Discrete and Air Segmented Automated Methods of Analy including Robots

An Essay Review (Second Edition) 1988.

Methods for the Examination of Waters and Associated Materials

Her Majesty's Stationery Office

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About This Series

This booklet is part of a series intended to provide both recommended methods for the determination of water quality, and in addition, short reviews of the more important analytical techniques of interest to the water and sewage industries.

In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, have issued volumes of methods for the analysis of water and sewage culminating in 'Analysis of Raw, Potable and Waste Waters'. These volumes inevitably took some years to prepare, so that they were often partially out of date before they appeared in print. The present series will be published as a series of booklets on single or related topics; thus allowing for the replacement or addition of methods as quickly as possible without need of waiting for the next edition. The rate of publication will also be related to the urgency of requirement for that particular method, tentative methods and notes being used when necessary.

The aim is to provide as complete and up to date a collection of methods and reviews as is practicable, which will, as far as possible, take into account the analytical facilities available in different parts of the Kingdom, and the quality criteria of interest to those responsible for the various aspects of the water cycle. Because both needs and equipment vary widely, where necessary, a selection of methods may be recommended for a single determinand. It will be the responsibility of the users—the senior technical staff to decide which of these methods to use for the determination in hand. Whilst the attention of the user is drawn to any special known hazards which may occur with the use of any particular method, responsibility for proper supervision and the provision of safe working conditions must remain with the user. The preparation of this series and its continuous revision is the responsibility of the Standing Committee of Analysts (to review Standard Methods for Quality Control of the Water Cycle). The Standing Committee of Analysis is a committee of the Department of the Environment set up in 1972. Currently it has 9 Working Groups, each responsible for one section or aspect of water cycle quality analysis. They are as follows:

1.0 General principles of sampling and accuracy of results
2.0 Microbiological methods
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General nonmetallic substances
6.0 Organic impurities
7.0 Biological monitoring
8.0 Sewage Works Control Methods
9.0 Radiochemical methods

The actual methods and reviews are produced by smaller panels of experts in the appropriate field, under the overall supervision of the appropriate working group and the main committee. The names of those associated with this review are listed inside the back cover.

Publication of new or revised booklets will be notified to the technical press, whilst a list of Methods in Print is given in the current HMSO Sectional Publication List No. 5.

Whilst an effort is made to prevent errors from occurring in the published text, a few errors have been found in booklets in this series. Correction notes, and minor additions to published booklets not warranting a new booklet in this series will be issued periodically as the need arises. Should an error be found affecting the operation of a method, the true sense not being obvious, or an error in the printed text be discovered prior to sale, a separate correction note will be issued for inclusion in that booklet.

L R PITTWELL
Secretary and Chairman

11 August 1988

Warning to Users

The analytical procedures given in this booklet should only be carried out by competent trained persons, with adequate supervision when necessary.

Local Safety Regulations must be observed.

Laboratory procedures should be carried out only in properly equipped laboratories.

Field Operations should be conducted with due regard to possible local hazards, and portable safety equipment should be carried.

Care should be taken against creating hazards for one's self, one's colleagues, those outside the laboratory or work place, or subsequently for maintenance or waste disposal workers. Where the Committee have considered that a special unusual hazard exists, attention has been drawn to this in the text so that additional care might be taken beyond that which should be exercised at all times when carrying out analytical procedures. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specifications. Specifications for reagents, apparatus and equipment are given in manufacturers' catalogues and various published standards. If contamination is suspected, reagent purity should be checked before use. Lone working, whether in the laboratory or field, should be discouraged.

The best safeguard is a thorough consideration of hazards and the consequent safety precautions and remedies well in advance. Without intending to give a complete checklist, points that experience has shown are often forgotten include: laboratory tidiness, stray radiation leaks (including ultra violet), use of correct protective clothing and goggles, removal of toxic fumes and wastes, containment in the event of breakage, access to taps, escape routes, and the accessibility of the correct and properly maintained first-aid, fire-fighting, and rescue equipment. Hazardous reagents and solutions should always be stored in plain sight and below face level. Attention should also be given to potential vapour and fire risks. If in doubt, it is safer to assume that the hazard may exist and take reasonable precautions, rather than to assume that no hazard exists until proved otherwise.

There are numerous handbooks on first aid and laboratory safety. Among such publications are: 'Guide to Safe Practices in Chemical Laboratories' and 'Hazards in the Chemical Laboratory', issued by the Royal Society of Chemistry, London: 'Safety in Biological Laboratories' (Editors Hartree and Booth), Biochemical Society Special Publication No. 5, The Biochemical Society, London, which includes biological hazards; and 'The Prevention of Laboratory Acquired Infection', Public Health Laboratory Service Monograph 6, HMSO, London.

It cannot be too strongly emphasised that prompt first aid, decontamination, or administration of the correct antidote can save life; but that incorrect treatment can make matters worse. It is suggested that both supervisors and operators be familiar with emergency procedures before starting even a slightly hazardous operation, and that doctors consulted after an accident involving chemical contamination, ingestion, or inhalation, be made familiar with the chemical nature of the injury, as some chemical injuries require specialist treatment not normally encountered by most doctors. Similar warning should be given if a biological or radiochemical injury is suspected. Some very unusual parasites, viruses and other micro-organisms are occasionally encountered in samples and when sampling in the field. In the latter case, all equipment including footwear should be disinfected by appropriate methods if contamination is suspected. If an ambulance is called or a hospital notified of an incoming patient, give information on the type of injury, especially if poisoning is suspected, as the patient may be taken directly to a specialized hospital.

Foreword

Since the early 1960's water laboratories have been required to perform increasingly large numbers of the commonly determined parameters such as ammonia, total oxidized nitrogen, chloride, chemical oxygen demand, biochemical oxygen demand, suspended solids, etc. Automation of various types has been applied to many of these determinations and has enabled these increasing sample numbers to be handled with improved efficiency.

Air segmented continuous flow analysers, discrete analysers and flow injection analysers have handled the bulk of this automation and improvements in both hardware and software have greatly improved reliability and reduced the risk of human error.

Using these forms of automation it has however not been possible to automate procedures that require large amounts of sample preparation, as for example chemical oxygen demand, biochemical oxygen demand, and suspended solids. In many laboratories today, such parameters are still determined manually with only minimal levels of automation applied to them, (eg. auto titrators, dissolved oxygen probes etc.). These methods when applied to large numbers of samples require analysts to perform repetitive operations which can lead to human error.

The application of laboratory robots appears to offer some solutions for these highly repetitive analyses. Although very much in their infancy in development they are able, in most cases, to perform sample preparation and handling techniques at rates similar to, or slightly faster, than humans. Operating throughout a working day and if possible overnight they can become a very effective laboratory aid.

This booklet contains:—

a. A table (Table 1) of determinands frequently analysed in the water industry by the techniques which follow.

b. Information on the types of discrete analysers in common use and methods applied to them.

c. The types of laboratory robots available, guidelines for selection and the methods to which they have so far been applied.

d. The second edition of the Air Segmented Continuous Flow booklet.

Discrete Analysers

1. Introduction

With the increasing sophistication of microcomputer technology, the range and versatility of discrete analysis has increased to the point where the techniques are alternatives to conventional continuous flow analysis as methods when considering automation. This is borne out by the wide range of instrumentation now available.

Currently available discrete analysis instrumentation may conveniently be sub-divided into three types: batch, random access and centrifugal. It is therefore important to distinguish between the terms "batch" and "random access" as generally applied to chemical analysis.

Multichannel continuous flow analyser equipment in its simplest form comprises the least flexible type of batch analyser. In practice, all samples tend to be analysed for all determinands, whether or not required. In contrast, in the simplest form of random access analyser only the samples actually scheduled for a specific analysis are analysed for that determinand.

The following sections describe the features of the three main types of discrete analyser using, where applicable, examples of instrumentation already established in the water industry. Limitations and problems commonly encountered when using discrete analysers are described in some detail in a later section.

2. Batch Analysers

These instruments are computer controlled discrete analysers which analyse the samples sequentially for the chosen detrimands, each set of samples and standards for one determinand being a batch. A batch, once entered via the computer keyboard and the analysis commenced, cannot usually be altered, eg. added to, until either the test is complete or unless the "run" is purposely aborted and recommenced.

Flow injection analysis is dealt with in a separate booklet in this series (Ref. 49).

Table 1. Some determinands frequently analysed in the Water Industry to which one or more of these techniques has been applied.

Key to Techniques:

A. Discrete Analyser B. Robots C. Air Segmented Continuous Flow
D. Flow Injection Analyser.

Determinands	Technique
Ammonia	A,C,D
Oxidized Nitrogen (Nitrate	A,C,D
(Nitrite	A,C,D
Phosphorus (usually ortho Phosphate)	A,B,C,D
Chloride	A,C,D
Sulphate	A,C,D
Sulphide	C,D
Sulphite	B
Cyanide	A,C,D
Alkalinity	A,C,D
Hardness	A,C,D
pH	A,B,D
Conductivity	A,D

contd.

Table 1. Some determinands frequently analysed in the Water Industry to which one or more of these techniques has been applied.—*continued*

Key to Techniques:

A. Discrete Analyser B. Robots C. Air Segmented Continuous Flow
D. Flow Injection Analyser.

Determinands	Technique
Colour	A
Turbidity	A
Fluoride	A,C
Permanganate Value/Index	C
Phenol	C
Boron	A,C,D
Silicon (or Silicate)	A,B,C,D
Surfactants	C,D
Iron	A,C,D
Aluminium	A,C,D
Manganese	A
Urea	C
B.O.D.	B*
C.O.D.	A,B,C,D

This table is intended as a guide to the more commonly encountered applications and is not an exhaustive list of determinands which can be analysed using these techniques. The majority of these procedures have been adapted from existing published methods in this series (Refs. 20-44 and 52).

Sodium, Potassium, Calcium, Magnesium and similar flame or AAS analyses can also be coupled to Discrete and Flow Injection analysers as can ICPS.

A typical instrument of this type comprises a computer module including keyboard, screen and disc drives, and a chemistry console the functions of which are controlled by the computer. In addition to control over the functioning of the chemistry console, the computer also deals with organising the workload in the most efficient manner, and all results production, data handling and storage, and may also be linked up to receive workloads from, and to transmit edited sample results to a main frame computer.

The chemistry console might typically be comprised as follows:

i. a sample wheel holding up to 99 sample cups surrounded by a cooled water bath

ii. a reaction wheel with spaces for up to 99 reaction tubes and provision where necessary of a heated water bath for reproducible reaction or final colour development

iii. a sample probe which transfers samples between the sample cups and reaction tubes

iv. air pressurized reagent bottles stored internally under ambient or refrigerated conditions, with individual reagent lines, solenoid dispense valves and dispense stations

v. mixers eg. pneumatically activated pairs of hollow rods releasing air bubbles in a controlled manner

vi. a spectrophotometer suitable for colorimetric/turbidimetric analysis.

For each method the following important parameters are preprogrammed:

Sample/diluent volumes, reagent dispense volumes, water bath temperature, degree of mixing, colour development time, spectrophotometer transfer volume and mathematical computations performed. Some of these may be altered via the keyboard for fine tuning method development.

* Presently, excluding the incubation stage.

3. Random Access Analyser

This type of instrument differs from the batch analysers considered previously in that only those samples within a group actually scheduled for a specific analysis are analysed for that determinand.

In the simplest form of random access analyser, the determinand order (ie. the order in which analyses are to be carried out) is user-controlled; after analysis for a particular determinand has been completed, the instrument pauses and requires operator intervention for replacement (if appropriate) of standards and reagents before proceeding to the next determinand. In more sophisticated instruments, provision is made for storage of multiple reagents and analysis continues with minimal operator involvement until all analysis requests are completed. Additional analysis requests and samples may be added after analysis has begun. It is this latter type of more sophisticated instrument which has found considerable use in water analysis.

Instruments based upon these principles may differ enormously in design, size and sophistication. Typically, a random access analyser consists of a CHEMISTRY CONSOLE and a COMPUTER.

The instrument computer and associated hardware (ie. disk drives, visual display unit, bar-code reader etc.) is used for some or all of the following functions:

(1) Basic control over operation of the chemistry console using stored methods generated by the user. Methods contain all information required for analsyis eg. sample/reagent volumes, reaction times, detection wavelength, expected range and performance, calibration and quality control data etc.

(2) Organisation of the analytical workload in order to maximise efficiency of operation (usually measured by tests/hour performed). This may involve analysis for several determinands simultaneously (with recalibration on a time basis). The order of analysis is continually updated by the computer to take account of additional analysis requests: many instruments have a facility for manual override of this function so that urgent samples may be given immediate attention. Analysis requests may be made directly via the instrument keyboard, using a bar-code reader or automatically downloaded from a Laboratory Information Monitoring System (LIMS) computer using an appropriate interface.

(3) Storage of raw data, including photometric and Kinetic information. Quality control data may be stored, with appropriate performance statistics generated automatically.

(4) Manipulation of analytical data, including calibration and reanalysis of samples falling outside pre-set limits (with automatic dilution where appropriate). This function may be fully automatic, or require operator validation.

The chemistry console typically consists of the following:

(1) **Storage** for reagents, samples and standards. The temperature of such stored solutions is usually maintained below ambient, so as to minimise losses and errors due to evaporation or decomposition.

(2) **Reaction Zone** This is often a wheel with spaces for up to 100 reaction tubes to which samples and reagents are added and mixed at a controlled temperature. The mixture is transferred to a photometer for analysis, following which the reaction tubes are rinsed and dried before re-use. Alternatively disposable cuvettes may be used directly for mixing and reaction thereby eliminating a possible source of cross-contamination due to inadequate rinsing (but at much greater expense). Transfer of liquids within the chemistry console may be via individual lines with non-return valves or by a single transfer arm (which may also be used for mixing). In all cases, carryover effects must be minimised by suitable rinsing/drying procedures.

(3) **Detector** This is usually a photometer suitable for standard photometric or turbidimetric analyses.

4. Centrifugal Analysers

The batch and random access analysers discussed above are both examples of SEQUENTIAL ANALYSERS ie. instruments where solutions for analysis are treated and measured one after the other. The centrifugal analyser represents the only fully automatic example of a parallel analyser ie. an instrument where solutions for analysis are treated and measured simultaneously.

In the way that Leonard Skeggs has been attributed with the initial development of the continuous flow analyser, Norman Anderson, then Director of the Molecular Anatomy Program at the United States Oak Ridge National laboratory, has been recognised as being the initial devoloper of the centrifugal analyser. His initial work took place in 1966 and was followed by a burgeoning commercial development by instrument makers. The first commercial systems appeared on the market in 1969, when acceptance into the clinical field was both rapid and enthusiastic. For a recent example for water analysis, see Ref. 52.

A centrifugal analyser in its simplest form consists of a circular plate on which are mounted a number of radial channels. Samples for analysis are placed in individual channels near to the centre of the plate, with reagent solutions placed near to the outside of the plate. This plate is loaded into a centrifuge and spun rapidly to ensure mixing and reaction, the analytical signal from each channel is monitored many times per second as the channel passes an appropriate analytical sensor (usually a spectrophotometer).

Instruments based upon this principle consist of a SAMPLE/REAGENT LOADER, an ANALYTICAL MODULE and a COMPUTER.

4.1 Loader—In order to use a centrifugal analyser individual aliquots of both samples and reagents are delivered into their respective compartments on a transfer disc by an accurate dispensing device. Each manufacturer has developed an automated system for sample and reagent loading. An example of a loader that is capable of loading a sample and two reagents is shown diagrammatically in Figure 1.

4.2 Analytical module—The analytical module consists of a rotor, a removable transfer disc, a drive motor, an optical system, a temperature controlling system and a means of synchronising the photodetector output with the microprocessor.

The rotor, which is the primary component of a centrifugal analyser, is made by sandwiching a teflon ring between a top and bottom ring of Pyrex, quartz or ultra-violet transmitting plastic, as shown in Figure 2. Rotors are available from different manufacturers with varying numbers of cuvettes that are suitable for a particular analyser. Each cuvette within the rotor has a bifunctional siphon to ensure mixing of reagents and samples within their respective cuvettes and to provide a means of emptying and cleaning the cuvettes between analytical runs (see Figure 3). Another development produced analysers with a disposable transfer disc which fits a much less complex rotor, shown in Figure 4. This disposable transfer disc eliminates carry-over and contamination between runs and thereby offers some advantage over the permanent rotor.

The size of motor used to drive the rotor assembly varies with the number of cuvettes in the rotor. Control of acceleration and deceleration of the rotor assemblies in various models is similar. Typically rotor speed is controlled by an auto-transformer and also a three position switch that is labelled "incubate", "accelerate" and "run". To initiate an analytical run, a loaded transfer disc is placed in the analyser with the rotor stationary.

The run is started by entering appropriate information controlling the analytical module. After all entries have been made, the instrument proceeds to the "incubate" mode that allows the rotor, transfer disc, reagents and samples to reach a preselected temperature before mixing occurs. Typically during this phase the rotor speed is 100 rpm. When the desired temperature has been obtained, the analyser enters the "accelerate" mode that increases the rotor speed to 4000 rpm and then comes to an abrupt halt. This acceleration and subsequent sudden stop takes place in a time span of 0.5 seconds. Finally, the analyser proceeds in the "run" cycle in which the rotor speed is maintained at 1000 rpm. It is at this stage that data collection is initiated.

The first analyser developed by Anderson had a monochromator system for making spectrophotometric measurements. More recently other types of optical measuring devices have been incorporated into basic systems, which can then measure transmittance, fluorscence, or light scattering of several parallel reactions. Since one of the most important reaction conditions is temperature, a centrifugal analyser must have a system for monitoring and controlling the temperature of the rotor and its environment. Temperature can be accurately and precisely measured and controlled by several different methods, all of which require some type of measurement sensor, which is usually a thermistor located near the rotor,

Figure 1 A centrifugal analyzer loader module capable of loading a sample and two reagents

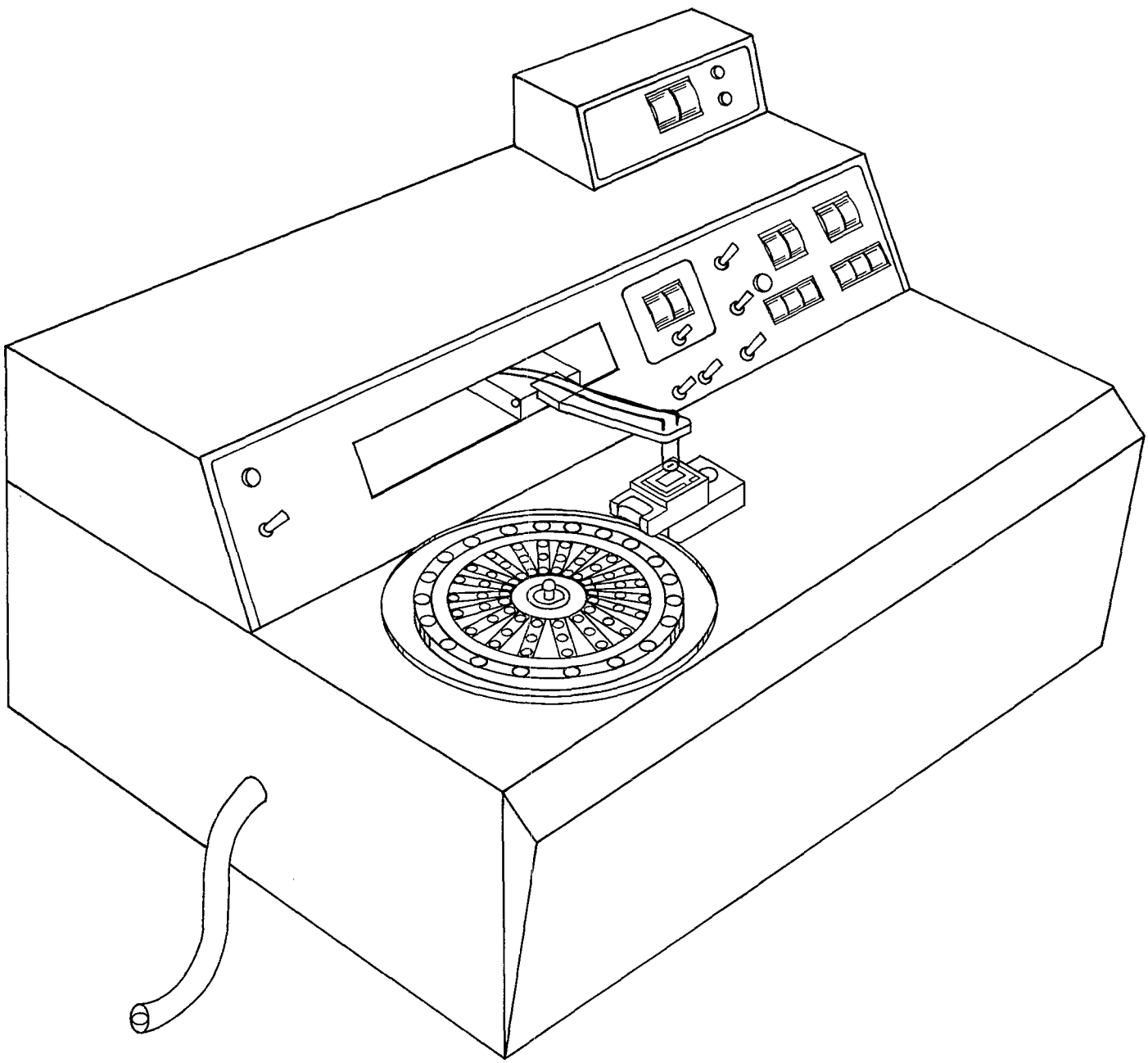
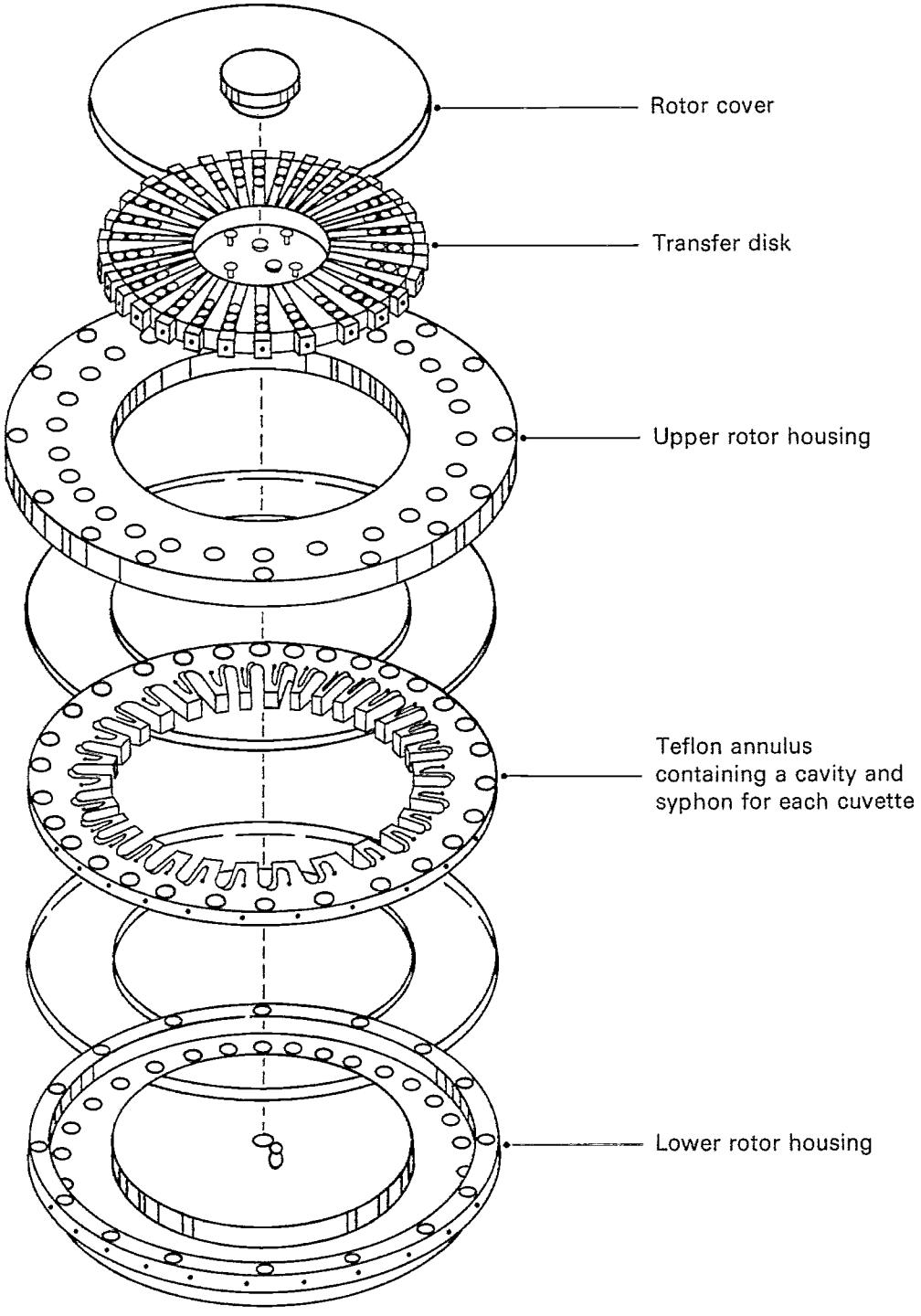


Figure 2 Schematic representation of a centrifugal analyzer rotor



within the rotor itself, or in one of its cuvettes. The techniques utilised for controlling the rotor temperature include electrical or radiant heating of the rotor, or control of the rotor environmental temperature with an air or liquid reservoir.

4.3 Computer—Analysis of raw data from the photomultiplier detector is accomplished using a computer. All of the centrifugal analysers that are commercially available have a built-in microprocessor, with up to 64 kbytes of memory. The analyser generates a continuing series of pulses, each corresponding to one sample space in the rotor, separated by dark intervals. In order to establish an initial starting point, there is also a small hole in the rotor that transmits light once per revolution of the rotor. This signal serves to synchronise the rotor speed and photomultiplier. It also signals the on-line computer when the dark-current readings and the cuvette readings are to be taken. The computer corrects for the dark current, calculates concentrations of samples and averages the results over many rotations. Built-in subroutines in the software, including calibration algorithms, allow for manipulation of the raw data.

5. Factors relating to the Applicability of Discrete Analysers

This Section summarises the relative merits of discrete analysers when compared with conventional methods.

1. Discrete analysers are versatile, easy to operate and able to deal with large sample numbers. Many are highly sophisticated requiring minimum operator intervention and may be operated unattended overnight.

2. Manual operations are minimised, thus reducing operator error, improving precision, and may improve limits of detection. Temperature control is possible.

3. Although initial capital expenditure is high, reduced analytical costs may be achieved.

4. Reduced usage of reagents and sample is possible. This is particularly beneficial when using expensive reagents or reagents of a toxic or hazardous nature.

5. Data handling systems are incorporated, offering a range of mathematical manipulations and reporting formats. These may include analytical quality control facilities and highlighting of results outside specified limits.

6. If instrument breakdown occurs, the problem of having "all one's eggs in one basket" may arise. A high level of routine preventative maintenance is therefore required in order to reduce instrument down time. This will include daily in-house maintenance together with the efficient, though expensive, service support from the manufacturers. Provision of adequate back up analysis should be considered.

7. Samples exceeding the normal calibration range can be easily repeated on a suitable range or automatically diluted and re-run.

8. Instruments should have minimum carry-over or cross contamination and incorporate sample heads which are self-cleaning and drying between each sampling operation. Regular cleaning or replacement of parts is necessary to reduce the possibility of carry-over due to droplets adhering to surfaces.

9. Methods for many determinands required by the Water Industry have been developed for discrete analysers (Table 1). However, where they have not been developed, considerable method development time may be necessary. Methods requiring distillation, dialysis and solvent phase separation are currently not practicable.

10. With these types of analysers, often a small volume of sample is mixed with a large volume of reagent. Most manual water chemistries, particularly those for potable water, require a large amount of sample combined with a small amount of reagent. Hence the ratio of maximum sample volume to final reaction mixture volume is important. Usually, machines are designed to permit up to 30% of sample volume in the final mixture, which will cause problems with those water chemistries requiring large amounts of sample. An instrument with below 15% relative sample volume will be severely restricted in the range

Figure 3 Diagram of a teflon annulus used to define the cuvettes

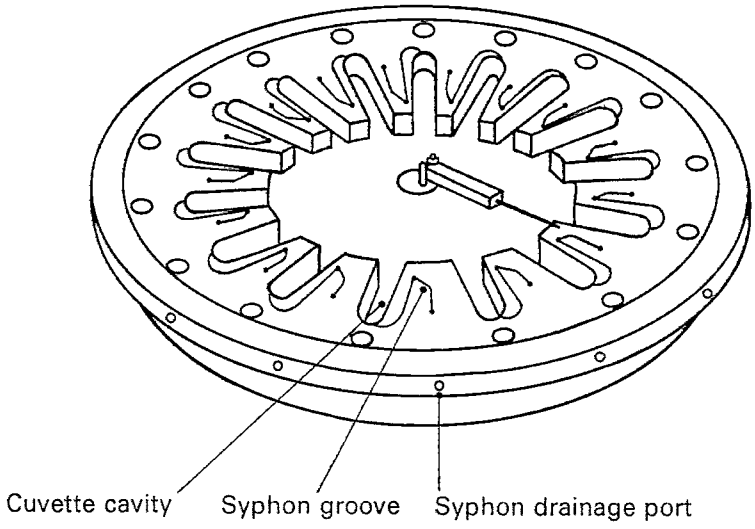
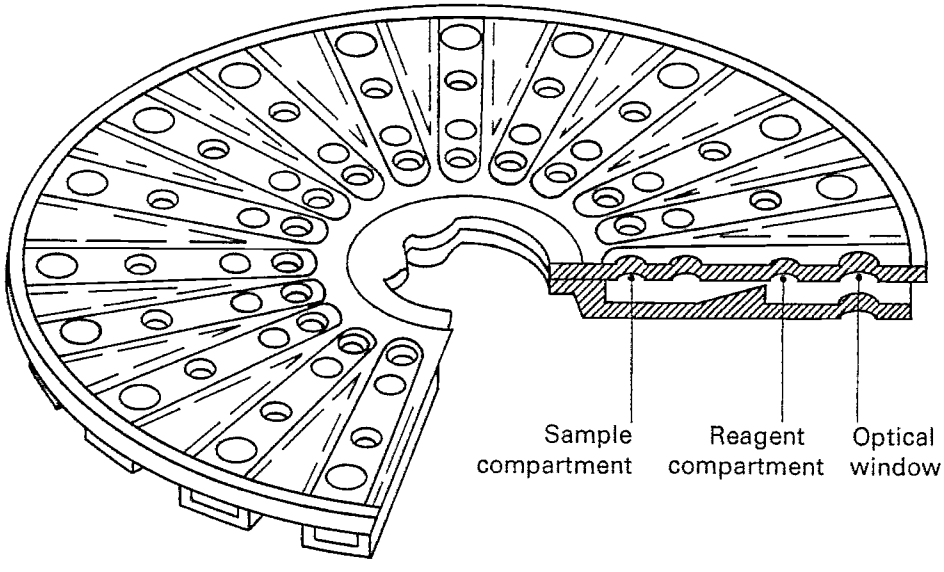


Figure 4 Cross section of a disposable transfer disk



of possible chemistries that can be used. Some instruments are available where a ratio of over 50% is possible. These have more flexibility in their applications and have improved sensitivity, which influences the limits of detection which can be achieved.

11. For some instruments, the number of reagents is limited to two or three. This may present a problem. One approach which can be used, is to make mixed reagents, provided these are stable or compatible for at least the duration of the test. The action of reagents on the components of the analyser must also be checked to ensure that there is no contamination from the apparatus. Particularly viscous reagents which cannot be easily pumped through narrow bore tubing, strongly corrosive acids or alkalis, and reagents which tend to precipitate in reagent valves or lines cause difficulties. These can be overcome in many instances by the use of resistant reagent lines and valve diaphragms, and by regular water purging after every usage.

12. The following problems have been reported for specific methods when run on certain instruments:—

i. Nitrate TON—Soda lime glass reaction tubes have been found to be contaminated with nitrate. This can be overcome by using borosilicate glass reaction tubes.

   In tests with nitrite standards, the Copper Hydrazine reductant reagent strength has been found to affect critically the shape of the calibration curve and analytical accuracy.

ii. Sulphate—the precipitating reagent must be shaken thoroughly before use, otherwise erratic results are produced. The final mixing stage in the turbidimetric method should be located as close as possible to the measurement stage.

iii. Ammonia—ammonia loss from sample cups occurs, this analysis should be carried out immediately the instrument is loaded with samples.

iv. Aluminium—pick up from glassware is common.

v. Silica—plastic reaction tubes must be used to avoid pick up from glass.

vi. Boron—special boron free glass or plastic must be used.

Robots

A widely accepted definition of a robot given by the Robot Institute of America is:

"A robot is a reprogrammable, multifunctional, manipulator designed to move materials, parts, tools, or specialised devices, through variable programmed motions for the performance of a variety of tasks".

In addition to the above definition, for the purposes of this booklet for an instrument to be classed as a robot it must meet the criteria below:—

"The robot must contain a grasping manipulator, or other specialised sample handling device capable of lifting samples, containers or associated parts from one part of an operating area to another;

and/or

the movements of the robot must be under user programmable control and the software shall be capable of accepting escape routines initiated automatically by the conditions encountered in the test such as obstruction, tight stoppers, exhausted samples, and so on".

Robotic methods have recently been applied to water analysis. It should be stressed that the use of robots does not imply the use of new techniques. The robot is merely a sample handling system enabling a previously proven manual technique to be automated. Among the fully robotized methods so far reported are: COD, pH, sulphite (SO_2), silica, ortho-phosphate, and the BOD method which has already been robotized except for the incubation stage. In no case has the technique been modified in any way from the published standard procedure; indeed as a result of automating these procedures the method conditions (eg heating and cooling times, electrode stability times and sampling conditions) can be adhered to more closely than before.

Although the robot may be a highly flexibile device, in any routine application the assembled equipment will be specified for the task or tasks being performed ie robot, controller, software, sample racks, peripherals etc. Also, depending on the application, the robot may be located on purpose made benching, in its own room, or behind safety or fume extraction screens.

Since in most cases robots exactly mimic human operators, their throughput rates may not always be significantly faster than those of operators using manual methods. However, they have the advantage that they are capable of operating unattended over a 24hr period.

The robots described by manufacturers as suitable for laboratory application include the following types:—

1. Anthropomorphic (or rotating jointed arm) Fig. 5
2. Cylindrical Fig. 6
3. Polar Fig. 7
4. Cartesian (or XYZ-3 dimensionally sliding arm) Fig. 8
5. SCARA (Single constraint automatic robot arm) Fig. 9
   (See also Refs. 48, 50 and 51)

Wrist action may also be incorporated into the arm head (see Fig. 5).

An additional form of robot usually confined to custom built apparatus consists of a carriage moving on a track which can include curves, gradients and remotely controlled junctions (much like a toy railway), which transports the samples in containers to a number of work

stations, automatic dispensers and the like. Manipulative robots of types 1 to 5 above may be coupled with such a system to transfer samples in containers to and from equipment such as heaters, spectrophotometers, and autosamplers. Combinations of this type can accommodate delays such as those found in methods like the BOD digestion.

Robot laboratories consisting chiefly of pumps, electrically operated valves, metering devices and a variety of sensors mounted on mobile arms (fitted with rinsing devices) are used for analysing waters requiring regular monitoring for several determinands at remote locations, results being transmitted by land line or radio (as well as recorded). Such devices can be equipped with automatic samplers which take reference samples for further analysis in the event of significant change being noticed.

The following operations have been adapted for robots:—

Liquid handling	eg. dilution and reagent addition dispensing homogenizing
Sample conditioning	eg. mixing heating cooling evaporating
Separation	eg. filtration partition centrifugation precipitation
Measurement	eg. of pH conductivity colour weight

Robots have also been used in the laboratory for handling hazardous samples—such as, opening vials which may be under pressure, or handling highly toxic, unstable, or radioactive materials. Thus in one sealed take COD digestion system samples are sealed into glass vials, heated for digestion, cooled and the vials opened, quantitatively emptied and finally analysed all by robot devices sited along a track along which the sample moves. Many similar arrangements can be devised. Although usually dedicated to a specific task or analysis, robot systems can be devised which, like some discrete analysers, perform a series of analyses on the same sample.

In selecting a robot or "robotized" technique for water analyses the following factors should be considered:

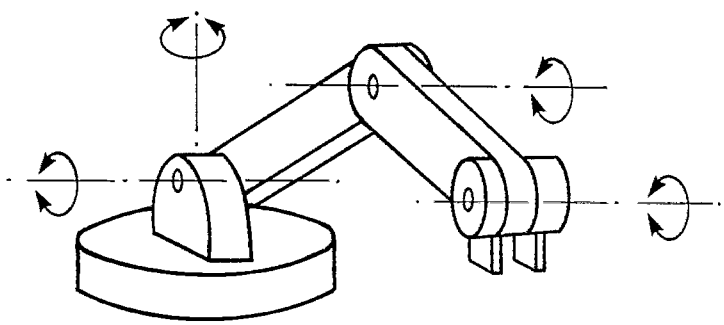
1. Has the technique been modified substantially from the standard procedure in order to automate it?
2. Is documented confirmation of comparability to the standard procedure available?
3. Is there a guaranteed source of consumables available from a reputable manufacturer?
4. Will the system perform the test unattended and out of hours?
5. Is there suitable benching available. Are there health and safety considerations in installing the system in a general laboratory which would require a separate room to be allocated? (See the note on hazards below)
6. Are there sufficient trained personnel to operate, programme and maintain the system correctly?
7. Do the sample numbers justify the capital expenditure?

Hazards. Although the following points are vital in manned operations they are just as important when using robots.

Consideration must be given to the consequences of vessel breakage, and equipment failure. Questions such as the following need study. Can corrosive substances fall onto electric wiring or insulation? Can flammable vapours be ignited by even a strong static spark? Can any piece of robot equipment cause injury, or provide a conducting path to the operator in the event of an arc-over?

Figures 5 to 9 Types of robot arm action

Figure 5 Anthropomorphic or rotating jointed arm



Wrist action variant (showing maximum degrees of freedom for one joint)

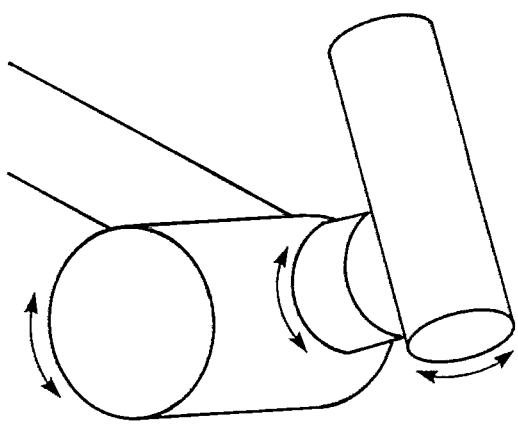


Figure 6 Cylindrical

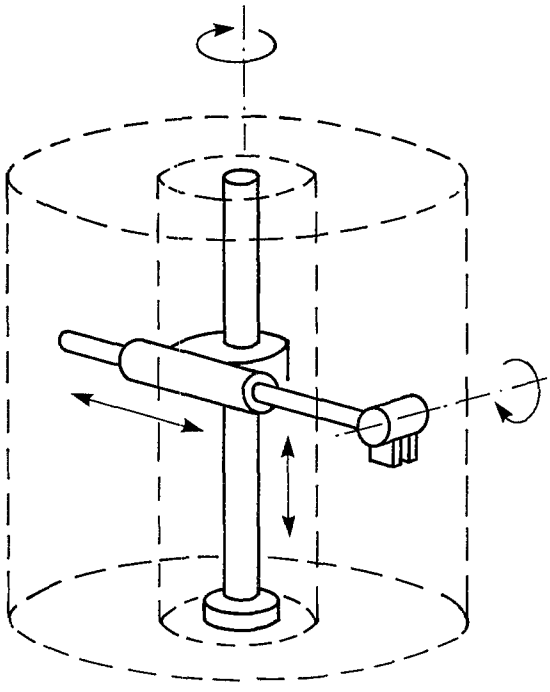
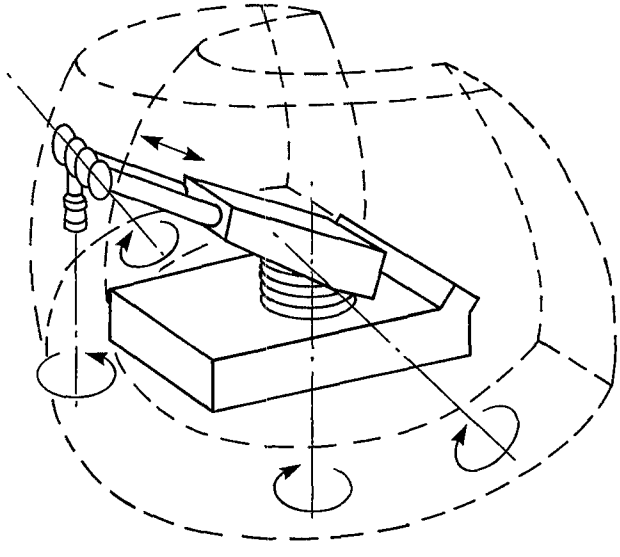


Figure 7 Polar



The arm slides in and out as well as rocking up and down and to and fro. The head may itself also rotate.

Figure 8 Cartesian or XYZ 3-dimensional sliding arm

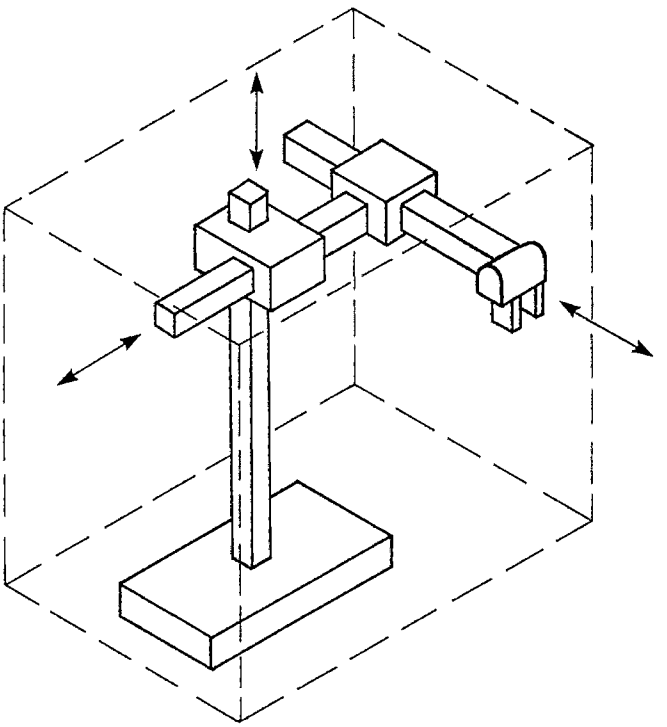
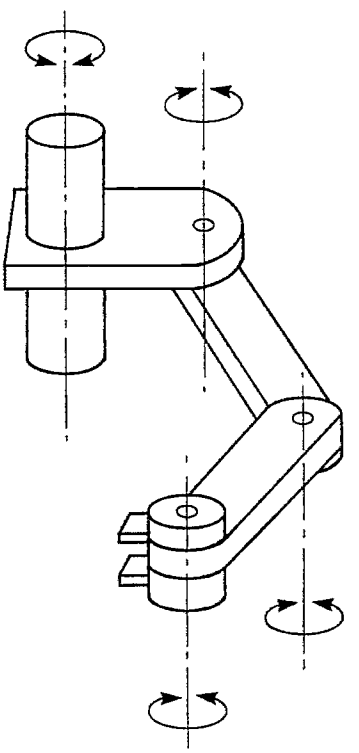


Figure 9 Single constraint robotic arm SCARA
(sometimes considered as a jointed arm with limited degrees of freedom)



Air Segmented Continuous Flow Automatic Analysis in the Laboratory (Second Edition)

(An Essay review by K W Petts)

1. Introduction

1.1 Historical (1)

Within the field of continuous automatic analysis the dominant influence has been the colorimetric work of Skeggs (2). His design of automatic analyser presented several novel features which have proved amenable to extensive further development and remain fundamental to the most widely used approach to automatic analysis by the continuous method. He designed and evaluated a continuous-flow system in which were performed the analytical operations and which also provided the means of sample transport as part of the instrument. The analyser consisted of a series of modules each performing a specific function, eg. sampling, sample transport, heating, dialysis and photometric measurement.

An operational feature of Skegg's analyser which has proved of profound significance in the subsequent success of his concept is that, in addition to sample and reagents, air is drawn into the analyser and produces segmentation of the liquid stream. This segmentation is of fundamental importance because it enables individual samples to retain their identity throughout the analytical process while at the same time permitting a high rate of sample throughout. Section 3.3 of this review gives an account of this segmentation of liquid by air.

Skegg's automatic continuous analyser was commercially introduced in 1957 and now adaptations of this system are found widespread in almost every facet of analytical chemistry; its range and flexibility have been extended by the introduction of additional modules. The original system was limited to colorimetry in the visible-light range as the detecting method, but units for flame photometry, UV spectrophotometry and fluorimetry are a few examples that are now commercially available. In principle the continuous-flow approach of this system does not impose any limitations on the choice of detection technique other than those inherent in design compatability. Consequently electrochemical and flame-ionization detection techniques are to be found used in conjunction with automatic continuous analysers. Details of the basic equipment of continuous flow systems are given in Section 3 of this review.

1.2 Present-day concept

In air-segmented continuous flow analysis a single sample or a number of single samples are converted into a continuous flowing stream by a pumping system and the necessary reagent additions are made by continuous pumping and merging of the sample and reagent streams. This stream is continuously segmented by air. Ultimately a treated sample is pumped to a flow-through measuring unit and thence to waste.

A processing rate of 20-80 samples per hour is normal and several samples are usually being processed at any one time between the sampling and measurement stages. However, there is usually no difficulty in associating each recorded detector response with a particular sample since the regular timing intervals between stages are controlled. The insertion of frequent standards in the sample sequence affords regular datum points. However, unless precautions are taken, interaction can occur in a continuous system thereby causing loss of discrimination between successive samples at the recording stage. Section 5.2 briefly outlines sample interaction and Section 6 relates the kinetic aspects of a continuously flowing analytical system which considers the factors that influence sample interaction and sampling rate.

2. Application in the Water Industry

Air-segmented continuous flow automatic analysis is now in widespread use in the Water Industry. The problems encountered in river pollution studies are extremely varied and the chemical factors causing the pollution are numerous. Thus numerous samples are analysed daily for the determination of many parameters in order to monitor the state of the river and

the effect on it of pollution waste. The determinations listed C in Table 1 (see page 7), are among those most frequently carried out by Laboratories analysing waters and effluents to which an air-segmented continuous flow automatic analysis technique has been applied.

Numerous papers have been published on the use of air-segmented continuous flow automatic analysis for these parameters. A bibliography produced by one manufacturer of this type of system (3) cites 300 literature references for the period 1967-73 related to the Water Industry. Method details can be conveniently expressed by a line diagram of the construction of the sample treatment system (the manifold) and subsequent analytical stages as the example in Fig 10 shows.

This essay review does not seek to provide detailed information on individual methods which are covered by other publications in this series.

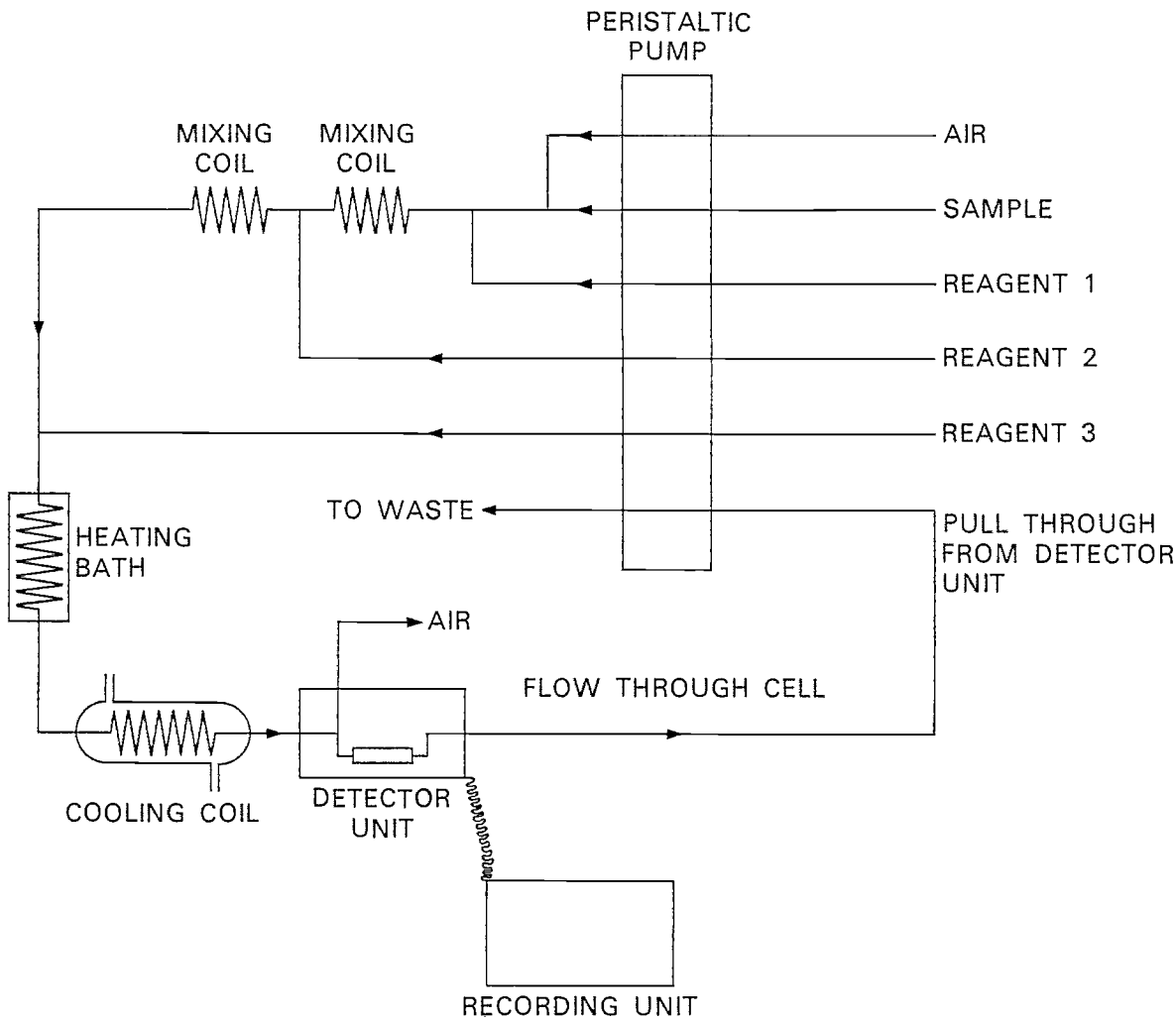


FIG 10 LINE (FLOW) DIAGRAM OF AN AIR-SEGMENTED CONTINUOUS FLOW AUTOMATIC ANALYSER

3. Basic Equipment of Continuous Flow Systems

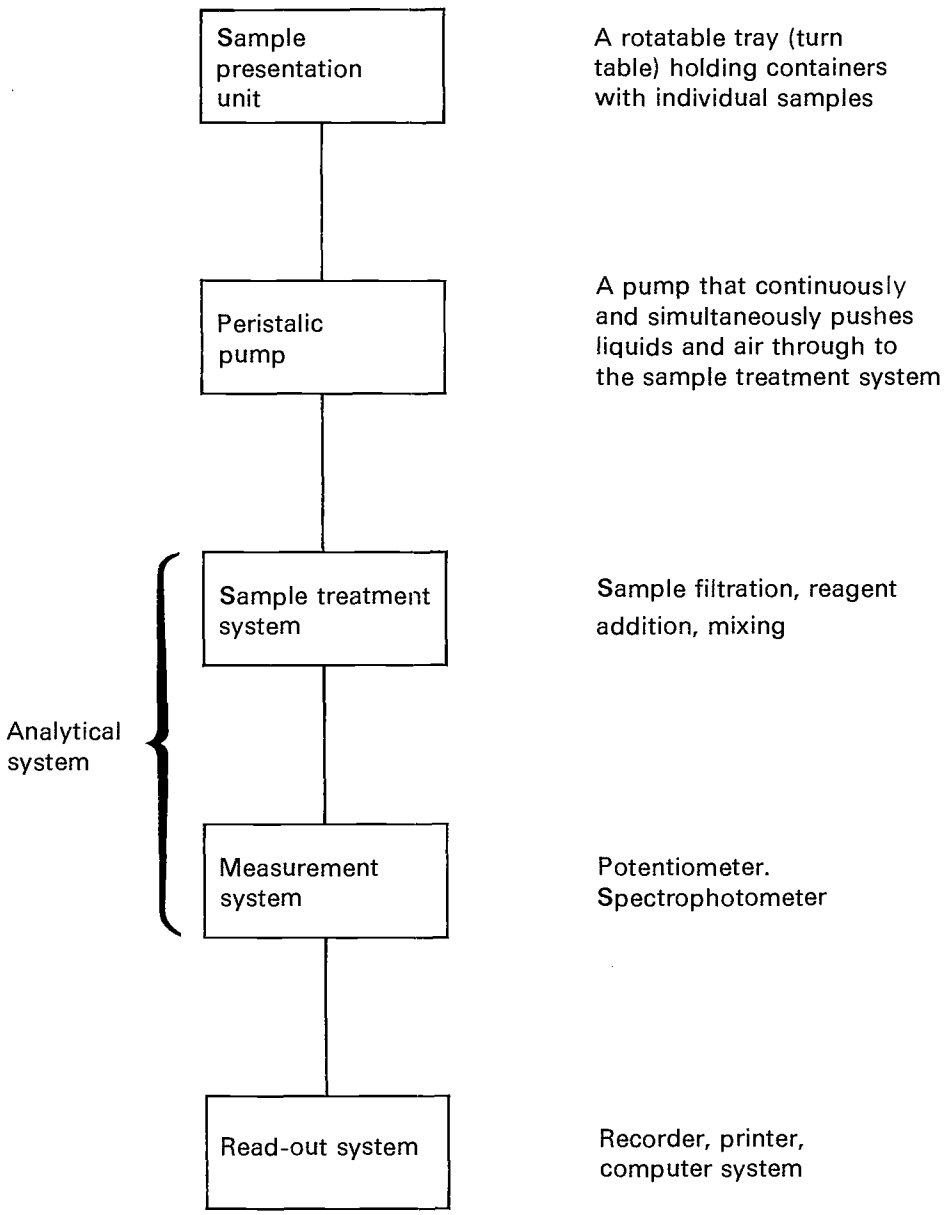
The block diagram Fig 11, shows the component stages of a typical automatic analysis system. A variety of such systems is commercially available.

3.1 Sample Presentation Unit

When a number of individual samples are to be submitted sequentially, each successive sample* is conveyed to the 'sample treatment system' by the peristalic pump (see Section 3.2). The sample presentation unit (the sampler) is comprised either of a plate with holes around the circumference to hold sample cups (Fig 12), or a number of sample tubes snapped together and wound into spools moving by a conveyor belt principle (Fig 13). Up to 400 samples can be placed on the latter type of sampler. Both are fitted with a sample probe, normally of stainless steel, but alternatively of glass or plastic, for withdrawing the sample.

* The term 'sample' refers to the solution being tested or the blank solution or a standard solution.

Figure 11 Basic equipment for continuous flow analysis



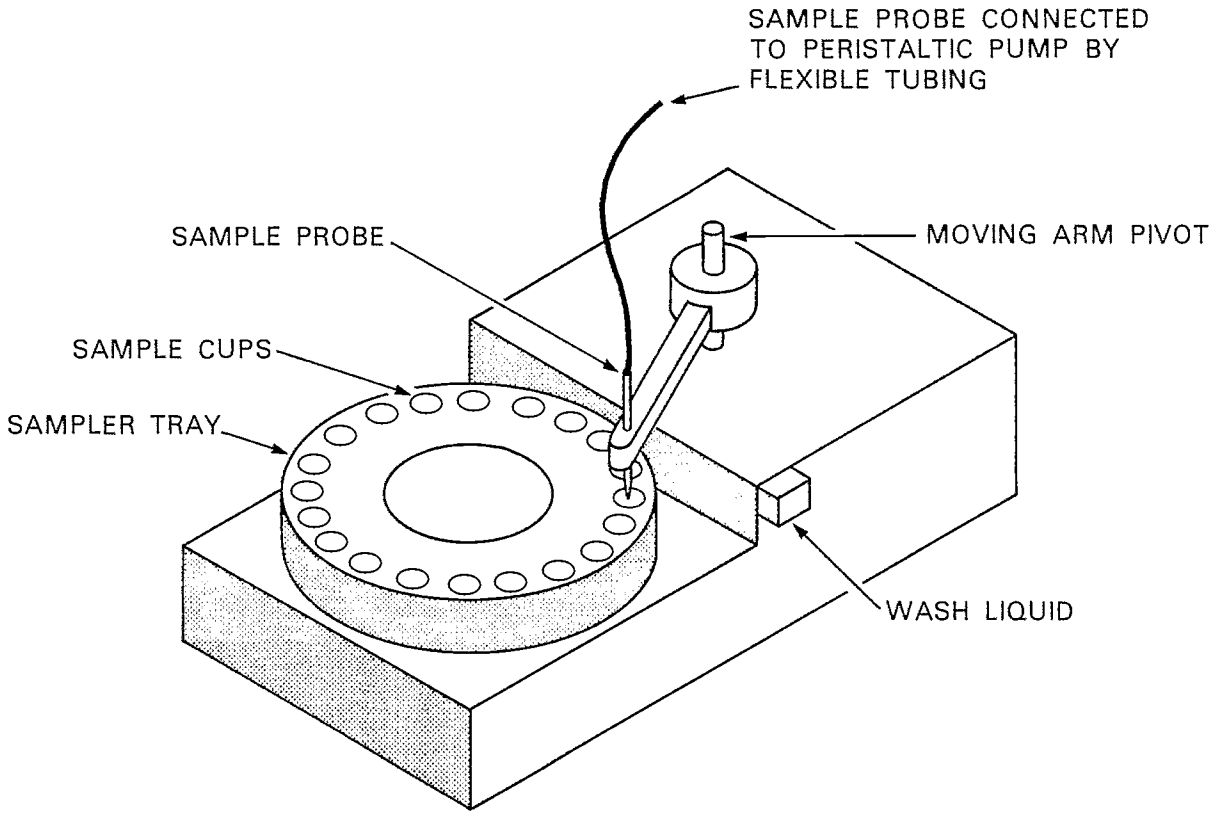


FIG 12 A SAMPLE CUP PRESENTATION UNIT

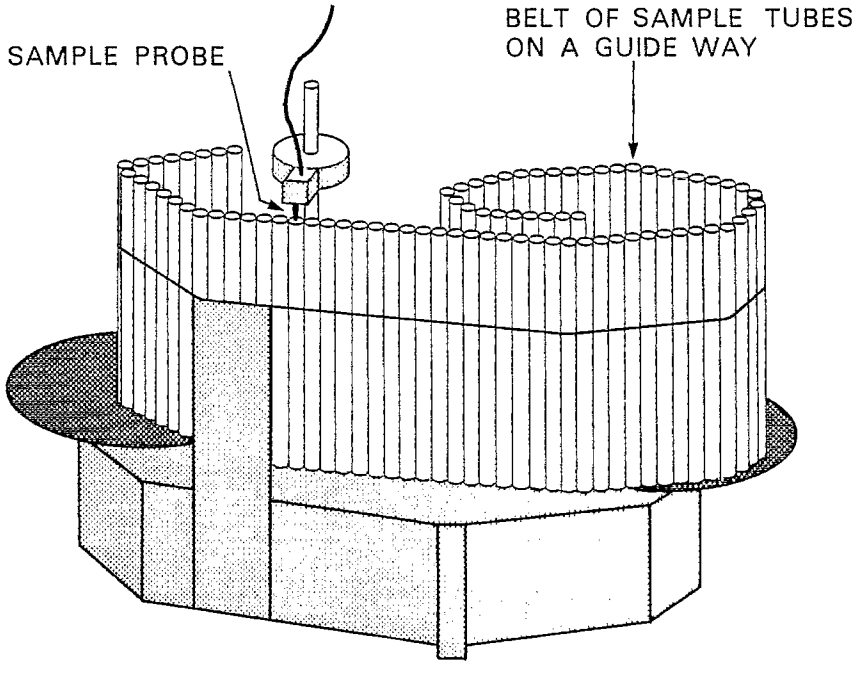


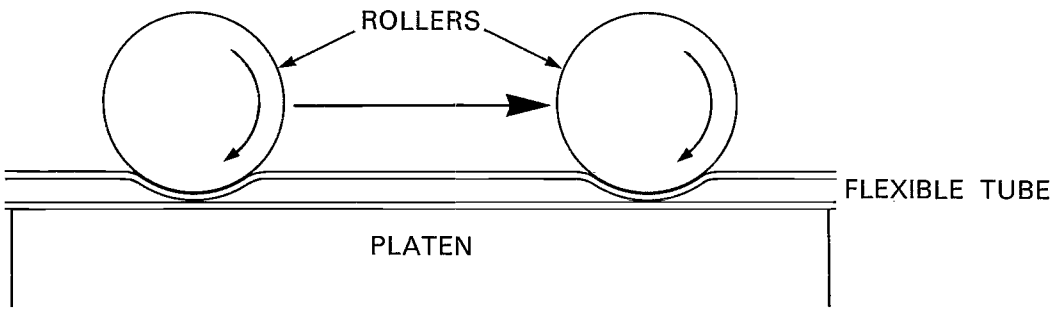
FIG 13 A SAMPLE TUBE PRESENTATION UNIT

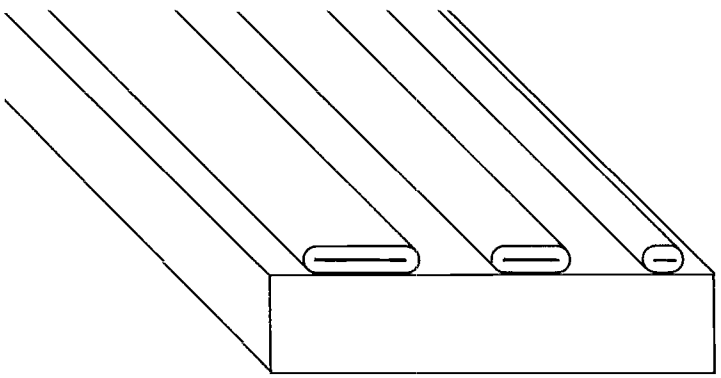
A receptacle is provided which contains water or other suitable 'blank' solution (the wash liquid) in which the sample probe rests between successive samples. This receptacle can be fed via the peristaltic pump to replenish constantly the wash liquid thereby minimizing interference from residues of previous samples and reducing the possibility of airborne contamination. The probe is lowered into each receptacle in turn for a fixed time and then withdrawn into the wash solution while the carrier is advanced to position the next sample under it. In most cases both the sampler plate and the probe are operated through appropriate cams and gears by a motor receiving impulses from a timer which controls the overall position. The sample is pumped into the sample treatment system by a peristaltic pump where it meets the reagent streams which are pumped through other plastic tubes. While the sampler plate is advancing to the next position the sample probe aspirates air during its travel to and from the sample and wash solution and therefore the action of the sampler must be rapid. If the solids content of a sample is to be included, a rotary mixer or vibrator can be added to the sampler, to maintain homogeneity of the sample prior to being pumped; although if this is done the possibility of blockages in the tubing of the system should be considered. The time for aspirating sample and wash liquid can be varied, as can the sample-to-wash ration, by operation of an adjustable cam or timer. The sample sizes used with sample cups are normally 0.5, 2.0, 3.5, 4.0, 5.0 and 8.5 ml and with sample tubes 6.0, 15.0, and 30.0 ml. When more than one determinand is to be measured from the same portion of sample, a sampler, that has more than one sample probe can be used or alternatively the sample can be divided using a 'stream splitter' placed in the sample line between the sampler and the peristaltic pump.

3.2 Multi-channel peristaltic pumps

This component is considered to be the vital part of a continuous flow automatic analyser. It serves three purposes: the introduction of both sample and reagents into the analyser, the transportation of solutions through the analytical system at a fixed speed, and the provision

PRINCIPLE OF PUMPING ACTION





PRINCIPLE OF PUMPING DIFFERENT RATES WITH THE SAME PUMP

FIG 14 PRINCIPLE OF THE PERISTALTIC PUMP

of air bubbles for liquid segmentation. The last is described in Section 3.3. The samples and reagents are introduced in appropriate flow ratios which can be pre-selected for each analysis by using tubing of the correct internal diameter for each solution. In one design the tubes, commonly known as pump tubes, are held taut and parallel beneath or above moving rollers. These rollers are motor-driven to bear successively on the pump tubes and push solutions in the pump tubes forward into the sample treatment system of the analyser, Fig 14 (top). The rollers are spaced at an equal distance apart so that the volume of liquid trapped beneath two rollers is constant for any one pump tube. In order that equal pressure is applied to all the pump tubes across the pump, the wall thickness of the pump tubes should be identical, Fig 14 (bottom). If the pumping speed is fixed, the rate at which each solution is pumped is determined solely by the internal diameter of the pump tube. Pump tubes are commercially available in a variety of qualities, sizes and materials so that they can be used to deliver liquids other than dilute aqueous solutions eg. strong acids and solvents. Almost all manufacturers colour code their pump tubes for identification of the internal diameter and flow rate. There are some solvents which are not successfully pumped by any of these pump tubes; pumping by displacement using liquid immiscible with the reactive solvent can overcome this problem, Fig 15. For instance, water has been used to displace chloroform and other organic solvents.

Because peristaltic pumps have the capacity to hold a number of pump tubes, in one case 28 and in another 60, it is possible to use a single peristaltic pump to perform several analyses.

FIG 15 DISPLACEMENT BOTTLE TECHNIQUE

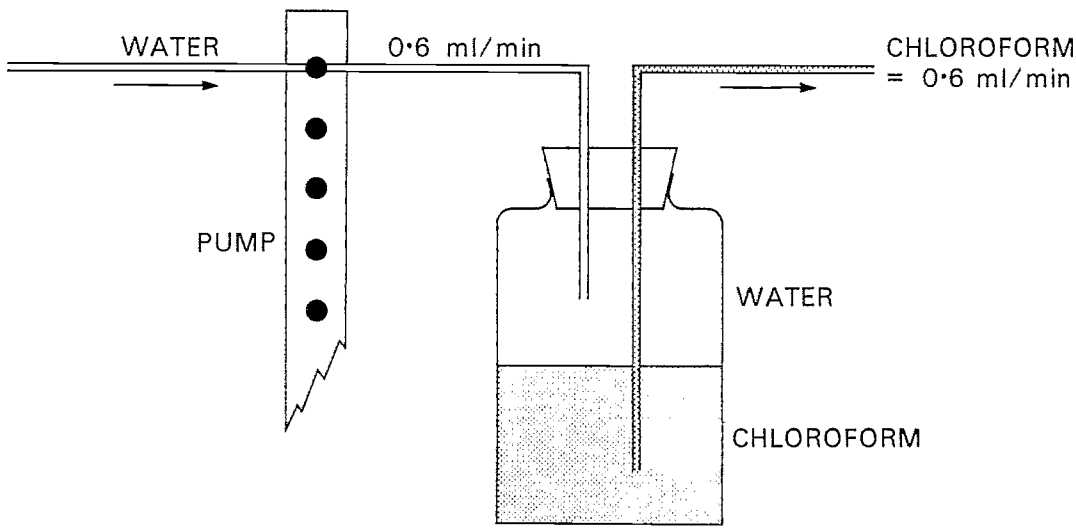
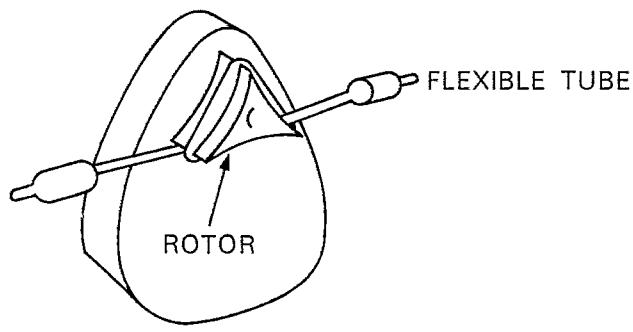


FIG 16 ALTERNATIVE DESIGN OF THE PERISTALTIC PUMP



An alternative design of pump is commercially available based on the principle that if a flexible tube is bent to a smaller radius than its bore and wall thickness relationship will tolerate, then it will kink and occlude the tube. A rotor is machined to form three tangential curves which produce three tiny radii, so that an elastic tube wrapped around the rotor will kink at three points. These kinks are moved by the action of the rotor and force the contents along the tube by positive displacement, Fig 16. With this type of pump the flow rate can be altered by changing either the motor speed or the internal diameter of the tube.

3.3 Segmentation of liquid by air

A continuous stream of liquid flowing through a tube exhibits a radial velocity profile, the flow being fastest at the centre and slowest at the tube wall where frictional retardation occurs. Material at the periphery mixes with that in the centre of the following liquid. Thus, if the continuous stream consists of a number of single samples in succession interspersed with wash solution, sample interaction could occur. The introduction of air bubbles into the continuous stream divides each aliquot of sample into segments (Fig 17) and restricts diffusion very nearly to within the segments of liquid contained between consecutive air bubbles. This assists both in maintaining the separation between samples and the mixing process to operate correctly, see Section 3.4.2.

The air bubbles are introduced into the stream of liquid at the earliest possible stage after the peristaltic pump. The air is drawn in from the atmosphere by the pump and it is advisable when analysing for low concentration levels of determinand to ensure that the air is made free of airborne contaminants eg. by passing the air through silica gel columns or through a dilute acid solution.

To achieve the maximum accuracy and precision, the air-liquid bubble-pattern must be regular and reproducible. To aid exact and reproducible proportioning, some pumps are now fitted with a device termed an air-bar which is essentially a controlled air-inlet valve by which air bubbles are added to the flowing streams in a precise and timed sequence to provide regularity of segmentation.

There will be occasions during the course of some sample treatment systems when the air bubbles are removed from the sample-reagent stream prior to a particular stage of treatment after which it is necessary to re-segment the stream with air bubbles as soon as possible in order to reduce sample interaction.

There are only three occasions when segmentation is not used:

(a) In tubes conveying regents to the sample treatment system.

(b) In narrow bore tubes through which liquid is passing at a relatively high velocity, as is the case with the tubing connecting the sample probe to the pump and also with the tubing transporting a de-bubbled stream to the detector.

(c) When a low sampling rate is employed.

The exact ratio of air to liquid in a segmented stream is not critical and it may well be changed as further reagents are added. usually the ratio of air to liquid is between 1 to 2 and 1 to 5.

3.3.1 Debubblers

Prior to its passage through the detection unit the liquid stream has to be de-segmented to remove any possible interference of the air bubbles with the measurement. If the detection unit's flow cell is not configurated to remove air-bubbles automatically, a glass de-bubbler unit as depicted in Fig 21 is satisfactory.

3.4 Sample treatment system

Normally at least one of the following chemical or physical treatments occurs during the analysis of a sample.

3.4.1 Dialysis

This is a separation technique which seeks to provide an interference-free determinand for analysis. In dialysis the sample stream, called the donor stream, flows over the recipient stream and is separated from it by a semi-permeable membrane, and the determinands are

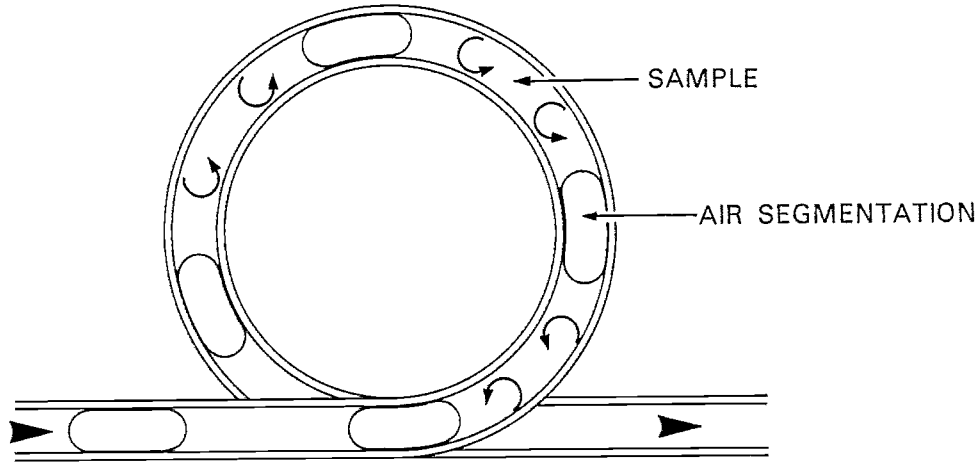


FIG 17a FUNCTION OF A TYPICAL MIXING COIL

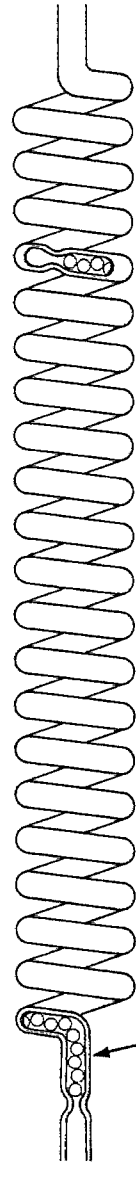


FIG 17b MIXING IN A BEADED COIL

transferred to the recipient stream by osmosis, leaving most interfering ions or molecules in the donor stream (Fig 18). Any membrane that is chemically compatible with the reagents can be used provided it allows the passage of the determinand and eliminates the interferants. The extent of dialysis is related to a number of factors; the thickness and pore size of the membrane, the size and nature of the molecule or ions, the contact time of the sample with the membrane and other factors such as the ambient temperature, pH of the donor and recipient streams, and the sample concentration. Samples and standards should have the same rate of dialysis and the ambient temperature should remain constant for the duration of the analysis. The dialysing contact time and area can be increased either by using more than one set of plates or by increasing the length of the dialysis path. It is not normally necessary to bring the process to completion, as during dialysis, although the percentage of a substance that dialyses is constant, the absolute amount is in proportion to the concentration. Donor and recipient streams must flow concurrently and as far as possible at the same rates in order to minimize sample interaction.

It should be noted that when dialysis is employed a reduction in sensitivity occurs, since only a percentage of the determinand diffuses through the membrane. However, this disadvantage may be turned into an advantage if a dilution step is required.

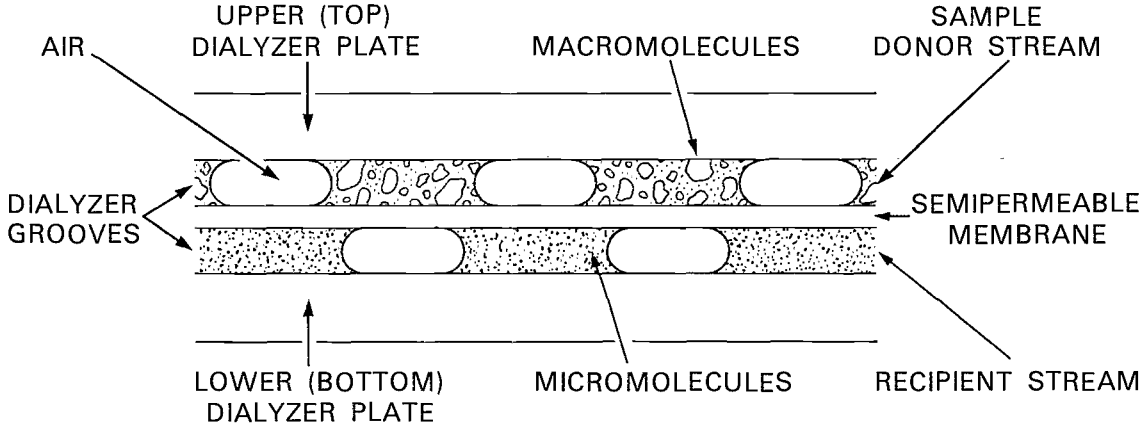


FIG 18 DIALYSIS UNIT

3.4.2 Reagent addition and mixing

The sample and reagent(s) merge in appropriate stages through T-connections (Fig 19). Normally after each addition of a reagent the two dissimilar merging streams are thoroughly mixed before the next reagent is added. This mixing is usually achieved by pumping both streams through a helix of glass tubing whose axis is mounted horizontally (Fig 17(a)) to give repetitive inversion of the two liquids. As a mixture rotates through a coil the air bubbles plus the rise and fall motion produces a homogenous mixture. The degree of

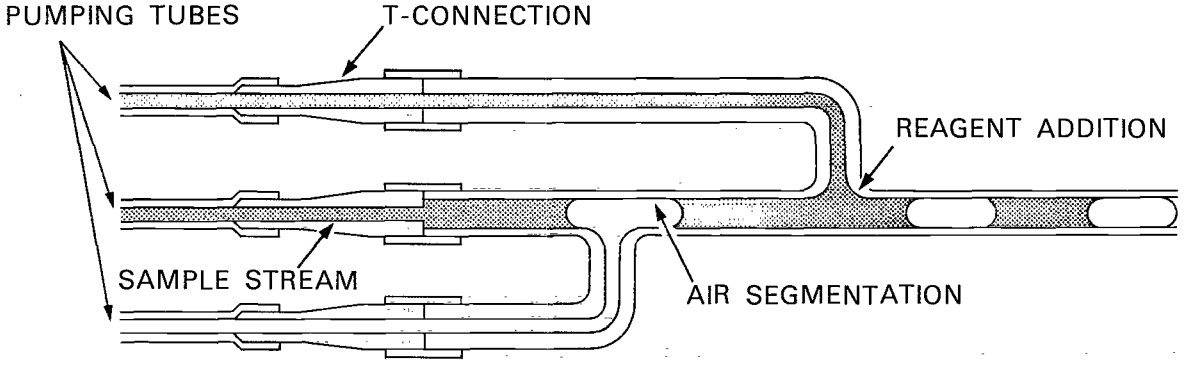


FIG 19 T-CONNECTIONS REAGENT ADDITION AND MIXING

homogeneity achieved is dependent on the number and size of loops contained in the coil. For this mixing to be effective the length of liquid contained between consecutive air bubbles should not be greater than about one quarter of the outer circumference of the mixing coil. These coils, the T-connections and the tubing used to transport solutions through the sample treatment system should be of glass to ensure the minimum amount of resistance to flow through them—to minimize adsorption onto the inner walls and to prevent breakup of the bubble pattern of segmented streams. An alternative design sometimes used for mixing and extraction with immiscible liquids is a bead packed vertical column (Fig 17(b)). The use of either of these types of coil introduces a small delay into the analytical process; there are times when long delays are required and for this purpose the use of special delay coils is necessary as described in Section 3.4.3.

3.4.3 Delay Coils

Delay coils are used to allow time for the reaction products to develop sufficiently for measurement. These coils consist of suitable lengths of glass-tubing which, for convenience, are coiled. Their lengths, which are dependent on the time delay required by the method, are also related to the liquid flow rate. They are usually mounted with their axis horizontally similar to ordinary mixing coils (Fig 17(a)).

A standard delay coil is a 40 ft length of tubing whose internal diameter is 1.6 mm and which has a 28 ml volume but the following coils having the same id are commercially available; length 20 ft, volume 14 ml, and length 10 ft, volume 7 ml.

The time delay is dependent upon the flow rate and may be calculated using the following formula:

$$\frac{\text{Volume of coil}}{\text{Flow rate of entering stream plus bubbles}} = \text{Time delay}$$

Two or more coils may be connected in series for a longer reaction time.

Water-jacketed coils are needed when a specific temperature must be maintained or cooling is required before the resulting product enters the measurement system.

3.4.4 Heating

If at any stage ot the sample treatment system a solution has to be heated eg. for a colour to develop, a glass delaying-coil is immersed in a temperature controlled bath. Oil is the liquid most commonly contained in the bath, and the grade of oil used depends on the temperature required. The emerging stream sometimes has to be cooled before it is transported to the next stage.

3.4.5 Digestion

Digestion with suitable reagents to break down the sample material and to produce the determinand in vapour or solution form can be carried out by passing the sample and/or reagents through a coil contained in a high temperature heating bath. However, there are commercially available digesters which have proved extremely efficient compared with the simple heated coil and are considered to be less hazardous, although the danger inherent in handling concentrated acids must not be overlooked. These commercial digesters have also been used for distillation and solvent evaporation.

3.4.6 Distillation

Distillation can be carried out using apparatus similar to that shown in Fig 20. The coil in which the sample is to be heated is kept in the bath which is maintained at the required temperature. The sample and reagents are pumped into the distillation coil where the liquid stream meets the carrier gas and flows in a thin continuous film down the lower surface of the coil with the air flowing over it. The vaporised components are transferred to the gas stream. At the bottom of the coil the residual unevaporated liquid is passed to waste through the vertical tube. The trapped volatile components emerge through the capillary where wash-water is introduced. The stream of gases and water passes through the water condenser and the required amount of condensate is resampled by the sample presentation system for analysis. Such a device has been used when analysing for determinands such as cyanide and alcohols.

3.5.7 Solvent extraction and phase separation

When solvent extraction is required, the two immiscible liquids (the sample and the solvent) are 'mixed' as described previously in Section 3.4.2 or in a beaded coil (Fig 17(b)). The resultant stream enters a separator (Fig 22) via a 'side-arm' positioned in its middle. The two liquids separate relative to their density characteristics and the required liquid is pumped to the next treatment stage, the other liquid going to waste (Figs 22(a), 22(b)). This separator can normally be of comparable size to the other components used in this system.

Normally it is found that the emulsification problem sometimes associated with manual solvent extractions is avoided in an automatic method, but the extraction efficiency is lower than that attained by shaking and this tends to inhibit the use of this technique when working at very low levels.

3.5 Measurement system

The treated sample is transported to this system for detection and measurement. Numerous detection systems are available since a continuous flow analytical system is very flexible and virtually any device capable of producing an electrical response to the presence of the determinand could be used. There is wide scope for ingenuity and this is one of the advantages of continuous flow analysis. A brief description of some of these detection systems is given below.

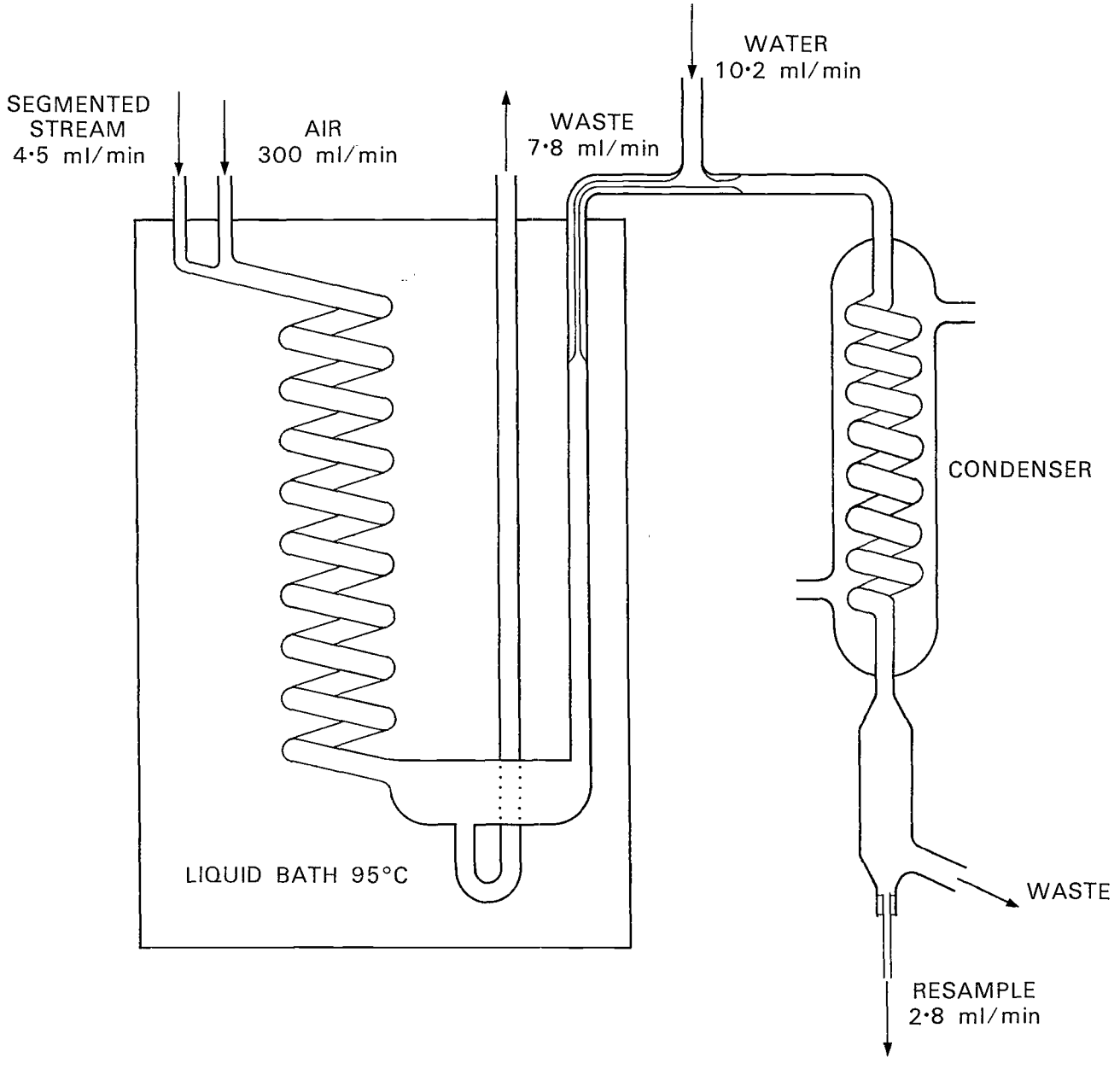


FIG 20 DISTILLATION UNIT

FIG 21 TYPICAL DETECTOR UNIT FOR COLORIMETRY

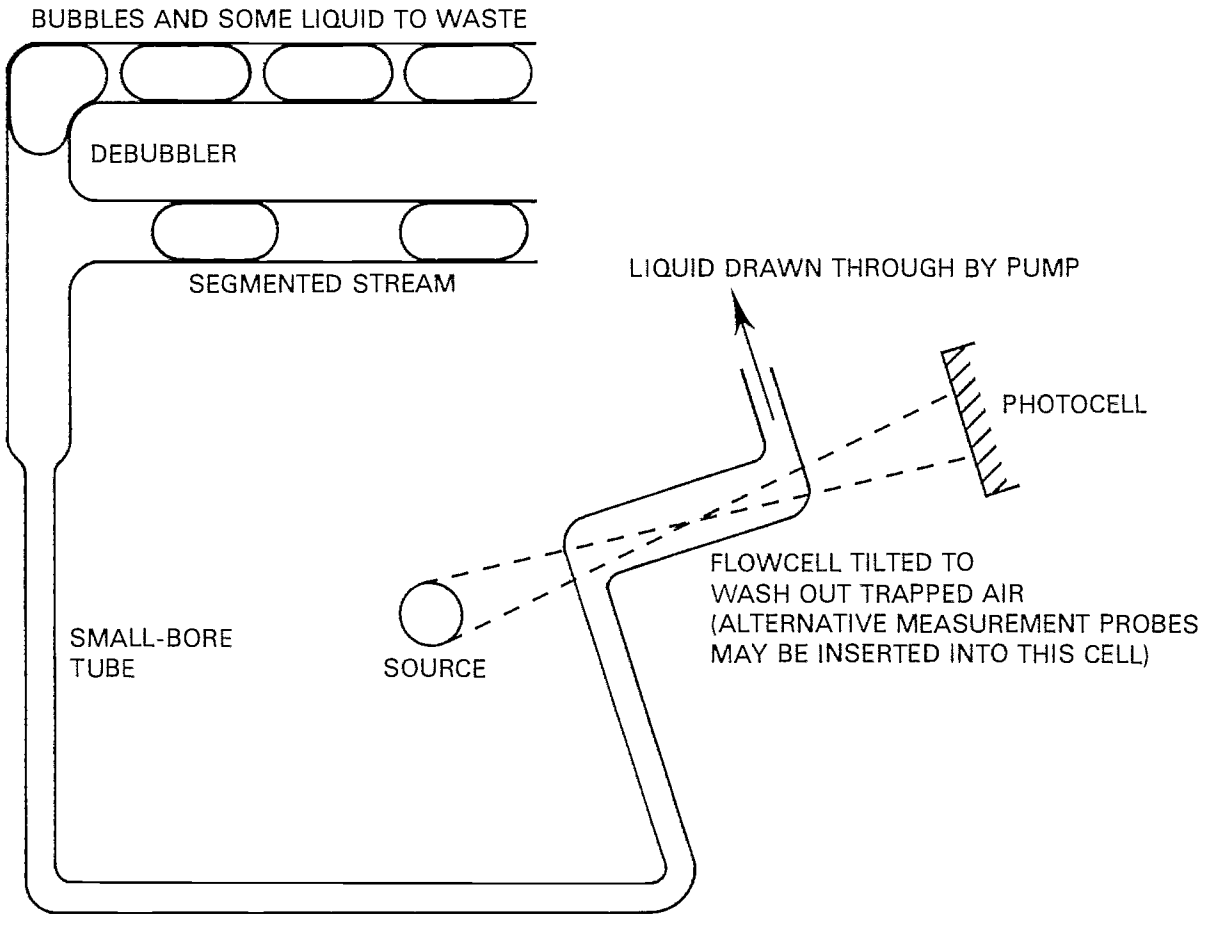


FIG 22 PHASE SEPARATION

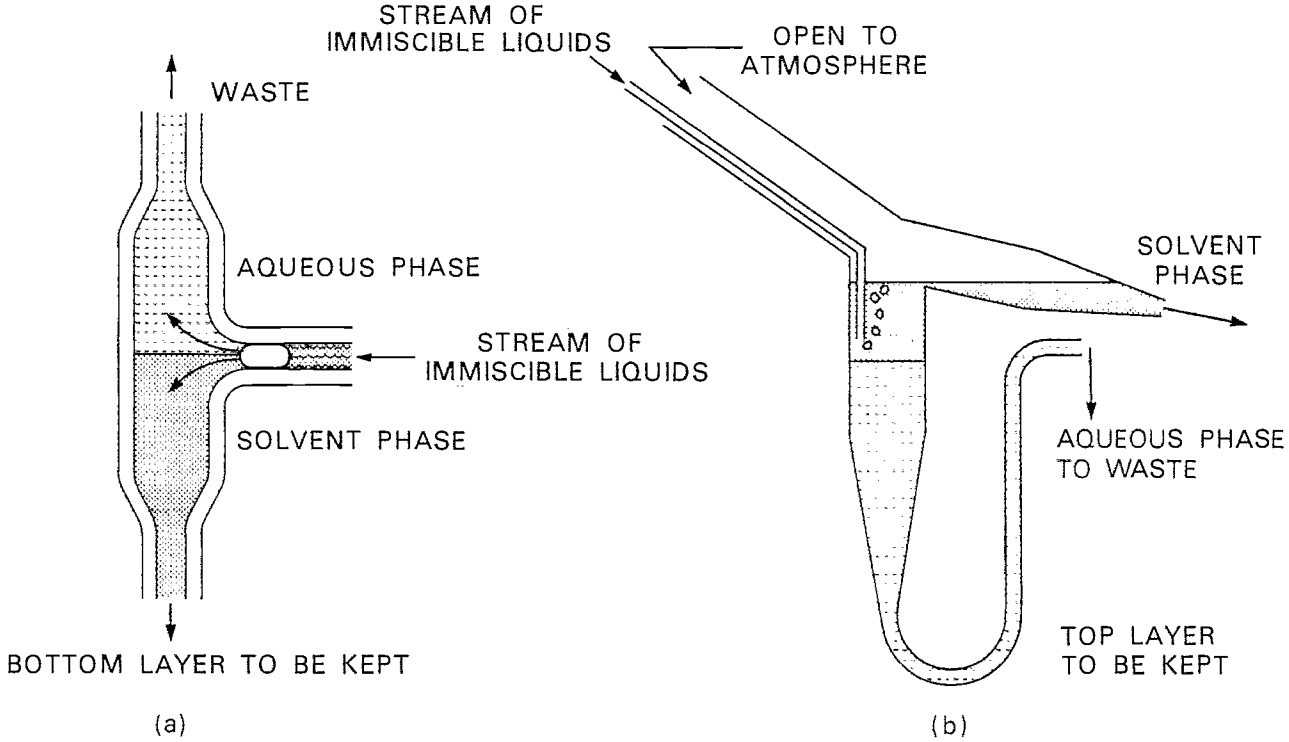


Figure 21 shows a typical debubbler for a colorimetric measurement. Other measuring devices which require a bubble free sample may be inserted into the detector cell. The detector cell volume should be small enough to contain less than the solution resulting from one sample at any one time, regardless of detector type used.

3.5.1 Colorimetric methods

Colorimetry is one of the most extensively used analytical techniques for both organic and inorganic materials and the automation of colorimetry has been intensively studied, perhaps more so than any other technique. In consequence there is a wide range of commercial instruments available for conducting continuous automated colorimetry. A typical cell assembly is shown in Figure 21. In order to avoid sample overlap and facilitate correct signal recognition the cell volume should be less than the amount of any single sample reaching the unit.

3.5.2 Electrochemical methods

The field of electrochemical analysis embraces a wide range of techniques including potentiometry, polarography, amperometry, conductimetry, coulometry, chrono-potentiometry and ion-selective electrodes, and by suitable choice of technique and experimental conditions a high degree of analytical sensitivity and specificity can be achieved. Electrochemical techniques have found considerable favour in the design of continuous and automatic methods, especially where trace components of a sample are to be determined and where their selectivity can reduce or eliminate the need for pretreatment stages, thereby simplifying the design of the automatic equipment.

3.5.3 Spectroscopic methods

Flame emission and atomic absorbtion techniques play a major role in elemental analysis, particularly at the trace level. Simplicity, speed, sensitivity and ability to perform several determinations on a single sample are the principal features which have provided the impetus for their extensive development. Such methods are obvious candidates for mechanization and automation because the step involving the flame is invariably short compared with sample-preparation and data-treatment stages.

3.5.4 Ionization methods

An analytical technique based on flame ionization detection of a gas, such as methane (used in the determination of carbon), is finding increasing use. The high sensitivity and linearity of the technique makes it suitable for low level analysis.

4. Interpretation of Results

Providing the determined concentration and system response exhibit a rectilinear relationship over the entire concentration range of the method, the most convenient approach to the measurement of the determinand concentration in a sample by a continuous flow analytical system is the direct comparison of the system response for the sample with that for a standard solution which has been subjected to identical experimental conditions. This standard solution will contain a known amount of the determinand and also identical concentrations of the other component substances contained in the sample.

In reality, however, the above conditions are seldom achieved in full. Thus, a realistic approach is to analyse a series of standard solutions which cover the whole concentration range of the method and produce a calibration curve of system response versus determinand concentration. This calibration will ascertain whether the system response to concentration is linear, non-linear or exhibits deviations from linearity. The system responses for the sample can be converted into concentration units of determinand by its reference to this calibration curve.

The other constituent substances of the sample need only be considered if they interfere with the measurement of the determinand; in which case they must either be eliminated or compensated for in the standard solutions.

Any calibration procedure should be effected at the start and finish of the sample analyses, but a further check may be desirable at regular intervals if the analytical run is considered to be lengthy. In this case, the samples will normally be analysed in groups and a calibration check standard followed by a blank solution will be inserted at the end of each group.

A suggested procedure for measurement using this arrangement is as follows: the baseline from which all the group response measurements are made is constructed by joining the responses of the last blank solution before, and after, each group of analyses whether samples or standards. The blank-corrected response of each calibration check standard is compared with the corrected initial calibration and if it is found that their concentrations are acceptably close to the initial values the initial calibration may be used over the entire analytical run of samples.

Occasions arise, however, where for various reasons (baseline drift and sensitivity change being prime examples) it may be necessary to accept output from a system which is not functioning normally. In these cases calibration procedures are less straightforward. The calibration may alter during the analytical run and this may be detected by responses of the calibration check standards differing significantly from their initial responses.

The response of the system's detector is usually displayed in its simplest form on a strip chart recorder as a peak or plateau. The conversion of this response due to a sample, into a concentration of determinand in the sample may be achieved in one of the four ways detailed below which are given in order of simplicity. However, each approach assumes there is *a rectilinear relationship between detector response and concentration over the concentration range of the method.*

1. No changes in baseline or calibration during the entire analysis of a batch of samples

(i) *Single-point calibration* Directly compare the sample response with that of a standard solution which contains a known amount of the determinand. Obtain a conversion factor from the standard solution response and multiply the sample response by the factor to give the concentration of the determinand in the sample,

eg. A 40 mg/litre standard solution = 80 chart divisions
Factor, F = 0.5
Sample response = 62 chart divisions
mg of determinand/litre of sample = 31

(ii) *Multi-point calibration* Calibration curve (line): obtain a calibration curve by plotting the detector responses against the determinand concentration of a series of standard solutions and producing the best straight line. The detector responses for the samples may be converted into concentration units of determinand by reference to the calibration curve.

Whichever approach is chosen, usually (ii), the calibration should be checked at the end of the analysis. However, it is often desirable to check the calibration at regular intervals throughout the entire batch of analysis, (a) for obvious reasons and (b) for reasons of changes in baseline and calibration. Approaches for the latter reason are detailed next.

2. Changes in baseline and calibration during the entire analysis of a batch of samples

(i) *Baseline drift but stable calibration* Proportional blank correction is applied across the number of samples within a group.

$$R = \left[P_s - B_1 + \frac{(B_2 - B_1) n_s}{n_t} \right] \times F$$

where R = result of sample in concentration units
P_s = peak (or plateau) of sample (ie. detector response)
B_1 = detector response of blank (baseline) before group of samples
B_2 = detector response of blank (baseline) after group of samples
n_s = position number of sample in the group
n_t = total number of samples in the group + 1
F = calibration factor (if linear and stable)

(ii) *Calibration drift but stable baseline*

(a) *For minimal drift (Analyst's decision)*

A good approximation to obtain a calibration factor for the samples in a group is to use,

$F = (F_1 + F_2)/2$

where F_1 = calibration factor from check standard before the group

F_2 = calibration factor from check standard after the group

(b) *For any size drift*

Use, $F = F_1 + [(F_s - F_1)\, n_s/n_t]$

where F_1 and F_2 are as in 2(ii)(a)

n_s and n_t are as in 2(i)

In both cases, the result $R = P_s \times F$ using previous notation.

Should both baseline drift and calibration (sensitivity) change substantially.

$$R = \left[P_s - B_1 + \frac{[B_2 - B_1]\, n_s}{n_t} \right] \times \left[F_1 + \frac{[F_2 - F_1]\, n_s}{n_t} \right]$$

Non-rectilinear relationships between system response and concentration, eg. % transmission values (% T) of light used as a measurement of the response, often tend to result in less accurate correction for changes in the calibration curve. In this case, the % T-values plotted on the logarithmic axis of semi-logarithmic graph paper may produce a linear calibration rendering the interpretation of calibration and sample results that much easier using the suggested procedure for rectilinear relationship situations.

The principles which govern the calibration of a continuous flow analysis system and their application to some commercially available systems, as well as a more detailed study of some of the points outlined in the subsequent Section 5, are set out in Reference 4.

5. Analysis of Samples

Ideally a sampling rate and sample/wash ratio will be chosen which provide the best possible precision and accuracy. In this case, sample interaction, defined as carry-over from one sample to the next, will be negligible or at least reduced to an acceptable minimum. However, there will usually need to be a compromise between: (i) sample interaction, (ii) the number and stability of samples to be analysed, and (iii) the accuracy required. Thus, some degree of carry-over will usually arise.

5.1 Sample-to-Wash Ratio and Sampling Rate

To determine the minimum time for which each sample must be pumped into the system, the time taken for the response on the measurement system to rise from its baseline value to one of steady state value for the maximum concentration standard is measured. A further 5 to 10 seconds is added to this time so that a definite plateau is given. The sum of these two times is the sampling time. The wash time is the time taken for the response to fall back either to the baseline or to some acceptably low response. If a sampling device which employs a timing cam is used, the choice of sample-to-wash ratio can be derived from the following:

(a) The sampling rate indicates the number of samples processed in one hour. Hence, a sampling rate of 60 means that 60 samples are processed per hour.

(b) The sample-to-wash time is expressed as a ratio in terms of units. Hence, a sample-to-wash ratio of 9:1 indicates that there are 9 sample units for 1 wash unit (the wash unit being unity).

(c) The relationship between sampling rate and the sampling cycle time is illustrated by the following examples:

Sampling rate (per hour)	Cycle time per sample (in seconds)
50	72
40	90
30	120

Thus if the sampling rate and the wash time (in seconds) are known (eg. 60 samples per hour with 6 seconds of wash time), the sample-to-wash ratio can be determined using the following formula:

$$\frac{\text{Cycle Time (sec)}}{\text{Wash Time (sec)} \times \text{Wash Units}} = \text{Sample units} + \text{wash units.}$$

For the example above:

$$\frac{60}{6 \times 1} = 10$$

Thus, sample to Wash ratio = (Total Units − Wash Units): Wash Units = (10−1): 1 = 9;1.

5.2 Sample Interaction**

Sample interaction, also known as cross-contamination or sample carry-over, is the term used to denote the situation whereby the response to any given sample is influenced by the tail of the response of the preceding one (Fig 23). It is observed on a recorded trace as incomplete separation of the two successive peaks (or plateaux) and it is related to the fact that a continuous stream of liquid flowing through a tube exhibits a velocity profile, the flow being fastest at the centre and slowest at the tube wall where the frictional retardation occurs, thus causing material at the periphery to mix with that in the centre of the following liquid (see Section 6). Provided the wash time is made long enough or blanks are inserted between samples, the carry-over is negligible, but if the sampling rate is increased and/or the wash time is reduced the carry-over effect may be severe, particularly when a concentrated sample precedes a diluted one. The degree of accuracy required for the particular analysis will determine the maximum acceptable degree of sample interaction and will, therefore, dicatate the choice of sampling rate and wash time to be used.

5.3 Sample Identification

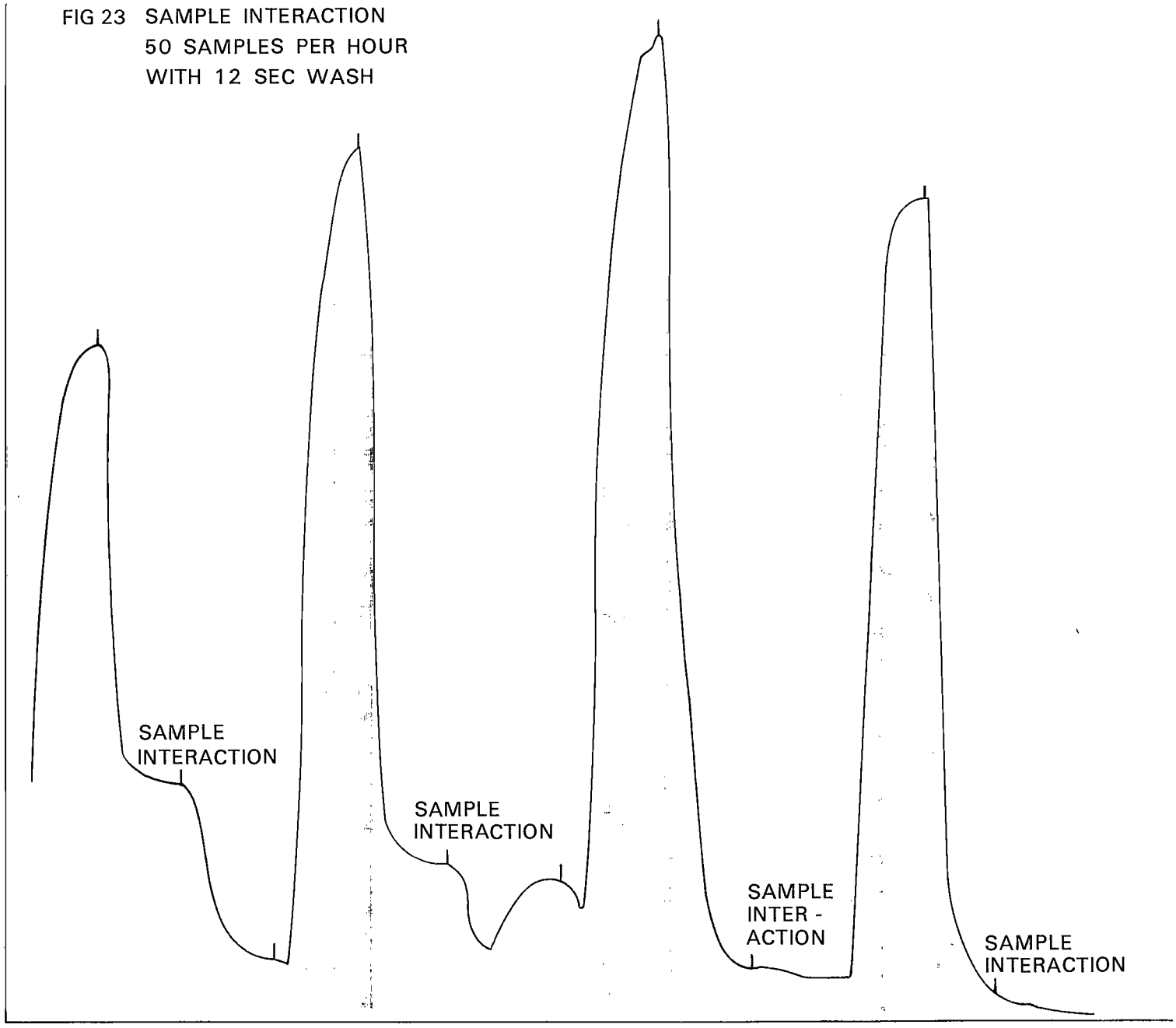
When large numbers of samples are to be processed it is essential to minimize the risk of confusion between samples. A record of the sampling order must be kept to ensure ease of identification of the responses given by the measurement system. A manually-produced record may be time consuming but it has to be regarded as a necessity. However, positive sample-identification mechanisms are available for certain sampling devices. Here, to each sample-tube is affixed a coded identification card; immediately following aspiration of the sample, the electronic reader identifies the code from the card and stores the information until the analytical result is ready for printing. The sample number is printed on the chart record for each sample and can also be included in a digital print-out. In addition, when computer facilities are available sample identification can be even more positive and permanent. A general map of the sampling positions can be programmed into the computer at the beginning of the analytical run.

5.4 Order of Analysis of Samples

When carry-over from one sample to the next is negligible, the order of their analysis is generally unimportant. The stability of the samples should then govern the order. But if carry-over cannot be made negligible, errors from this source should be controlled by analysing samples in an order that minimizes determinand concentration differences between

** For a theoretical treatment of sample interaction see section 6.3

FIG 23 SAMPLE INTERACTION
50 SAMPLES PER HOUR
WITH 12 SEC WASH



successive samples and standards. It should be noted that if all samples have approximately the same determinand concentration, or can be grouped into blocks of samples of similar determinand concentration, the error arising from carry-over effects will be nearly constant and will be allowed for in the calibration. If the samples have widely differing concentrations, the wash time may need to be increased so as to reduce carry-over effects to an acceptable minimum for the worst case, when a very low value immediately follows a very high one.

The number of analyses between calibration checks should be governed by the stability of the calibration and the required accuracy of results. As a general guide, groups of 15 analyses between each calibration check are suitable, but the number may be varied in the light of local knowledge. These 15 analyses are usually made up of samples and quality-control tests (see Section 9).

It is recommended that when new methods of analysis are derived one of the loading patterns given in Section 5.4.1 is used.

5.4.1 Examples of Sampling Unit Positions for Samples and Standards

To illustrate the considerations made in Section 5.4 the following examples are offered of the order in which samples and standards might be processed for analysis with systems exhibiting rectilinear system response with concentration (4).

Carry-over Negligible				Carry-over Present			
Calibration stable		calibration unstable		Calibration stable		Calibration unstable	
Test No.	Solution	Test No.	Solution	Test No.	Solution	Test No.	Solution
1	Blank	1	Blank	1	Blank	1	Blank
2	Calibration Std 0.2 Cm*	2	Calibration Std 0.2 Cm*	2	Blank	2	Blank
3	Calibration Std 0.4 Cm	3	Calibration Std 0.4 Cm	3	Calibration Std 0.4 Cm*	3	Calibration Std 0.4 Cm*
4	Calibration Std 0.6 Cm	4	Calibration Std 0.6 Cm	4	Calibration Std 0.2 Cm	4	Calibration Std 0.2 Cm
5	Calibration Std 0.8 Cm	5	Calibration Std 0.8 Cm	5	Calibration Std 0.8 Cm	5	Calibration Std 0.8 Cm
6	Calibration Std 1.0 Cm	6	Calibration Std 1.0 Cm	6	Calibration Std 0.6 Cm	6	Calibration Std 0.6 Cm
7	Blank	7	Blank	7	Calibration Std 1.0 Cm	7	Calibration Std 1.0 Cm
8-20	Samples 1-13	8-20	Samples 1-13	8	Blank	8	Blank
21	Sample 1**	21	Samples 1**	9	Blank	9	Blank
22	Control Std+	22	Control Std+	10-22	Samples 1-13	10-22	Samples 1-13
23	Blank	23	Calibration Std++	23	Sample 1**	23	Sample 1**
24-37	Samples 14-27	24	Blank	24	Control Std+	24	Control Std
38	Sample 14**	25-37	Samples 14-26	25	Blank	25	Calibration Std++
39	Control Std+	38	Sample 14**	26	Blank	26	Blank
40	Blank	39	Control Std+	27-39	Samples 14-26	27	Blank
41	Repeat cycle 8-23	40	Calibration Std++	40	Sample 14**	28-40	Samples 14-26
		41	Repeat cycle 7-23	41	Control Std+	41	Sample 14**
				42	Repeat cycle 8-24	42	Control Std+
						43	Calibration Std++
						44	Repeat cycle 8-25

* Where Cm is the greatest concentration that the calibration is intended to cover

** A check of sample reproducibility

\+ A standard synthetic solution of known concentration corresponding to about 0.8 Cm (see Section 9)

++ Selected from the range of calibration standards in the light of local knowledge of the determinand concentration of samples, or a 1.0 Cm standard

The above orders of analysis are affected after the preliminary stabilization and initial setting of the systems response to a baseline and for sensitivity which is discussed in the ensuing section.

5.4.2 Preliminary stabilization and initial response settings

The solvent, eg. water (distilled or deionized), that originates from the same source as that used to prepare the calibration standards, is continuously analysed until the system response is adequately stable. The time required to obtain this stability can vary for different

determinands. The initial response settings can then be affected by setting the 0.0 Cm standard (blank) to zero response and the 1.0 Cm standard to full scale response if changes in response to these standards over the duration of the entire set of sample analyses is negligible. If, however, changes in response are expected it is better to set the blank slightly abover zero and the 1.0 Cm standard to slightly below full-scale.

5.5 Shutdown Procedure

To keep the equipment in good working condition, the proper shutdown procedure, including any necessary flushing out, must be observed. As a general guide, water is pumped through the tubing of the system via the reagent lines for a time which is equivalent to that required to stabilize the system (see 5.4.2 above) further details of the shutdown procedure will be given in the specific method.

6. Kinetic Aspects of a Continuously Flowing Analytical System (1,9)

The performance of an analyser which processes discrete samples at intervals is related to the dynamics of the flowing stream and an understanding of the dominant factors is important in optimizing the design of continuous methods. A continuous stream of liquid flowing through a tube exhibits a velocity profile, the flow being fastest at the centre and slowest at the tube surface where frictional retardation occurs. Material at the periphery mixes with that in the centre of the following liquid and is the cause of sample carry-over. Segmentation of the stream by air-bubbles reduces carry-over by providing a barrier to mixing but it does not entirely prevent it, because mixing in the surface layer can still occur. Nevertheless, carry-over occurs mainly in unsegmented streams and in terms of air-segmented continuous flow systems this implies the initial sample-line before air-segmentation and after debubbling before entering the detector. The need for quantitative correlation of the magnitude of carry-over as a function of the kinetic parameters of the analyser has prompted definitive studies by several groups (5, 6, 7, 8).

Ideally, a conventional continuous flow system should fulfil the following requirements.

6.1 Rapid response

Response time is the sum of the dead time and the transition time. Dead time is the time between sample introduction and initial detector response. Transition time is the time required for the detector response to transit from its initial value to a steady state value or to a predetermined percent of steady state value. It is desirable to minimize both, but the reasons for doing so and the methods used differ. A long dead time is not in itself of great importance since it does not affect the sampling rate. However, it does imply a complex system and therefore a larger degree of sample interaction and an associated increase in the transition time, which does affect the sampling rate. Long lengths of transmission tubing between component parts should be avoided as far as possible. Improvements in the chemistry of the method either allow a shorter processing time for a particular step or even permit the omission of one or more of the treatment steps.

Transition time itself depends upon two factors—speed of detector response and degree of sample interaction. Speed of detector response depends on the rate of change of solution within the cell where volume for the flow cell and flow rate are the important factors to be considered. The demands of continuous flow analysis had led to the evolution of flow through cells having very small hold-up volumes. However, when micro cells are used the presence of bubbles or small particles of solid matter in the flowing stream is far more of a hazard.

On the other hand, sample interaction depends upon the design of the system from one end of the processing stream to the other. The size and geometry of the processing units, the detector and the connecting lines all affect the interfacial mixing, as does the presence of stagnant regions (joints) or traps in which the sample might be held by absorption, adsorption or chemical reaction (see Section 7.2). Non-wetting capillary tubing should be used for unsegmented portions of the flowing sample stream (but not reagent lines).

6.2 High frequency of sample throughput

Thiers and coworkers (6), were the first to make general observations on mutual sample interactions; their work which was later confirmed and extended by Walker and co-workers (8) remains the basis of quantitative consideration in determining the highest practicable sampling rate. Other workers (10, 11, 12, 13) have produced modifications to continuous

flow systems to increase the sampling rate (which is usually around 40 samples per hour). All these innovations, however, lead to a complex and inevitably more expensive instrumentation.

A newer approach to obtaining an increased sampling rate is flow injection analysis (49) (FIA). With this concept there is no air segmentation, the sample is introduced as a plug via a valve or syringe directly into the reagent-carrying stream, mixing is mainly by diffusion-controlled processes, and the response curves do not reach the steady state plateau, but have the form of sharp peaks. The absence of air-segmentation leads to a higher sample throughput. The presence of a sample-carrier interface, over which concentration gradients develop during the course of analysis, has opened up new analytical possibilities for continuous flow analysis. The reproducibility is good and there is no sample carry-over. There is no need to introduce and remove air bubbles, and an expensive high-quality pump is not necessary. A report by Betteridge (14) cites 48 references to FIA among which is the initial work of Ruzicka and Hansen (15).

6.3 Linearity of reponse

With changes in sample concentration is desirable since this facilitates the use of a direct readout form of presenting results. Non-linearity of results can have a chemical basis or it may be inherent in the detector and the determination of optimum working conditions which give a linear response can be a time consuming task.

In evaluating the performance characteristics of a continuous analyser, two parameters have been demonstrated to be fundamental, the lag phase (L) and the half-wash time ($W_{½}$)(6); they afford a correlation between the approach to steady state, fraction of steady state reached in a given time and the interaction between samples. The half-wash time is the time for the detector to change from any value to half that value; the lag phase is defined in the ensuing discussion.

For the shapes of the detector-response curves obtained in continuous flow analysis, it was shown (6, 8) that during the transient state (ie. baseline steady state to sample steady state and again from sample steady state to baseline steady state) the apparent concentration at any time (C_t) follows kinetics which are first order with respect to the difference between the apparent concentration and steady state (C_{ss}) concentration, as in the following equation.

$$\frac{dc}{dt} = K\,(C_{ss} - C_t)$$

in the case of the rise curve and likewise, for the decreasing part of the response curve

$$\frac{dc}{dt} = KC_t \text{ (here } C_{ss} = 0).$$

The value of $W_{½}$ is calculated directly from the slope of the linear portion of a plot is termed the lag phase L, and is expressed numerically as the value of the intercept of the linear portion on the time axis. The full curve structure is the inverse of that of the rise curve.

The half-wash time, which has been shown (8, 15) to originate in non-segmented parts of the stream, describes the exponential part of the transition curve. The lag phase, which is said to be normally due to air-segmentation (16, 17), precedes the exponential part.

Sample interaction (Fig 23) can be quantitatively expressed by using $W_{½}$ and L. If the between sample time is t_b, the value of the expression $(t_b - L)/W_{½}$ gives a measure of the interaction of a sample with the following one. For values of $(t_b - L)$ $W_{½}$ of 1, 2, 3, 4 ... the degree of interaction with the following sample is 50, 25, 12, 10 .... % and this interaction appears additively in the response for the following sample. Clearly the smaller the values L and $W_{½}$ the lower the degree of interaction; or conversely, for a given acceptable percentage interaction the lower the values of L and $W_{½}$, the faster the available sampling rate.

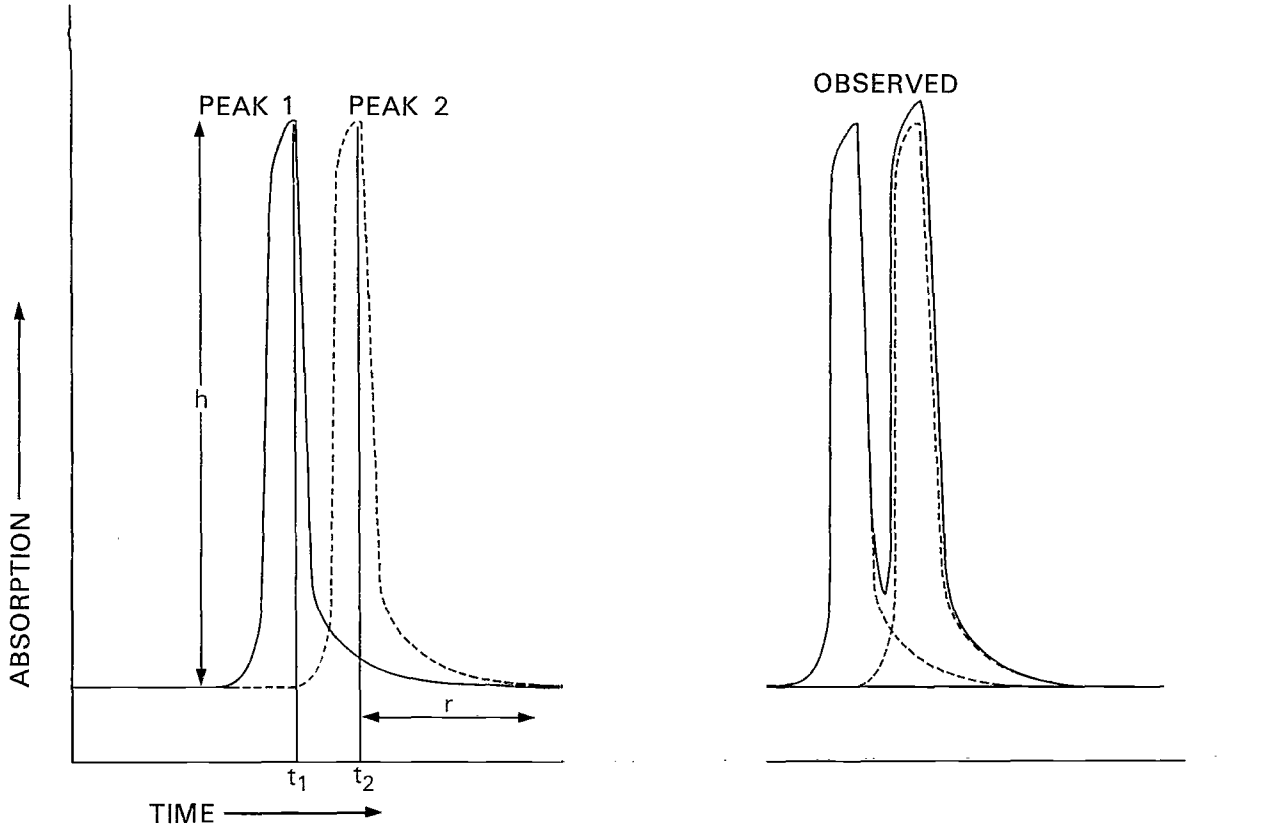


FIG 24 A TYPICAL ABSORPTION CURVE ILLUSTRATING THE EFFECT CARRY OVER WILL HAVE ON A SUBSEQUENT CURVE (SEE ALSO FIG 23)

The effect of carry-over can be measured as the following considerations indicate: consider the typical absorption curve in Fig 24. The problem of carry over is caused by the fact that at the time (t_2) when the height of peak 2 is read there is still residual absorbtion 'r' due to peak 1. For any particular manifold and pumping rate we have

$r = kh$

where 'k' is the carry over constant, h is the absorbtion at time t_1 and r is the residual absorbtion at time t_2.

The value of 'k' will depend on the nature and complexity of the manifold, the viscosity and chemical composition of the reagent and the pumping rate. For a particular situation the value of 'k' can be determined practically by simply running a standard solution which gives near full scale deflection and then running water until the baseline is restored. The constructions in Fig 25 enable 'h' and 'r' to be measured and hence 'k' to be calculated.

Normally the value of 'k' is in the range 0.005 to 0.02.

Once 'k' is known it is simple to correct any results obtained for carry over as follows. Let the peak heights for samples S_1, $S_2 \ldots S_n$ be h_1, $h_2 \ldots h_n$. Then the correct peak heights are:

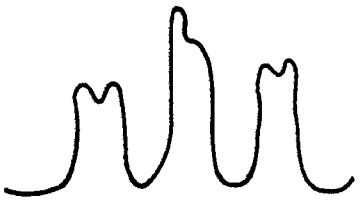
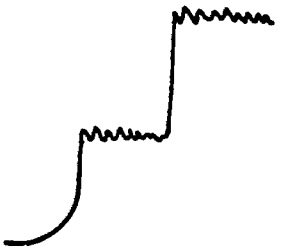
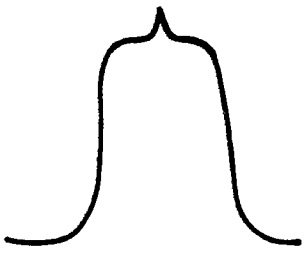
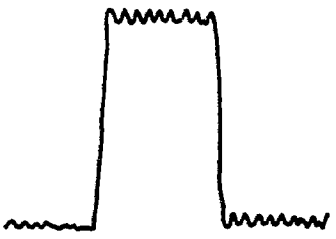
$S_1 = h_1$
$S_2 = (h_2 - kh_1)$
$S_n = (h_n - kh_{n-1})$

It should be noted that standard solutions should be corrected in exactly the same manner.

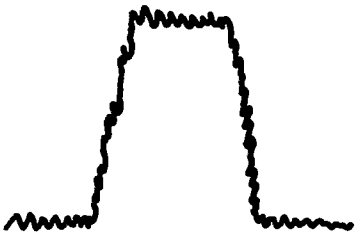
7. Fault-Finding Guide

In general, faults in continuous flow analysis systems can be observed as an irregularity or anomaly in the readout. See Pictorial Fault Finding Guide (Figure 25). Therefore a recorder chart trace should be retained even when computing facilities are also used, since a recorder trace is the only visible record of what has actually happened during the analytical run. However, some computer data handling systems may have a simulated detector response facility.

Figure 25 Pictorial fault finding guide

Recorder trace	Probable cause
1. "Dead space" spots 	Two pieces of glass tubing joined together by a "sleeve" over them are not properly aligned
2. Crinkled or split dialyser membrane 	Observed when initially calibrating the detector with a standard solution; caused by a dry membrane and ageing membrane
3. Dead spot on potentiometer slide wire of the recorder 	Dust particle or grease
4. Air bubble lodged across flow cell entrance 	Varying air-to-liquid ratio in conducting tubing

5. Oscillation on baseline and standard

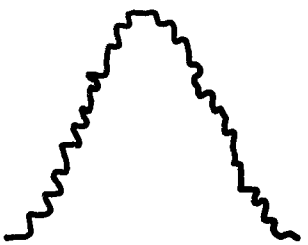


Poor quality reagents or air bar out of sequence

6. Spikes on baseline

1. Electrical interference
2. Pulling liquid through the flow cell a little too fast, creating a minute air bubble
3. A pump tube not pumping
4. A leaking joint at the debubbling stage
5. Small (hairline) crack in the glass of the flow cell

7. Stepwise peaks



Insufficient gain (amplification) on the recorder

8. Noise (oscillation)

Slow (S)

1. Intermittent localised over-heating in heating bath
2. The presence of particulate matter in the tubing causing surging and changes in flow rates
3. Back pressure causing blockage

Fast (F)

1. Worn pump tubes causing irregular pumping
2. Unstable or dirty reagents
3. A relatively large particle of solid in flow cell

Rapid (R)

Too much gain on the recorder, but may be a combination of all three (S,F and R)

System malfunctions normally fall into three categories:

1. Electrical, optical and mechanical.
2. Hydraulic.
3. Chemical.

7.1 Electrical Malfunction

If electrical components fail it is usually of an obvious nature, ie. colorimeter light-source, photomultiplier failure, or recorder malfunction. Typical malfunctions are due to:—

1. Fading phototube (photocell)
2. Dirty optics
3. Cracked lens
4. Old or dirty lamp
5. Misalignment of optics
6. Dirty interference filters.

Regular maintenance is advisable for all the electrical components as well as the mechanical components. A service contract with the appropriate manufacturer is the most positive means of achieving this.

7.2 Hydraulic Malfunction

Hydraulic malfunction is the principal source of failure in continuous flow systems and when ill-defined recorder peaks (plateaux) or erratic traces are observed, it is the hydraulics which should be checked first. If air segmentation is employed, the first step is to observe the bubble-pattern since it is mandatory to maintain a regular bubble-pattern throughout the sample treatment system of the process. The air bubble introduced during the actual sampling does not normally affect the regularity of bubble-pattern.

An irregular bubble-pattern may be caused by:—

(a) Partial or total pump failure; check all the inputs to the system and the pull-through output are functioning by introducing a small air-bubble into each and observing its passage. Check also for split tubes and leaking joints.

(b) Worn or damaged air-inlet tubing (where fitted); observe the pattern of air bubbles following the air inlet and if pulsating sharply or producing air-bubbles of irregular sizes replace the air-inlet tubing.

(c) Air inlet dirty; if replacing the air-inlet tubing [(b) above] is ineffective the inlet may be dirty or greasy. If so, disconnect the injector and disturb the dirt or grease with wire then clean the inlet in strong detergent solution, dilute acid solution or a suitable solvent.

(d) Other injectors (inlets); malfunctions at other injection points in the system do not necessarily produce irregular bubble patterns but they may well disturb the normal liquid/air ratio. These malfunctions are usually caused by blockage or partial blockage resulting from particular matter suspended in the flowing liquid or a slow build up of particulate matter. The sample inlet line is prone to partial blockage when used to sample sewage regularly. The net result could be an increase of one reagent or air against a decrease of another reagent (this could apply equally to the sample) giving rise to poor peak responses at the readout.

The cleaning procedure for these injectors or inlets described in (c) above should be used.

(e) Failure of the dialyser membrane (where fitted); providing the membrane used satisfies the requirements detailed in 3.4.1 it will operate satisfactory unless it is blocked (the pores are clogged), crinkled, ruptured or allowed to become dry for any length of time. The response at the read-out will be affected by all of these conditions but not necessarily in the same manner. Blocked membranes will probably give no response at the readout, crinkled membranes normally give the same response of sensitivity as a normal membrane but with reduced precision and ruptured membranes can either allow more 'diffusion' of the determinand thereby increasing the response at the readout or allow more interferants through resulting in reduced response as well as poor precision (and accuracy). These changes in response may not remain constant throughout an analytical run. Dry or badly aligned membranes tend to produce crinkled or ruptured membranes which should be replaced. In fact normal membranes themselves are usually replaced after 40 h of use.

(f) On occasions, air bubbles may become trapped at some point in the flowing analytical stream resulting in irregular flows which lead to poor system responses. The most common areas where this occurs are (i) in 'dead spaces' brought about by bad jointing of two pieces of tubing. They must be butted together as close as possible, and (ii) at the entrance of the measurement unit, eg. an air bubble may become lodged in a flow cell or may rest on the membrane component of a selective ion electrode. In both cases this may result in an oscillating system response or sharp deflection on the readout. However, if this air bubble interference is infrequent it can easily by removed, by pinching the pull-through tube for ca 2s; but the sample treatment system should be redesigned if the problem is consistently present. An air bubble lodged in the detector cell will cause a sudden marked loss of sensitivity.

7.3 Chemical Malfunction

Problems with the system chemistry arise as a rule from using unstable or poor quality reagents whether they are working reagents or the reagent stock, although changes in ambient temperature may also present problems. Reagents normally remain stable for a longer period of time when they are stored in a refrigerator between 0° and 5°C in between use, but problems with ambient temperature can only be controlled by minimizing the external effects that cause them. The two most obvious effects are baseline drift and sensitivity change, but loss of linearity may also result.

7.4 Sample Receptacle Contamination

Contaminated sample receptacles will produce problems for low-level analysis in the form of positive peaks at the readout for blank solutions. Most sample receptacles are disposable but if they are to be reused they should be cleaned with an appropriate cleaning agent, eg. detergent or acid, and then stored in a dilute solution of it, ensuring of course that the cleaning agent chosen will not interefere with the determination of the particular determinand, eg. do not use nitric acid if the determminand is nitrate and do not use detergent if the determinand is phosphate.

Sample receptacles for re-use which are not stored in a solution of the cleaning agent should be dried at 70°C after washing, to remove the problem of algal growth that might occur in them.

7.5 Maintenance Programme

The operator should produce a maintenance programme to be used routinely so that malfunctions that could occur during day-to-day operation are reduced to a minimum. This 'prevention is better than cure' policy requires less labour when the instrument is in continuous operation and the maintenance programme should include operations such as (1) lubrication of moving parts (2) checking electrical contacts (3) replacement of dialyser membranes, pump tubes and other tubing, and (4) cleaning of air injectors and other inlet connectors.

8. Factors Relating to the Applicability of Automatic Analysis (18)

(a) The Time involved in setting up an automatic analyser usually means that its cost can be justified only when the number of samples exceeds some minimum. The value of this minimum depends on individual circumstances but is about 20 samples per analytical run for a single determinand.

(b) Experience has shown that the replacement of manual analysis by automatic analysis generally leads to better precision of analytical results. The improved precision often gives lower detection limits than manual analysis using a similar procedure.

(c) If a laboratory reaches the position where the ma jority of its analyses are made automatically and the number of analysts employed has been chosen on this basis, problems are likely to arise if one or more of the instruments cease to function properly. It is essential, therefore, to ensure that replacement units and/or the services of an instrument technician are readily available.

(d) The inherent consistency with which automatic analysers carry out their operations makes it possible to automate procedures that would not be feasible for manual analysis, eg. methods requiring very precise control of experimental conditions.

(e) Many automatic analysers are in fact only semi-automatic because the results produced by the instruments must be mathematically processed by the analyst to obtain the concentrations in samples. This process can be unduly time-consuming in an

otherwise automated procedure. Increasingly, instruments have the facility for automatic calibration so that the results from them can be provided directly in concentration units. If correction for drift in the response of the instrument or more complex calculations are necessary, digital computers can be used on-line or off-line. Special purpose microprocessors are also available for acquiring and operating on signals from instruments and producing final print-outs of analytical results.

9. System Control

When an analytical procedure has been put into routine use there remains a need to maintain a continuous check on analytical errors. The use of a control chart is a simple and convenient means of maintaining this check. References 19, 45, 46 and 47 explain in detail the principle and use of control charts. One standard synthetic solution of known concentration corresponding to about 0.8 Cm (where Cm is the greatest concentration that the calibration is intended to cover), termed 'control standard', is used as a minimum of control.

The control standard, which is prepared from a different standard stock solution from that used to prepare the calibration standards, but stored under the same conditions, is analysed with every block of samples within each batch and the results are plotted on a control chart with the true concentration of the control standard as the mean value.

The control chart will have horizontal lines inserted corresponding to (a) the mean value, μ, expected for the result, and (b) the limits $\mu + 3\sigma$ where σ is the standard deviation, to provide objective statistical criteria for interpretation of the chart; these limits are called the 'action limits'. It is also useful to insert two lines on the control chart of limits $\mu + 2\sigma$ to act as 'warning limits'.

10. Hazards

In operating a system due account should be taken of the following possible hazards:

The peristaltic pump and, in many cases, other part of the apparatus are connected to the electric mains. Their construction should be such that even in the event of a major spillage, such as might arise from the knocking over of a reagent bottle or an undetected leak, an operator is in no danger of being connected electrically to the mains.

The peristalitic pump may well have exposed moving parts which might trap loose items of clothing.

Reagents should always be treated with proper care and attention should be paid to the possibility that the bursting of a tube or the breaking of a connection may lead to a reagent, perhaps hot, being sprayed out under presssure.

Comprehensive instructions cannot be given because much will depend upon the analysis being undertaken and the equipment used. However, the following advice will be useful in most cases.

(i) Electrical leads should be supported a little above the work-bench.

(ii) Individual components, or groups of components, should be placed in trays, so as to contain minor spillages.

(iii) Reagent bottles, both in use and spare, should be kept in a cupboard under the work-bench.

(iv) There should be a plastic safety shield between the operator and the apparatus.

(v) There should be adequate drainage for waste liquids to avoid the possibility of air locks and biological growths occurring in the exit tubes draining some components.

Analytical Quality Control

There are two reasons for analytical quality control when using techniques such as those described in this booklet. Firstly, there is the need to make random checks on the overall accuracy of the entire analytical procedure including sample preparation, and secondly there is the need to guard against either instrumental drift or sudden partial failure of the equipment which cause eroneous results. (See the Chapters on Problems which may occur and on Fault finding, for examples).

Once the method has been put into normal routine operation many factors may adversely affect the accuracy of the results obtained. It is recommended that tests be made to check for the various types of error that may occur. As a minimum, a control standard sample should be included in each batch of samples, provided a sufficiently stable sample can be obtained, which is taken through the entire procedure. The results should be plotted on a control chart. (See References 19, 45, 46, 47 and Section 9 of Part 2).

As a check on slow drift and sudden partial failure, it is recommended that AQC standards be included at regular intervals throughout the batch. If any significant variation is noted the cause should be investigated and check samples analysed to ascertain their true analyses.

If interference effects are suspected, include spiked samples along with the real samples and also synthetic standard samples containing varying amounts of the suspected interferent.

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Address for Correspondence

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The Standing Committee of Analysts
The Department of the Environment
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This booklet consists of two reviews with several items common to both. The review on Air Segmented Continuous Flow Analysis was approved in 1979 and is repeated here with minor revision. Members responsible are listed in the lists below. Note that due to reorganisation of responsibility for instrumental methods the working Groups referred to are different.

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