate containing no EDTA to one containing 0.5 mM EDTA, and vice versa. Applied potential = 0 V vs SCE, time scale= 1 min/column, sens.= 1/5 (4.7 uA f.s.d.).

the reverse direction the current fell to the steady state value in one minute. Evidence on the lack of any appreciable turbulence is given by a minimal fluctuation in the steady state current of only about 2% at analytically useful flow-rates.

3.1.3. Polarographic behaviour unique to a flowing system.

Electrochemical phenomena occurring on the surface of an electrode immersed in a solution is governed by two factors, namely the velocity of mass transfer to the electrode and the velocity of the electrode processes proper; the former therefore accounts for the difference in polarographic behaviour between measurements made in a static and a flowing system. Some understanding of the transport of matter in stirred solutions (convective diffusion) began with the qualitative Nernst diffusion layer concept followed much later by quantitative hydrodynamic theory for calculation of the convective diffusion to and hence the current at a rotating disc electrode by Levich.[95] Similar treatments for other solid electrode configurations have also been reported since; for example, a micro-conical electrode by Jordan,[96] a spherical electrode by Strafelda and

Kimla[97] and a tubular electrode by Blaedel and Klatt.[98]

Convective diffusion to a liquid electrode was studied by Kolthoff[99] who derived an equation for the current at a rotated DME:

$$i = 230nCD^{\frac{1}{2}}\left[m^{\frac{2}{3}}t^{\frac{1}{6}} + 103D^{\frac{1}{2}}(mt)^{\frac{1}{3}} + 7{\cdot}45\,u_o^{\frac{1}{2}}\,(mt)^{\frac{1}{2}}\right] \qquad (1)$$

where u_o denotes the speed of the solution at the electrode surface and all other terms have their usual meaning. When C is expressed in millimolar units and u_o in cm/sec., the value of i is obtained in uA. The first two terms in equation (1) i.e. the terms independent of solution speed constitute less than 10% of the total current, and therefore for large values of u_o, equation (1) simplifies to,

$$i = 1714nCD^{\frac{1}{2}}u_o^{\frac{1}{2}}(mt)^{\frac{1}{2}} \qquad (2)$$

In both the original and simplified forms, the limiting current is proportional to the concentration.

In the absence of a specific theoretical treatment for the case of a DME in a flowing stream, attempts will be made to interpret data obtained with such a system, using the theoretical treatment of the closely related case of a rotated DME bearing in mind the limitations arising from such a correlation. For example, whereas it was assumed with justification that the speed u_o of the solution at the electrode surface of a rotated DME is uniform over the whole electrode surface, it cannot be justified for a DME in a flowing stream where the solution speed at the surface will vary depending on whether that particular surface is directly confronting the solution stream or is sheltered by the other half of the mercury drop from the solution stream. Experimental parameters such as the direction of solution flow with respect to mercury flow, i.e. whether the solution flows in a direction perpendicular or parallel to the direction of mercury flow and if parallel whether it is in the same or opposite directions as the mercury flow, will have an effect on the convective mass transfer to the electrode surface, as had been experimentally shown.[69]

In accordance with equation (1) it was found that the current is a linear function of C (Figure 3.1.3) and $u_o^{\frac{1}{2}}$ (Figure 3.1.4), and in accordance with the simplified equation (2), the current is practically independent of the height of the mercury head (h) (Figure 3.1.5) the slight increase in current with

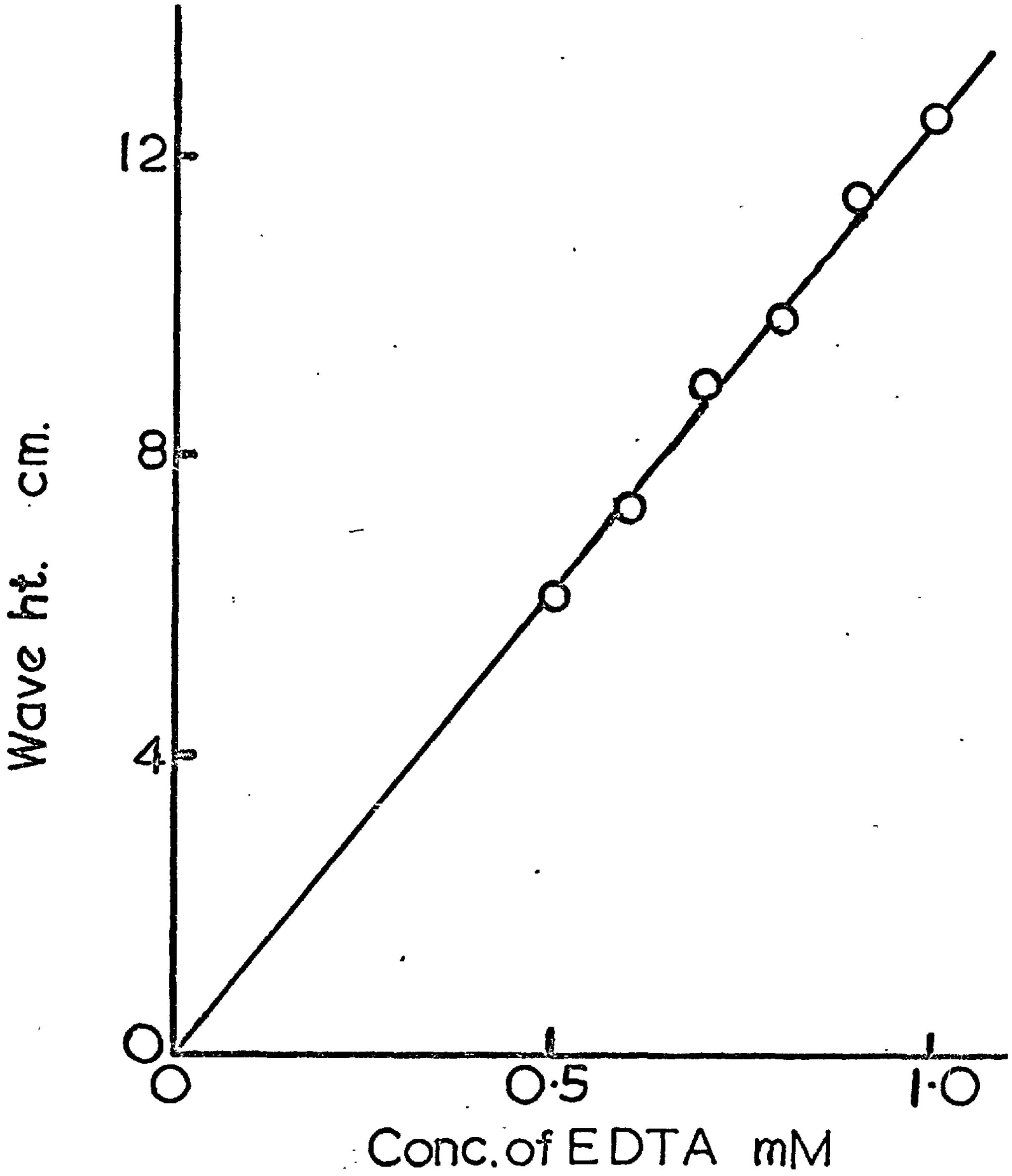


Figure 3.1.3 Dependence of limiting current on depolariser concentration in a flowing stream. 0.5 to 1.0 mM EDTA in 0.18M ammonia/0.05M ammonium nitrate, flow rate= 1.2 ml/min.

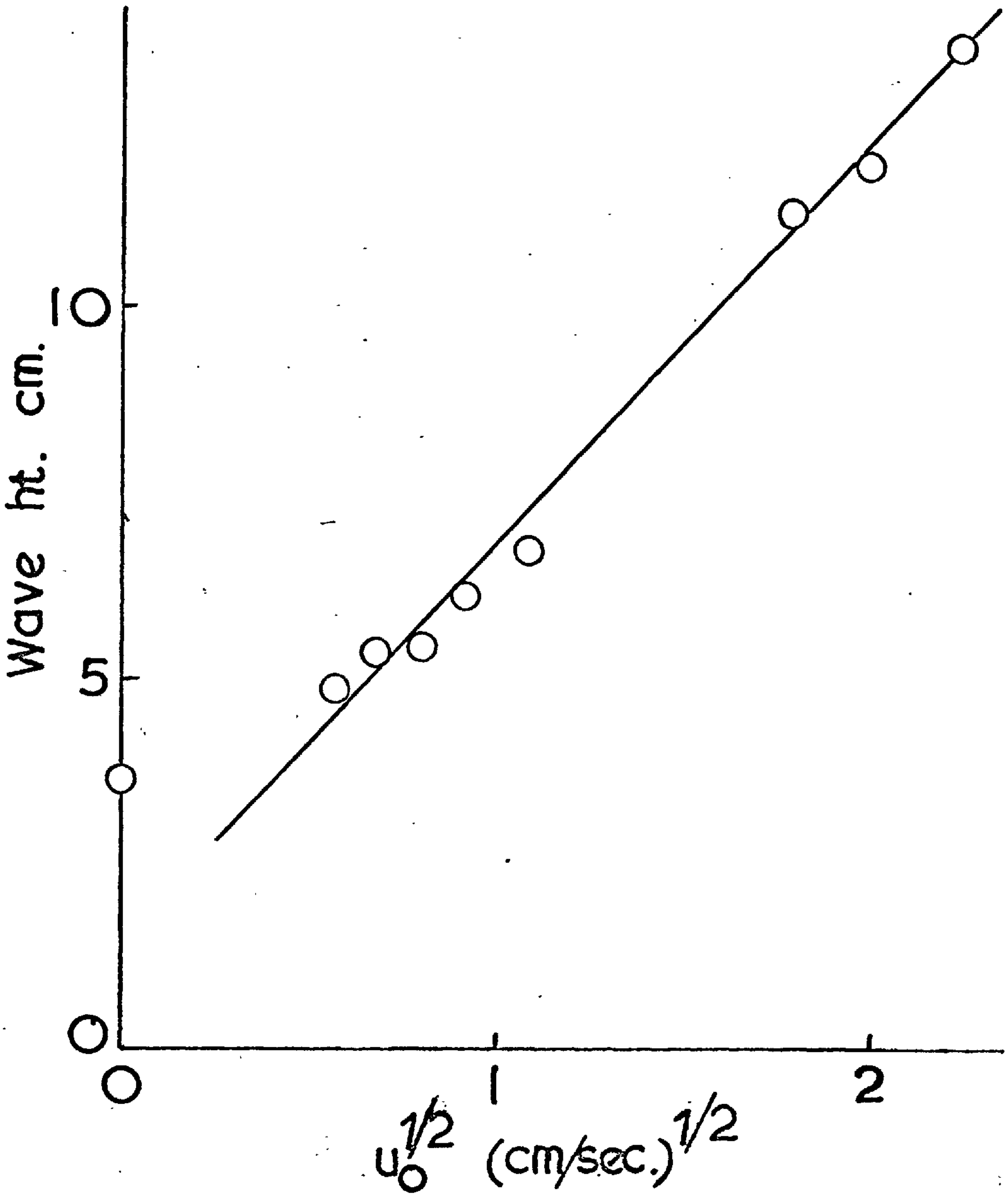


Figure 3.1.4 Dependence of limiting current on solution flow-rate. Solution stream consists of 1.67 mM cadmium in 0.1M potassium chloride.

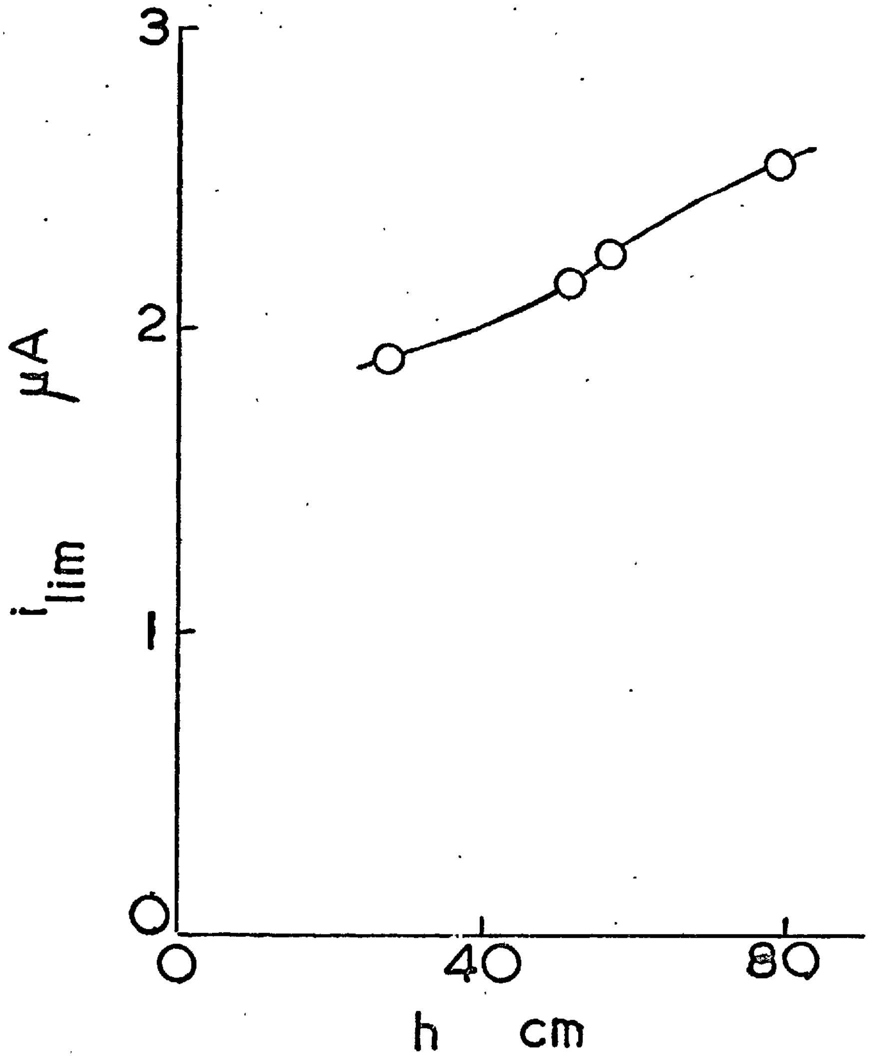


Figure 3.1.5 Dependence of limiting current on height of mercury head. Solution stream consists of 0.5 mM EDTA in 0.22M ammonia/0.06M ammonium nitrate. Applied potential= 0V vs SCE, flow rate= 0.6 ml/min.

h being the small influence of the h dependent first term in equation (1).

These results indicate the rather large extent to which the theory of a rotated DME can be applied to the case of a DME in a flowing stream and therefore suggest a similar line of treatment for the latter taking into account, if need be, the differences between the two cases as outlined earlier.

Due to its simpler instrumental set-up, the DME in a flowing stream has an advantage over the rotated DME for analytical purposes, etc.; and in fact even for theoretical studies, it now seems that the DME in a flowing stream would be a better system to study since in equation (1) whilst the validity of the equation had been checked experimentally for most parameters, the linear dependence of the current on $u_o^{\frac{1}{2}}$ had not and cannot be confirmed since the speed of the solution at the mercury surface (u_o) is different from the speed of rotation of the electrode (u) and is therefore unknown. This is fortunately not so in the case of the DME in a flowing stream and therefore makes experimental verification feasible, as in Figure 3.1.4.

The values of m and t have been shown to remain approximately constant up to quite high flow rates,[69] but at extremely high flow rates (greater than 20 cm/sec.) the natural dropping of the mercury is disturbed and both m and t decrease with increasing flow rates, thus contributing to a decrease in current. A plot of limiting current vs. flow rate extended over extremely high values therefore is peak-shaped.[72] Equation (1) or a similar treatment for the specific case of a DME in a flowing stream will not account for this behaviour and would be rather complicated, if not impossible, to do so. The region where the natural dropping of the mercury is disturbed, however, occurs at such flow rates far beyond the range useful for analytical and theoretical purposes that the phenomenon is of no disadvantage whatsoever. Even a current value measured at such high flow-rates would be totally meaningless due to the enormous turbulence present.

3.1.4. A simple continuous polarographic analyser for metal ion analysis.

The principle of the analyser is to follow the concentration of metal ions by measuring the decrease in the anodic limiting current of an appropriate complexone, due to a decrease in the concentration of the latter through reaction with the metal ions. The limiting current is measured at a fixed applied potential.

The method is specially suited for electro-inactive metal ions or those which are electroactive but their electrochemical behaviour is unsuitable for analytical measurements. For electroactive ions with well-defined and simple polarographic behaviour, it is simply necessary to measure their limiting currents as a direct measure of their concentration.

Figure 3.1.6 shows some polarograms of complexones measured in a flowing stream; the reproducibility and the regularities of the drops can be noticed and the waves are well-suited for analytical measurements. Since the limiting current is linearly related to sample size at constant flow-rate and since the plot passes through the origin (Figure 3.1.3), calibration of the analyser may be achieved with a single standard sample and unknown samples may subsequently be read out directly.

A potential lying on the limiting current plateau of the anodic wave is applied to the DME and in the pH 8-10 region and in most supporting

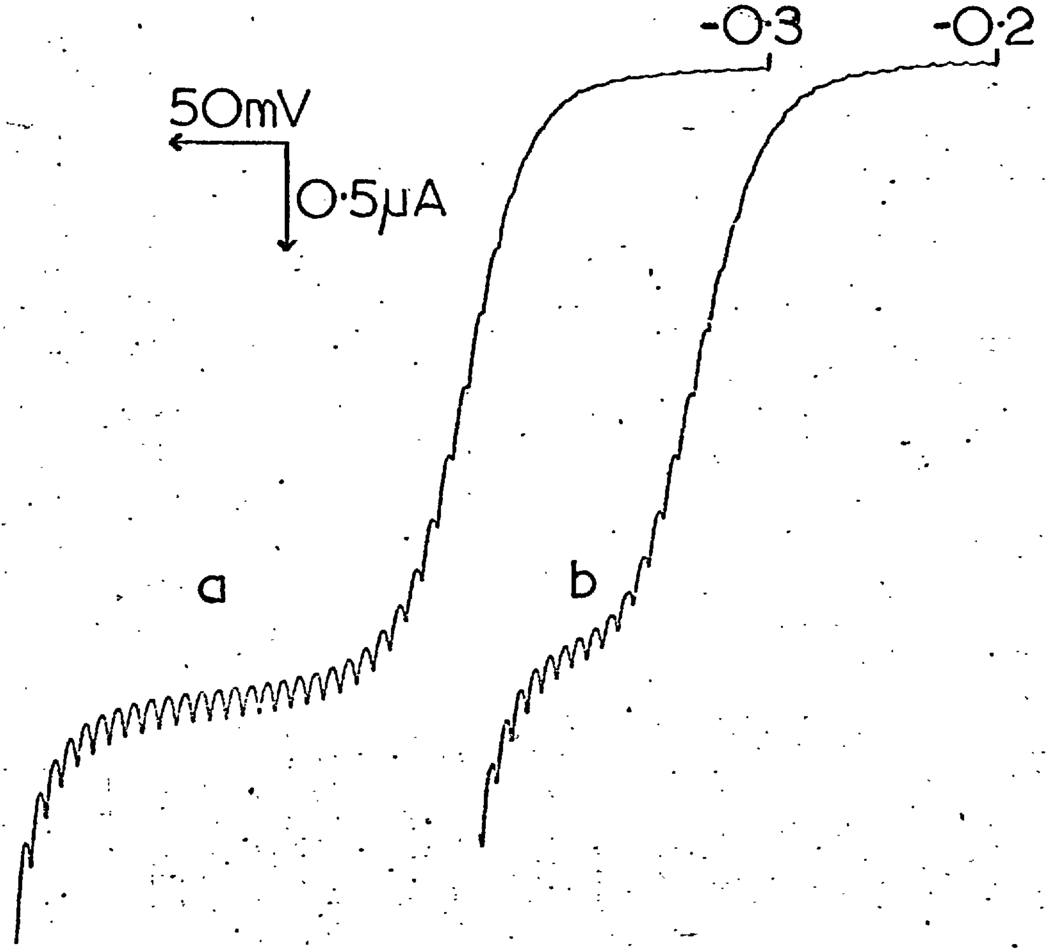


Figure 3.1.6 Anodic d.c. polarograms of 0.5 mM (a) EDTA and (b) EGTA in 0.2M ammonia/0.2M ammonium nitrate obtained in a flowing stream (0.6 ml/min.).

electrolytes a potential of 0V vs. SCE is found to lie more or less on the limiting current plateau of most complexones, and is therefore convenient in that no potential source is necessary and one simply shorts the DME and the reference electrode through a current recorder. Even if 0V lies on the rising portion of the waves it can be "shifted" on to the plateau by changing the potential of the reference electrode and this is achieved by adjusting the concentration of depolariser (potassium chloride) in the reference electrode. A "shift" of as much as about 100 mv can be achieved in this way since 0V vs. a 0·1N calomel electrode (0·1NCE) is equal to +0·092V vs. SCE.

Deoxygenation of the solutions before polarographic measurements was found to be necessary even though in principle measurements of anodic limiting currents from the zero current value should not be affected by cathodic currents due to reduction of oxygen. However, the presence of cathodic currents in the polarograms had been found to distort the anodic waves of the complexones. All solutions employed were therefore deaerated by bubbling nitrogen gas in the reagent bottles before and during the analysis. Deaeration of samples in the sample cups however was not found to be essential since only a small volume (1-2 ml) is employed and the oxygen present was insufficient to produce the above effect. An equilibration with nitrogen in the mixing and reaction coil also removes most of the traces of oxygen present.

A flow diagram for a simple continuous polarographic analyser is given in Figure 3.1.7. It simply consists of two solution lines, one for the sample from an automatic sampler and the other for the reagent, which are pumped to merge into a single stream and this is met and segmented by a stream of nitrogen gas before entering the horizontally placed helical coil for mixing and reaction. The reagent normally is a multicomponent solution containing the appropriate complexone in an appropriate buffer (supporting electrolyte) and also containing masking agents etc. if necessary, all under optimum conditions of concentration, pH, ionic strength and so on. The solutions may also be pumped separately if necessary.

When a liquid flows inside a tube, there exists a quiescent layer 0·05-0·1 mm in thickness, along

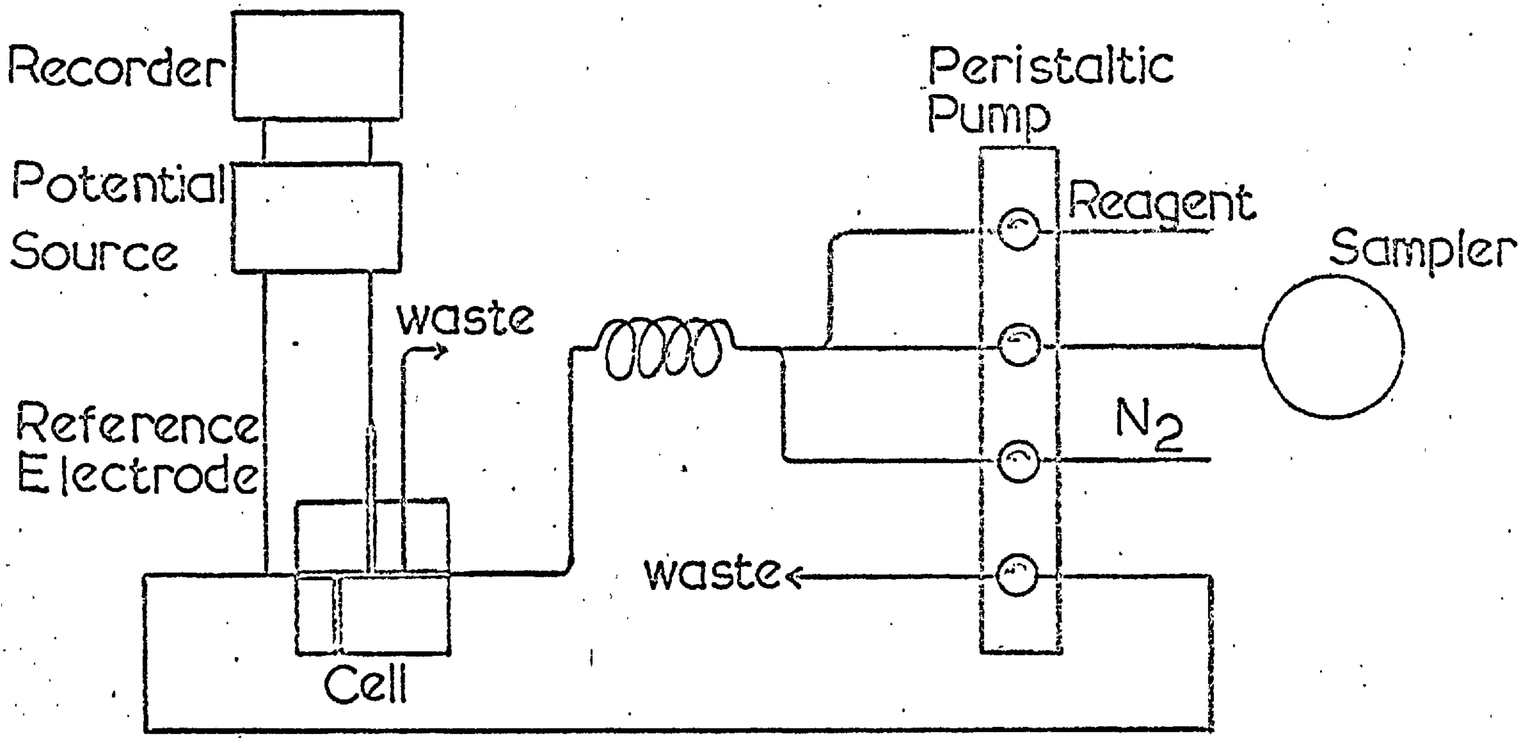


Figure 3.1.7 Schematic of a simple continuous polarographic analyser.

the walls of the tube which is an obvious disadvantage for continuous analysis. This layer persists even upon vigourous agitation or rapid turbulent flow and can come to equilibrium with the rest of the stream only by a very slow diffusion process. The introduction of a gas bubble (gas segmentation) brings into play a surface tension force on the walls of the tube and the quiescent liquid layer is swept clean as the bubble moves forward. Another function of gas segmentation is to separate the liquid stream into tiny aliquots so that thorough mixing and reaction is achieved by repeated inversions in the helical coil (mixing and reaction coil). Gas segmentation also helps to keep consecutive samples separate during sample change over, in addition to removing traces of oxygen in the solution stream when nitrogen or any other inert gas is used for segmentation.

The solution, after thorough mixing and reaction in the helical coil, then enters the polarographic flow-through cell where the gas is first removed before the limiting current is measured at the DME to which an appropriate potential is applied (if necessary) from an external potential source. The solution is then pumped back to waste. A continuous record of limiting current is made on a suitably damped current recorder.

Table 3.1.1 shows some representative results on the analysis of calcium, magnesium and (calcium + magnesium) samples using the continuous polarographic analyser system just described. EDTA was used as the complexometric reagent and the analysis was performed at pH 9•5-10 in an ammonia/ammonium nitrate buffer. Calibration of the instrument was done with a single standard sample after which unknown concentrations are read out. A brief procedure is given below.

Table 3.1.1. Some representative results obtained with the simple continuous polarographic analyser system.

Sample	Present (millimolar)	Found (millimolar)
Ca	0•400	0•410
Ca	0•600	0•599
Ca	0•800	0.814
Ca	1•000	1•016
Ca	1•400	1•386
Mg	0•400	0•403
Mg	0•600	0•610
Mg	0•800	0•812
Mg	1.000	1•006
Mg	1•200	1•210
Ca + Mg	0.200	0•198
Ca + Mg	0•400	0•405
Ca + Mg	0•600	0•601
Ca + Mg	0•800	0•814
Ca + Mg	1•000	1•015
Ca + Mg	1•400	1•376

Procedure

The flow plan is as given in Figure 3.1.7. The reagent consists of a solution of 10^{-3} M EDTA in 0•44 M ammonia/0•12 M ammonium nitrate. Both the reagent and sample are diluted twice at the DME. The applied potential to the DME is OV vs. SCE and analysis is performed at the rate of twenty samples per hour since the transition time (for 100% transition) is *ca* 2 minutes. A standard sample is interposed among the unknown samples for calibration of each type of sample.

3.1.5. A continuous polarographic analyser for simultaneous analysis of calcium and magnesium.

The principle of the analyser is to follow the concentration of the sum of calcium and magnesium by measuring the decrease in the anodic limiting current of EDTA due to a decrease in concentration of the latter through reaction with the metal ions and to follow the concentration of calcium alone in the sample by measuring the decrease in the anodic limiting current of EGTA due to a decrease in concentration of EGTA through reaction with calcium alone in the sample. The reactions are carried out at pH 9•5-10 in an ammonia/ammonium nitrate buffer and in the second reaction (with EGTA) a masking agent, sodium tripolyphosphate (ref. Section II, Chapter 2), is present to eliminate any interference by magnesium. The limiting anodic currents of both complexones are measured at the same fixed applied potential which lies on the limiting current plateau of both the waves.

A scheme of the continuous polarographic analyser is given in Figure 3.1.8. The fundamental principles of this analyser are the same as the simple continuous polarographic analyser described earlier. However, in this case, since a simultaneous analysis for two components in the sample is required, a dual sensing and recording system would normally be necessary. But this is avoided by introducing a valving technique which makes it possible to present the two systems (one for analysis of the sum of calcium and magnesium using EDTA, and the other for the analysis of calcium alone in the sample using EGTA) alternately to a single sensing and recording system. This is achieved by using a cam-controlled tap system to control the EDTA, EGTA, distilled water and sample lines (Figure 3.1.8). The buffer and nitrogen gas streams are allowed to flow uninterrupted whereas

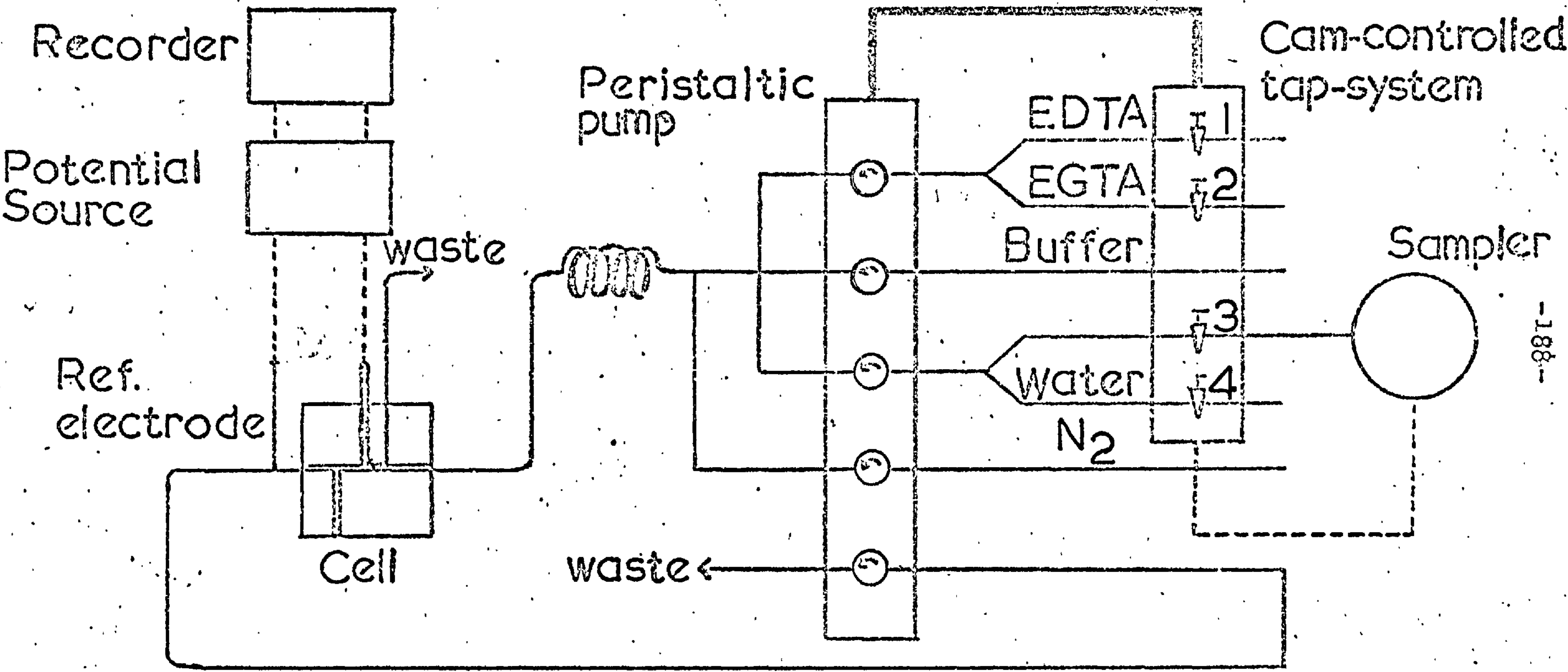


Figure 3.1.8 Schematic of the continuous polarographic analyser for the simultaneous determination of Ca & Mg. Solution pump tubings=0.03" i.d.(0.32 ml/m), N_2=0.056" (1.2 ml/m), waste=0.035" (0.42 ml/m). Thick solid lines and broken lines represent mechanical and electrical connections respectively.

the cams are cut and programmed so that four operations are performed per complete revolution of the cams, namely,

(i) Taps T_1 (EDTA), T_4 (distilled water) open and taps T_2 (EGTA), T_3 (sample) close simultaneously,

(ii) Tap T_4 (distilled water) closes, tap T_3 (sample) opens simultaneously while taps T_1 (EDTA) and T_2 (EGTA) remain as in (i),

(iii) Taps T_2 (EGTA), T_4 (distilled water) open and taps T_1 (EDTA), T_3 (sample) close simultaneously, and finally

(iv) Tap T_4 (distilled water) closes, tap T_3 (sample) opens simultaneously while taps T_2 (EGTA) and T_1 (EDTA) remain as in (iii).

The sequence occurs in the order given and is repeated after every complete revolution of the cams.

Figure 3.1.9 is an actual recorder tracing obtained as a result of the four operations. The limiting currents are numbered to correspond to the operations just described so that the rise from zero current value to limiting current (i) corresponds to introduction of EDTA and distilled water into the system by means of operation (i). The fall in current to (ii) corresponds to a decrease in

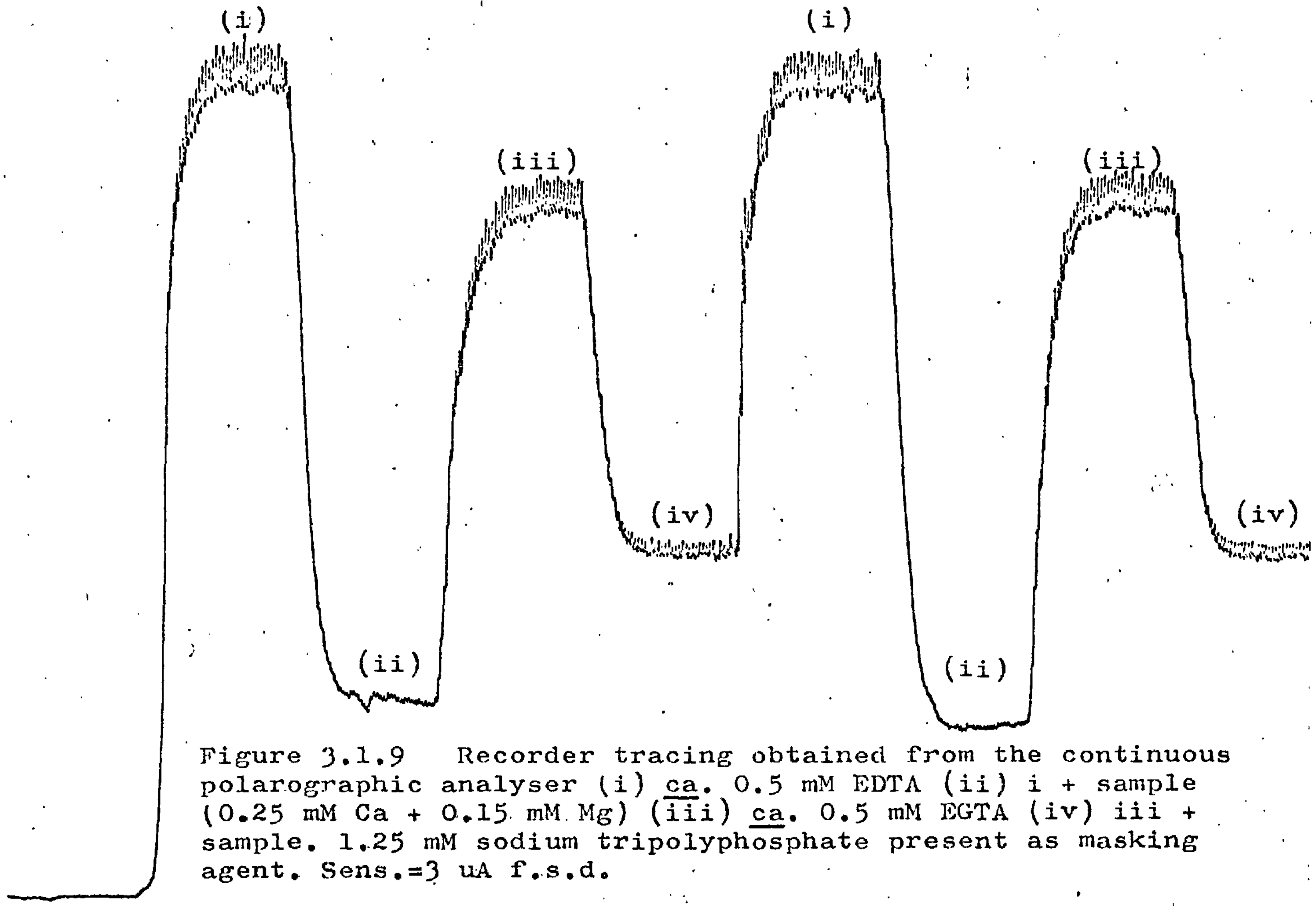


Figure 3.1.9 Recorder tracing obtained from the continuous polarographic analyser (i) ca. 0.5 mM EDTA (ii) i + sample (0.25 mM Ca + 0.15 mM Mg) (iii) ca. 0.5 mM EGTA (iv) iii + sample. 1.25 mM sodium tripolyphosphate present as masking agent. Sens.=3 uA f.s.d.

concentration of EDTA due to reaction with calcium and magnesium in the sample when the distilled water is replaced by the sample in operation (ii), the decrease in current from (i) to (ii) being proportional to the total concentration of calcium and magnesium. The introduction of EGTA (plus sodium tripolyphosphate as masking agent) and distilled water into the system by means of operation (iii) causes a rise in limiting current from (ii) to (iii); the following fall in current from (iii) to (iv) corresponds to a decrease in concentration of EGTA due to reaction with calcium alone in the sample, when distilled water is replaced by the sample in operation (iv). The decrease in current from (iii) to (iv) is proportional to the concentration of calcium alone in the sample (ref. Section II, Chapter 2).

The instrumental arrangement is also such that the automatic sampler is made to change samples at the end of every complete revolution of the cams, as described in the "Experimental" section. In Figure 3.1.9 however, the recording had been repeated with the same sample in order to show the reproducibility of the analyser.

Reproducibility of the analyser over a long period of time was tested by repeated analysis of a single sample containing 1·1 millimolar calcium and 0·3 millimolar magnesium, the analyser being set to analyse at the rate of 8 minutes per analysis for a period of approximately 3 hours. The results obtained are shown in Figure 3.1.10 where the zig-zag curves show the variation of the experimental points about their mean value (straight horizontal lines). The upper curve represents the concentration of total calcium and magnesium whilst the lower curve represents the concentration of calcium alone in the sample. Corresponding current scales are also given on the graph. Data obtained by a statistical analysis of the figures is given in Table 3.1.2.

Table 3.1.2. Precision of the analyser over long periods of time.

	Ca+Mg	Ca
No. of analyses	20	20
Concentration present	1·40 mM	1·10 mM
Average current decrease	1·47 uA	0·87 uA
Std. deviation (conc.)	0·020 mM 1·4%	0·027 mM 2·5%
Std. deviation (current)	0·02 uA 1·4%	0·02 uA 2·5%

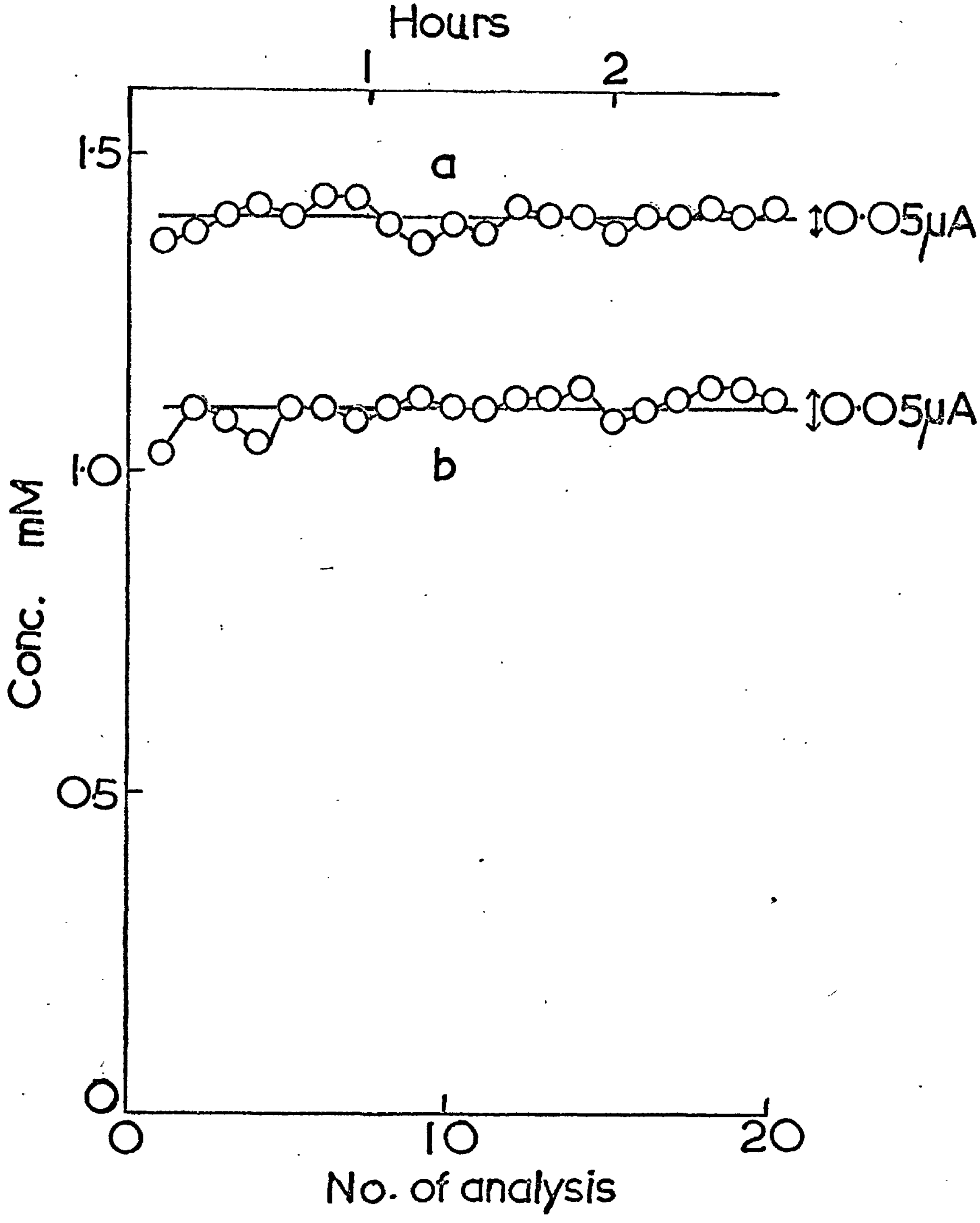


Figure 3.1.10 Reproducibility of the continuous polarographic analyser.
(a) (Ca+Mg) and (b) Mg.

The transition time varies slightly between 1.0 and 1.5 minutes depending on a number of factors such as the degree of synchronisation achieved of the cams, the magnitude of the current decrease, etc. Analysis may therefore be performed at the rate of 8 minutes per analysis amounting to 2 minutes per operation for the four operations needed for the analysis.

Experimental

Instrumentation:

A description of the polarographic flow-through cell has been given earlier. A System Heyrovsky Polarograph type LP 55A (Czechoslovakia) is used as a source of applied potential and current measurements are made with a Servoscribe recorder (Smiths Industries Ltd.) which is damped by means of a 2000 uF capacitor connected in parallel.
A 15-channel peristaltic pump (Technicon AutoAnalyzer Proportioning Pump, Technicon Instruments Corp., Chauncey, New York) is used to pump liquid and gas streams. Standard AutoAnalyzer pump tubing, transmission tubing, connectors, helical coils, etc., are used for construction of the flow system. The manifold is constructed from $\frac{1}{8}$" aluminium plate on

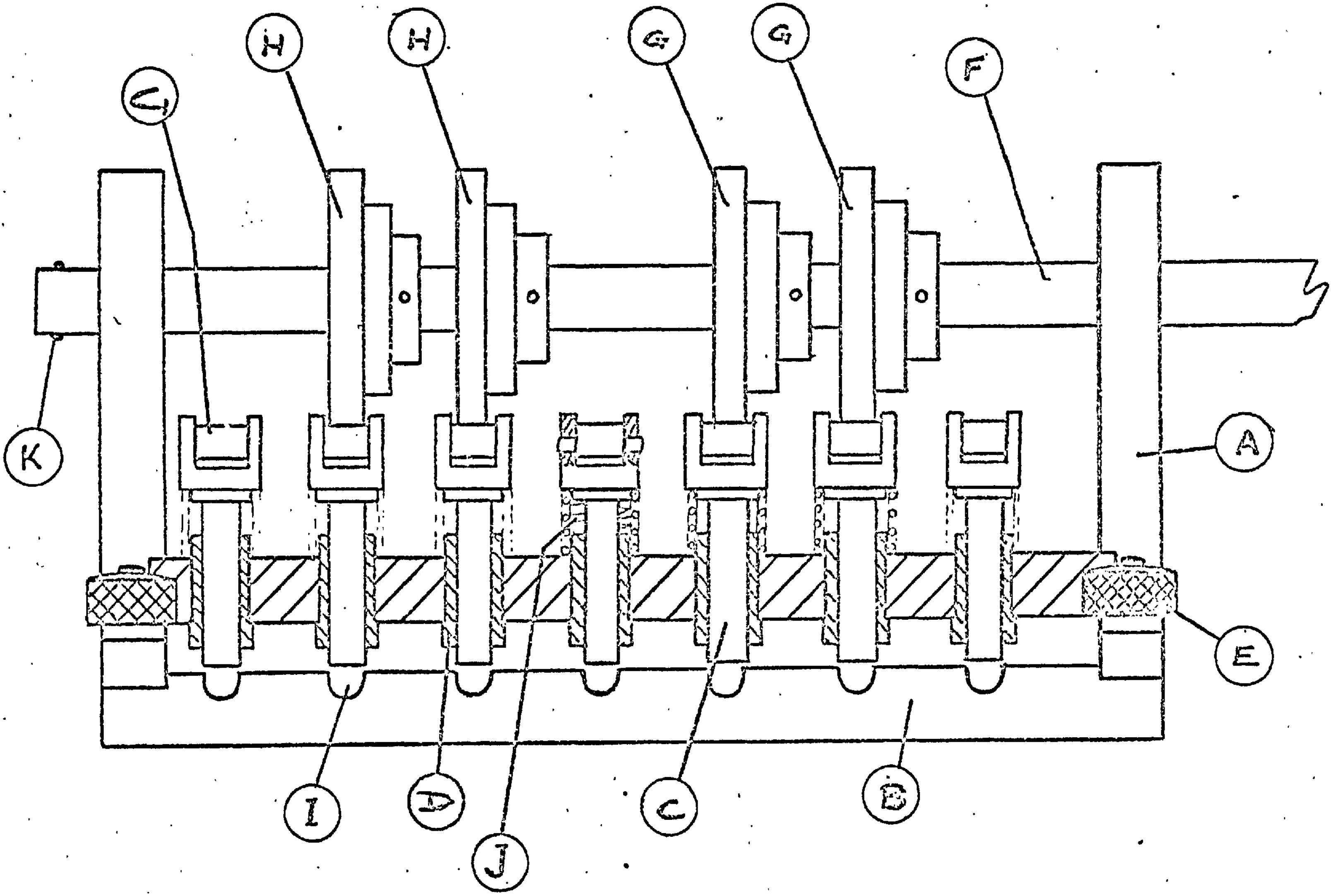
which a flow-system as given in Figure 3.1.8 is set up; the manifold is fitted on the pump so that the pump tubings are sandwiched between the platen and pump rollers.

Cam-controlled tap assembly

The cam-controlled tap assembly, shown in Figure 3.1.11, is constructed on the manifold plate at the input end of the peristaltic pump. Simply, it consists of a series of cams (G,H) which control the push rods (C) which in turn act on elastic tubings placed in the grooves (I) and therefore control the flow of solution streams.

The mounting (A) and base plate (B) of the cam assembly are constructed of aluminium. The grooves (I) are of 5/16" diameter and 3/16" diameter mild steel push rods (C) work inside steel sheaths (D) and are fitted with springs (J) for rebounding action. Hardened steel rollers (C_1) are provided on the heads of the push rods for smooth response with the cams. Cams (H) control the flow of EDTA and EGTA and have two "dwell" positions, a "rise" and a "fall" each; cams (G) controlling the flow of distilled water and sample have four "dwell" positions, two "rises" and two "falls" each. The

Figure 3.1.11 Cam-controlled tap system.



positions of the cams are adjustable and are synchronised to perform the four operations already described. The cams constructed of brass are mounted on a silver steel shaft (F).

Power for driving the cams is taken from the peristaltic pump motor itself since the two are to work in close conjunction. The drive is taken from the universal joint clutch of the peristaltic pump through 90° mitre gears into a 3-speed constant mesh gear box and then via a sliding dog clutch on to the cam shaft at (K). Speeds of $\frac{1}{4}$, $\frac{1}{8}$ and 1/16 revolutions per minute of the cams are available from the gear box, providing analysing rates of 4, 8 and 16 minutes per sample respectively.

Synchronisation of operations with sampler

An AutoAnalyzer Sampler II (Technicon Instruments Corp., Chauncey, New York) is modified so that the cams which control the sampling and washing times of the sampler are totally dispensed with and electrical connections from the microswitch activated by these cams are directly connected to a microswitch at the cam-controlled tap system. An additional cam is mounted at (L) on the cam-shaft of the cam assembly so that at every complete revolution of the tap controlling cams, i.e. after every complete analysis, the microswitch is switched on and activates the sampler to change over to the next sample. The time that the cam presses on the microswitch determines the time that the sampler probe remains out of the sample and is kept to a minimum (2 and 4 seconds at cam-speed settings $\frac{1}{4}$ and $\frac{1}{8}$ respectively).

One notable problem in connection with sample change-over is the fact that after sample change-over the new sample has to displace the previous sample in the length of tubing from the sampler probe up to the connection with the pump tubing. This can contribute considerably to the lengthening of the attainment of steady state (transition time) and is avoided by reducing the volume of such trapped sample by using very fine capillary tubing (0·005-0·015" i.d.) for that section.

The instrument is now ready for continuous automatic polarographic analysis of calcium and magnesium simultaneously.

Procedure

The flow-plan and instrumental arrangement are as described and as shown in Figure 3.1.8. The buffer solution is an ammonia buffer of composition

$0{\cdot}66$ M NH_3/0.18 M NH_4NO_3. The concentration of both EDTA and EGTA is 1·50 millimolar, the latter also containing ca 4·5 millimolar sodium tripolyphosphate as masking agent. Samples containing up to about 1·4 millimolar total calcium and magnesium may therefore be analysed using this system. Proceed as follows:

(1) Fill entire system with distilled water by pumping in the latter through all the lines except the nitrogen line.

(2) Adjust cams so that they are in the start position, that is, ready to follow operation (i) described earlier.

(3) Place 2-3 ml each of the samples in sample cups and arrange the cups on the sampler plate. Use distilled water or some known samples in the first one or two sample cups to enable the analyser to attain analysing conditions (i.e. to fill up solution lines with appropriate reagents) before actual analysis. Interpose standard samples amongst the unknowns at appropriate intervals.

(4) Deoxygenate all solutions, except the samples, by bubbling nitrogen into the reagent bottles. Connect reagent bottles to appropriate solution lines.

(5) Adjust the following instrumental settings:

Applied potential = 0 V vs. NCE (or other experimentally determined value).

Cam-speed = $\frac{1}{8}$ rev./min.

Chart-speed = 2 mm/min.

Sensitivity = as determined experimentally.

The DME used has the following capillary characteristics: m = 1·38 mg/ sec. and t = 5·07 sec. under open circuit condition.

(6) Switch on the sampler, pump and recorder simultaneously and a continuous record of the complete analysis of calcium and magnesium in all samples will be obtained at a rate of 8 minutes per sample.

<u>Performance of the analyser</u>

Table 3.1.3 gives some typical results obtained with the analyser.

Repeatability of the analytical results had been thoroughly tested; for example in one instance as illustrated by samples 7-10 in Table 3.1.3 the four repetitions of the analyses were carried out on three different occasions on three separate days. The results are found to be entirely repeatable. The results in Table 3.1.3 also give an indication of the accuracy of the analyser; but it must be

Table 3.1.3. Some typical results obtained with the continuous polarographic analyser for the simultaneous analysis of calcium and magnesium.

Sample No.	(Ca+Mg)					Ca				
	Present mM	Found mM				Present mM	Found mM			
		1	2	3	4		1	2	3	4
1.	0·70	-	0·73	0·69	-	0·60	-	0·60	0·60	-
2.	0·80	0·76	0·82	0·82	-	0·60	0·60	0·59	0·60	-
3.	0·90	0·88	0·94	0·94	-	0·60	0·62	0·61	0·60	-
4.	1·00	1·01	1·03	1·06	-	0·60	0·63	0·61	0·59	-
5.	1·10	1·09	1·12	1·14	-	0·60	0·57	0·62	0·58	-
6.	1·30	1·32	1·26	1·26	-	0·60	0·56	0·63	0·60	-
7.	1·40	1·40	1·40	1·44	1·40	0·80	0·78	0·78	0·81	0·76
8.	1·40	-	1·40	1·44	1·40	0·85	-	0·86	0·85	0·86
9.	1·40	1·40	1·40	1·41	1·40	0·90	0·88	0·88	0·90	0·88
10.	1·40	1·40	1·40	1·41	1·40	1·00	1·00	0·99	1·00	0·98

mentioned in all fairness that a maximum error of 0·04 mM in the table corresponds to an error in current measurement of only about 0·05 uA and therefore more accurate results are to be expected with more accurate current measurement.

3.1.6. Conclusion

The development of a simple continuous polarographic analyser for metal ions, and a more complicated one for simultaneous automatic analysis of calcium and magnesium both employing complexometric principles, illustrates the feasibility of the application of complexometric principles to continuous polarographic analysis providing the latter with a firm foundation for wide applications.

Attempted application of Kolthoff's equation on the limiting current obtained at a rotated DME to the case of a DME in a flowing stream has been successful and therefore suggests a similar line of treatment for the latter. Dependence of the limiting current at a DME in a flowing stream on concentration and square root of the solution speed and virtual independence on the height of the mercury head have been experimentally proved. Limitations in making a correlation between the two cases have been discussed.

SECTION III

CHAPTER 2

INVESTIGATION OF SOME CONTINUOUS ELECTROCHEMICAL SENSORS OTHER THAN THE DME

3.2.1 Introduction

As with voltammetry in stationary solutions, the development of sensors other than the DME for continuous voltammetric sensing is carried out for two main reasons, namely to increase the sensitivity and to expand the usable potential range especially in the positive region over the DME. An additional factor in the case of continuous sensing is to find a geometrical configuration of the electrode more suitable for continuous analysis.

With these in mind, the present chapter describes an investigation of two mercury-type electrodes - a hanging mercury-drop electrode (HMDE) and a mercury-plated tubular platinum electrode (MTPE) - and a non-mercury type solid electrode, namely, a tubular platinum electrode (TPE), as continuous voltammetric sensors with an emphasis on the monitoring of the anodic oxidation of complexones.

3.2.2. Hanging mercury-drop electrode (HMDE)

A HMDE whilst preserving most of the merits of the DME offers new possibilities from both the theoretical and practical points of view. Elimination of capillary noise and decrease of charging current compared with a DME are some of the advantages in the latter point of view. Its analytical use in the field of stripping voltammetry is very well known. The use of a HMDE for continuous sensing however has never been reported.

Experimental

The HMDE used is an E410 Microfeed Device which is an integral part of the BM 5-03 Metrohm Inverse Polarography equipment (Metrohm Ltd., Switzerland). It consists of a displacement piston which projects into the mercury-reservoir space via a rubber seal and is actuated by a precision micrometer screw. The mercury droplet size can be adjusted by means of the engraved scale on the micrometer head.

The cell used is exactly the same as the polarographic flow-through cell described in the previous chapter. The peristallic pump, polarograph and recorder used has been described previously. (Section III, Chapter 1)

Results

The complexones give normal S-shaped voltammetric curves with a limiting current plateau. At flow rates of 0.42 ml/m (6 cm/m) the current recordings are completely noise-free with minimum damping used and measurement of current is easier due to absence of current oscillations. The reproducibility of the limiting current and of the half-wave potential has been investigated for the complexones and standard deviations of 0.01-0.03 uA for the limiting current and 0.003 - 0.004 V for the half-wave potentials are obtained from five different voltammetric curves each of <u>ca</u>. 5 x 10^{-4}M solutions of the complexones (EDTA and EGTA) in an ammonia buffer pH 9.5. The flow rate is 0.42 ml/m (6 cm/m) and drop-size 0.66 mm diameter. The results are obtained with a single mercury drop, without renewal between the individual measurements. This independence on the history of the drop is due to the soluble nature of the electrode reaction products which are swept clean by the flowing stream.

The rate of increase of the limiting current (i_{lim}) with increasing surface area (A) of the mercury drop is found to be much higher than that of the residual current (i_r) resulting in a net increase in sensitivity. The ratio i_{lim}/i_r increases from 20 to 50 with the increase in surface area of the drop from <u>ca</u>. 0.5 to 3 mm^2 when a solution of 10^{-3} M EDTA in ammonia buffer is measured at a flow-rate of 0.42 ml/m (6 cm/m). The half-wave potential remains unaffected, the shape of the wave is not altered and no increase of noise in the recordings are observed within the range studied. A plot of i_{lim} vs $A^{3/4}$ gives a straight line passing through the origin in accordance with Levich's equation[95] for the current at a liquid drop where mass transfer is by convective diffusion.

The relation between the limiting current and concentration is found to be linear for the complexones in the concentration range studied (10^{-4} to 10^{-3}M).

Practical analyses had been performed with sucess on the determination of metal ions.

Conclusion

On the basis of the above experiments it can be stated that the HMDE is suitable for continuous voltammetric sensing when the electrode reaction products are soluble and do not contaminate or dissolve in mercury. The situation is therefore ideal when anodic currents due to anodic oxidation of complexones are measured. Increased sensitivity is obtainable by increasing the size of the drop without the introduction of ill-effects such as noise. At constant flow-rate the limiting current is proportional to concentration; the limiting currents are very reproducible and the electrode shows no "memory" effect for the case of the complexones.

3.2.3 Tubular platinum electrode (TPE)

Even though the use of a platinum micro "by-pass" electrode for continuous analysis was suggested as early as 1947[68], the TPE through which a solution flows was introduced for analytical purposes only in 1963 by Blaedel and his coworkers[100]. The TPE has the advantages of being well suited geometrically for continuous electrochemical measurements in flowing streams and of high sensitivity. From Levich's theoretical analysis of convective diffusion to the surface of a tube[101] which gives the total flux (J), as, $J = 2.01\pi CD^{2/3}R^{2/3}X^{2/3}V_o^{1/3}$ (1)

Blaedel et al derived the following equation for the limiting current (i) at a tubular electrode,

$$i = 5.24 \times 10^5 \, nCD^{2/3} X^{2/3} V_f^{1/3} \qquad (2)$$

Where R and X are the radius and length of the tubular electrode, and V_o and V_f are the linear and volume flow-rates respectively; all other terms have their usual meaning in voltammetry. They proved this equation using a ferrocyanide/ferricyanide system. In a later paper, again by Blaedel[98], an equation for the current-potential relationship for reversible charge transfer processes at the TPE is presented, again proving it with the ferrocyanide ferricyanide system.

In this chapter the anodic and cathodic potential limits of a TPE in different supporting electrolytes over a wide pH range are defined and usable potential ranges indicated. A study of the anodic oxidation of complexones at the TPE in a flowing stream is also presented.

Experimental

The cell assembly is similar to the one for a mercury-plated tubular platinum electrode (Figure 3.2.3) except that the tubular electrode is directly connected to the solution line from the pump and the mercury-containing unit is dispensed with altogether.

The tubular electrode used is a platinum cylinder 1.5 cm long cut from seamless platinum tubing (1.0 mm i.d). The cutting is done carefully so that a smooth ending is obtained otherwise turbulent flow will be introduced.

The apparatus used has been described earlier (Section III, Chapter 1).

General electrode treatment consists of an occasional cleaning of the electrode first in alcoholic caustic potash to remove greasy substances, then in hot cleaning mixture (sulphuric acid + sodium dichromate) and then in hot nitric acid and finally in distilled water. Immediately before every measurement, the electrode is pretreated by repeated successive anodisation and cathodisation at +2 and -2V respectively while a solution of 0.1M perchloric acid is pumped through. After a final anodisation step the current is allowed to decay to a small value at the cathodic dissolution potential of the platinum oxides (ca. -0.1 V vs SCE). The electrode is now ready for use. The electrode is stored in 8F nitric acid whenever not in use.

All solutions are thoroughly deaerated by bubbling through nitrogen in the reagent bottles before measurement.

Results and discussion

(a) Useful potential range

The range of potentials accessible to the electrode is limited by the nature of the ions present in the supporting electrode and on the pH of the solution. Figure 3.2.1 shows some residual current curves at the TPE in different supporting electrolytes over a wide pH range. The curves are obtained by an anodic and a cathodic scan both from the potential of zero current after the current has been allowed to decay to a constant value at the potentials of oxides formation and dissolution respectively.

Figure 3.2.1 shows that practically no use can be made of the cathodic region, whereas quite low residual currents and more or less flat base lines should permit anodic measurements to be made. The cathodic and anodic discharge potentials and the useful anodic potential ranges for the different supporting electrolytes at different pH values may be seen from the figure.

(b) Anodic oxidation of complexones at a TPE

The anodic voltammetric behaviour of complexones at a rotating platinum electrode (RPE) was studied by Vydra and Stulik[102] who also applied the principle for biamperometric indication in chelometric titrations[103].

Anodic voltammetry of complexones at a TPE was studied with a view to possible application in continuous analysis using complexometric principles (Section III, Chapter 1).

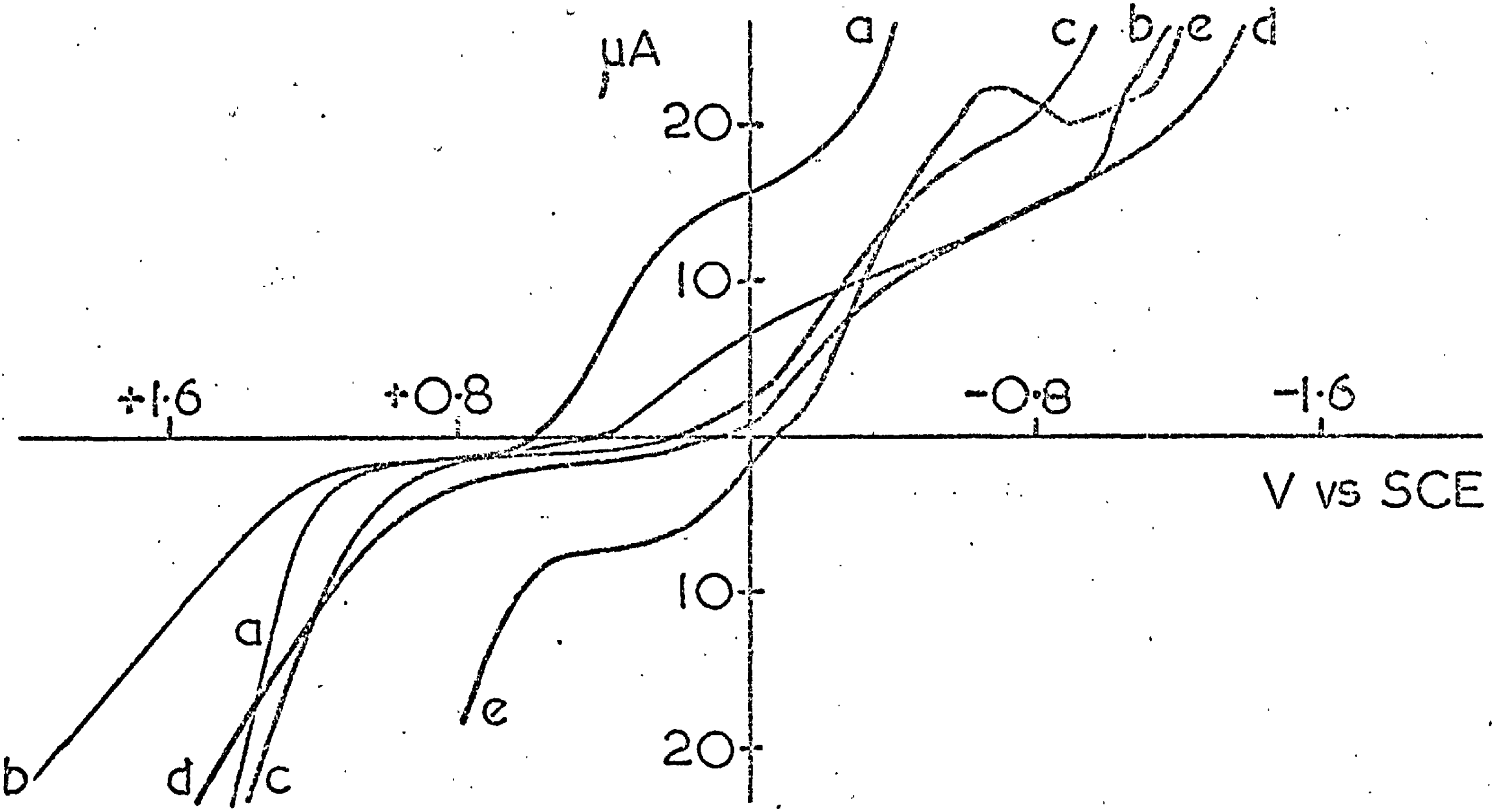


Figure 3.2.1 Some residual current curves at a TPE: (a) 0.2M perchloric acid (b) 2.0F acetic acid/0.2F sodium acetate (c) 0.5M potassium nitrate (d) 0.2M sodium tetraborate (e) 0.2M sodium hydroxide.

Figure 3.2.2. (a) shows the voltammograms of the complexones EGTA and CDTA. EDTA gives an ill-defined wave. Even though a high sensitivity is achieved the currents are found to be very much time dependent as shown in Figure 3.2.2. (b) and therefore irreproducible. This dependence on time is probably due to the accumulative adsorption on the electrode surface, of the various electrode reaction products which consist mainly of different amines and formaldehyde (decarboxylation products of the complexones) and their condensation products.

Conclusion

Although a sensitive voltammetric response is obtained with a TPE to the anodic oxidation of different complexones, the observed voltammetric behaviour is not suitable for analytical applications. In principle, the TPE has been shown to be suitable for use in the anodic region over a wide pH range.

3.2.4 Mercury-plated tubular platinum electrode (MTPE)

The first and only report on the use of a MTPE as a voltammetric sensor appeared in late 1967 in a paper by Olson and Oesterling[104] who studied the effect of mercury-film thickness on cathodic reductions of some metal ions and also showed a linear dependence of the diffusion limited currents on concentration of metal ions. A study on the reproducibility of the voltammograms as well as the

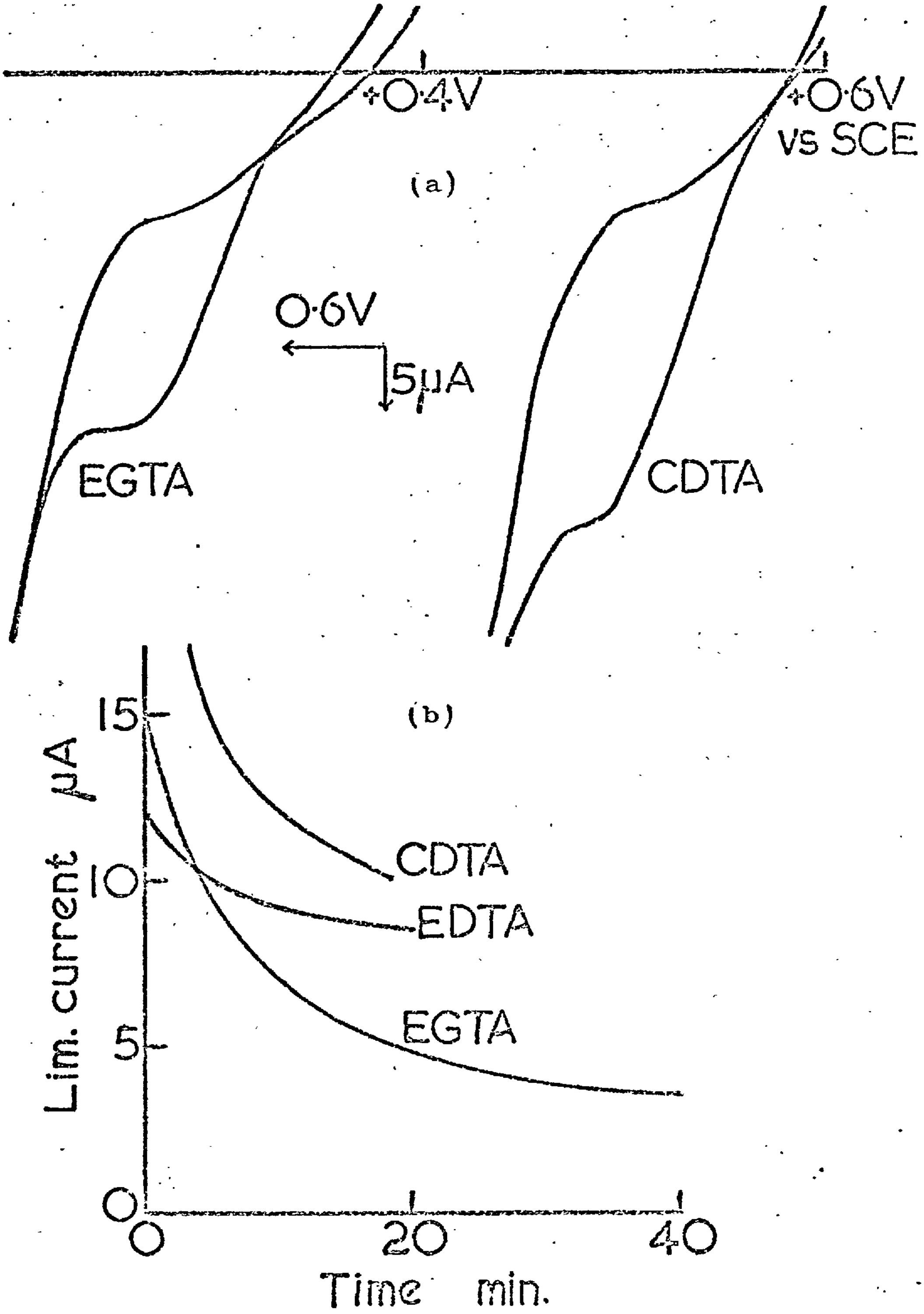


Figure 3.2.2 Some voltammograms of complexones in 0.2M perchloric acid at a TPE shown together with the curves of supporting electrolytes alone. Lower graph shows the time dependence of limiting currents.

diffusion-limited current values was not reported by the authors. It is obvious that the electrode will not be suitable for electrode reactions which yield insoluble reaction products and which are either adsorbed or dissolved in the mercury film, such as in the cathodic reduction of metal ions to the metals. Long-term measurements without occasional surface renewal or reconditioning by stripping etc. would give erratic results and therefore unsuitable for continuous analysis where long-term measurement is one of the principle objects.

If complexones show a suitable voltammetric response at the MTPE, this serious limitation of the electrode may be solved in many cases by the use of analytical methods employing complexometric reagents in which the concentration of the sought-for substance is obtained indirectly from the concentration of the complexones which may be easily monitored with the MTPE.

A cell-design suitable for a convenient mercury plating process as well as for continuous analysis is presented and an evaluation of the electrode for indirect complexometric analysis is given.

Experimental

The tubular electrode used is a piece of platinum cylinder 3mm i.d. and 10 mm long cut from a length of seamless platinum tubing. The cell is designed to provide for independent pumping of the solution and mercury streams and also to provide for both forward and backward pumping of the mercury stream which are necessary in the plating process. The cell assembly for the MTPE is shown in Figure 3.2.3. The tubular electrode is mounted in an upright position and is connected at both ends to plastic nipple connectors via tight-fitting polythene sleeves. The upper nipple connector fits in a rubber stopper at the lower end of a 15 mm i.d. glass tubing provided with a side arm which goes to waste. The glass tube forms the reference electrode compartment and a saturated potassium chloride salt-bridge with sintered-glass separation is inserted. The lower nipple connector from the electrode is connected by means of a tight-fitting polythene sleeve to an h-shaped glass tube, one arm of which is filled with mercury connected via polythene tubing to an external levelling reservoir. The other arm connects to the solution line from the peristaltic pump and is provided with a screw-or pinch-clip.

Of three methods available for plating the platinum surface with mercury, namely electroplating, abrasion and wetting, the wetting method described by Enke[105] has been used. For a successful application of the method a clean surface free from any surface oxides is necessary. The electrode is first degreased in alcoholic caustic potash, then

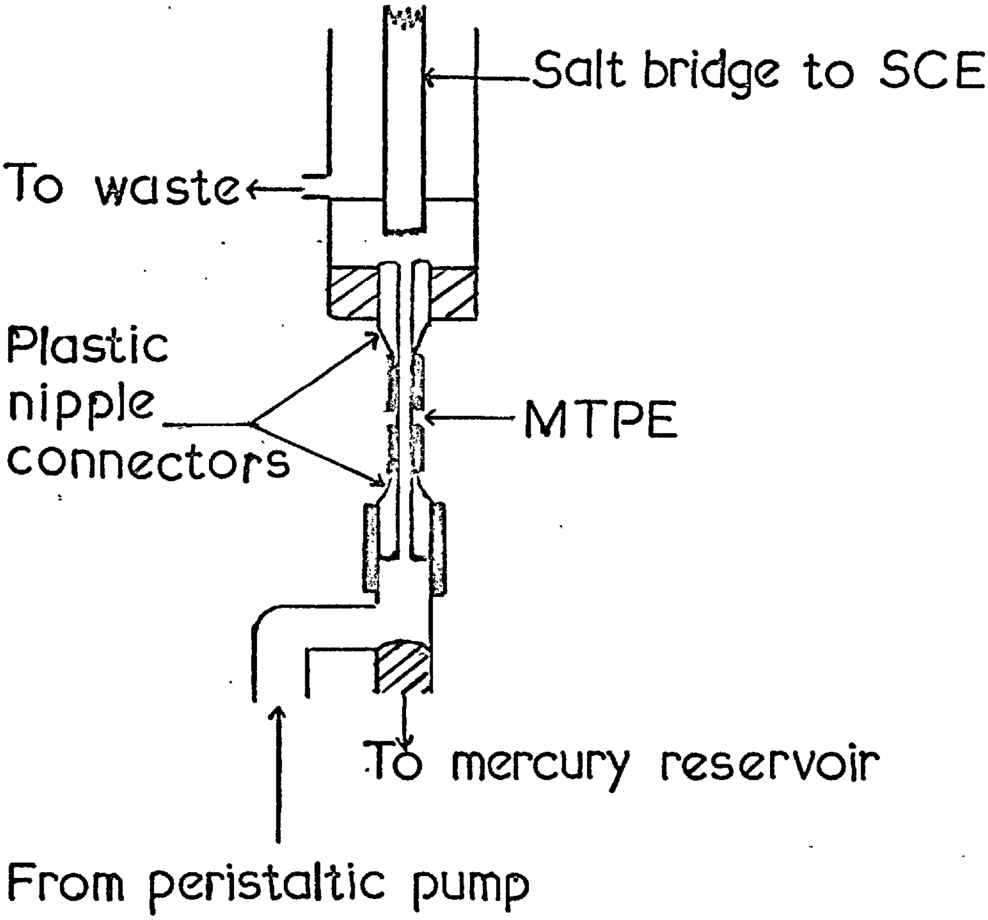


Figure 3.2.3 Cell assembly for MTPE.

cleaned in hot nitric acid and finally rinsed with distilled water. It is then fitted in the cell assembly as shown in Figure 3.2.3. A solution of 1M perchloric acid is pumped through the cell at a rate of 0.32 ml/m while the electrode is cathodised to the point of vigorous hydrogen evolution for about 15 minutes by applying a potential of -3 to -4V vs a SCE (or a large TPE). Then the pumping of the solution is stopped, the screw-clip closed and very pure high-grade mercury is introduced to make contact with the entire inner surface of the tubular electrode by raising the mercury reservoir, whilst the large negative potential is still maintained. Such stationary contact is maintained for a period of 15-30 minutes and then a forward and backwardmotion of the mercury in the tubular electrode is applied for a few times, again using the mercury reservoir for pumping. The mercury is then brought back to the junction level. The process may be repeated as often as necessary. The elctrode is filled with mercury whenever not in use.

The efficiency of the coating is tested by running a current-potential curve for a solution of deaerated 0.1M sodium sulphite at 0.32 ml/m. The plating is considered adequate if the residual current is only about 1.0 uA at a potential of -1.0 V vs SCE, and if the anodic and cathodic discharge potentials are in the region of +0.1 V and -2.0 V vs SCE respectively.

All solutions are thoroughly deaerated before and during measurement using deoxyenated nitrogen. The "oxygen-free" nitrogen (British Oxygen Ltd.,) is first bubbled through a train of four gas washing bottles containing a 2% solution of the sodium salt of anthraquinone-2-sulphonic acid in 2N sodium hydroxide and containing granulated zinc, before bubbling through the reagent solutions via very efficient gas dispersers.

The apparatus used has been described in the previous chapter.

Results and discussion

The cell described provides a very convenient means of plating mercury on the tubular platinum electrode. The rapid and simple procedure for plating is also very reliable and efficient in spite of the large surface area and the peculiar geometrical configuration of the platinum base.

The main problem associated with the use of the MTPE is found to be the large residual currents which result from the reduction of slight traces of oxygen at the mercury surface. Even after the most thorough deaeration using "deoxygenated" nitrogen a residual current as high as 8uA is obtained for a solution of 0.1M perchloric acid flowing at 0.32 ml/m.

Trace concentrations of oxygen present in the solution is sufficient to produce large currents at the large surface area of the electrode. This suggests the use of the electrode as a continuous voltammetric sensor for trace analysis of oxygen. It is however a disadvantage in the measurement of other substances and for this reason even an efficiency test for mercury plating has to be made with a solution of sodium sulphite (which reduces any oxygen present) instead of using the conventional electrolytes such as potassium nitrate or perchlorate.

Anodic voltammetric waves are obtained for the complexones EDTA and EGTA in an ammonia/ammonium nitrate buffer pH 9.5 which may be attributed, as with a DME, to the formation of mercury (II) complexonates. The waves are reasonably well-defined on the plateau but are slightly elongated presumably due to some influence of the large cathodic residual current (reduction of oxygen). Reproducibility of the waves as well as the limiting currents have been investigated and a standard deviation of 0.001V in the apparent half-wave potentials and of 0.08 uA (1.4%) in the limiting currents are found for a solution of 10^{-4}M EDTA in 0.3M ammonia/0.1M ammonium nitrate at a flow-rate of 0.32 ml/m. Sensitivities of the order of 56.5 uA per millimolar are achieved for the complexones.

Dependence of limiting current on concentration of the complexones is found to be linear in the concentration range studied (2 to 10 x 10^{-5}M); the limiting current of a fixed concentration of complexone is also found to decrease linearly with increasing concentration of added metal ion and therefore well suited for complexometric analysis with continuous voltammetric sensing. The limiting currents in these experiments are measured from zero current since a suitable residual-current base-line does not exist.

Conclusion

The use of a MTPE for continous voltammetric sensing proves to be a feasible proposition. Being a large electrode, high sensitivity is naturally achieved; its advantages over other large mercury-type electrodes such as a mercury-pool electrode is its ideal geometrical configuration analysis, precisely reproducible surface area and resistance to vibrations etc. (The surface of a mercury pool is affected even by changes in potential). The preparation of the electrode has also been shown to be a simple process.

Anodic voltammetric behaviour of complexones at the MTPE is found to be feasible for analytical applications, for which purpose a more rigorously designed assembly for exclusion of oxygen from the system is found to be necessary.

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