Air Segmented Continuous Flow Automatic Analysis in the Laboratory 1979

An Essay Review

Methods for the Examination of Waters and Associated Materials

London Her Majesty's Stationery Office £2 net

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K W Petts

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Contents

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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 a properly equipped laboratory. 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 others. Lone working, whether in the laboratory or field, should be discouraged. Reagents of adequate purity must be used, along with properly maintained apparatus and equipment of correct specification. 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.

There are numerous handbooks on first aid and laboratory safety. One such publication is *Code of Practice for Chemical Laboratories* issued by the Royal Institute of Chemistry, London. Another such publication, which includes biological hazards, is *Safety in Biological Laboratories* (editors E Hartree and V Booth), Biochemical Society Special Publication No 5, The Biochemical Society, London.

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. It cannot be too strongly emphasized 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 any 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.

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 the 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. If in doubt it is safer to assume that a hazard may exist and take reasonable precautions rather, than to assume that no hazard exists until proved otherwise.

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About this series

This booklet is one of a series intended to provide recommended methods for the determination of water quality. In the past, the Department of the Environment and its predecessors, in collaboration with various learned societies, has 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 individual methods, 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 being issued 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 analytical chemist, biologist, bacteriologist etc, to decide which of these methods to use for the determination in hand. Whilst 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 Analysts is one of the joint technical committees of the Department of the Environment and the National Water Council. It has nine 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 Instrumentation and on-line analysis
3.0 Empirical and physical methods
4.0 Metals and metalloids
5.0 General non-metallic substances
6.0 Organic impurities
7.0 Biological methods
8.0 Sludge and other solids analysis
9.0 Radiochemical methods

The actual methods etc 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 method are listed inside the back cover.

Publication of new or revised methods 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, and the current status of publication and revision will be given in the biennial reports of the Standing Committee of Analysts.

TA DICK
Chairman

LR PITTWELL
Secretary

20 July 1977

Air Segmented Continuous Flow Automatic Analysis in the Laboratory 1979

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 throughput. 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 compatibility. 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 this 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. Table 1 gives a list of some of the determinations most frequently carried out in a Water Authority laboratory to which an air-segmented continuous flow automatic analysis technique can be applied.

Table 1 Determinations most frequently carried out in the water industry

Chemical oxygen demand	Sulphate
Permanganate value	Phenol
Chloride	Cyanide
Silicate	Sulphide
pH	Anionic surfactants
Alkalinity	Non-ionic surfactants
Ammoniacal nitrogen	Iron*
Nitrite nitrogen	Copper*
Nitrate nitrogen	Nickel*
Phosphate	Aluminium*
Fluoride	

Excluding those marked with an asterisk, which with other metals are normally determined by atomic absorption spectroscopy, the majority of the remaining determinations can employ an air-segmented continuous flow colorimetric technique. Numerous papers have been published on the use of air-segmented continuous flow automatic analysis for these parameters. The latest 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. 1 shows.

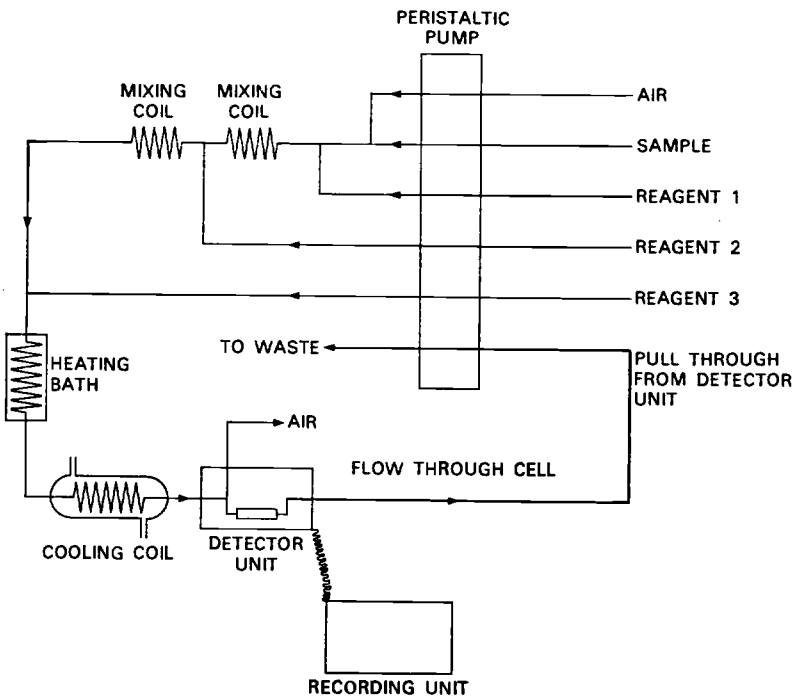

Figure 1 Line (flow) diagram of an air-segmented continuous flow automatic analyser

This essay review does not seek to provide detailed information on individual methods which are covered by other publications in this series. The Reports of the Standing Committee of Analysts give the publication programme. Additionally the Second Report mentions applications to marine analysis.

3 Basic Equipment of Continuous Flow Systems

The block diagram Fig 2, shows the component stages of a typical automatic analysis system. A variety of such systems is commercially available.

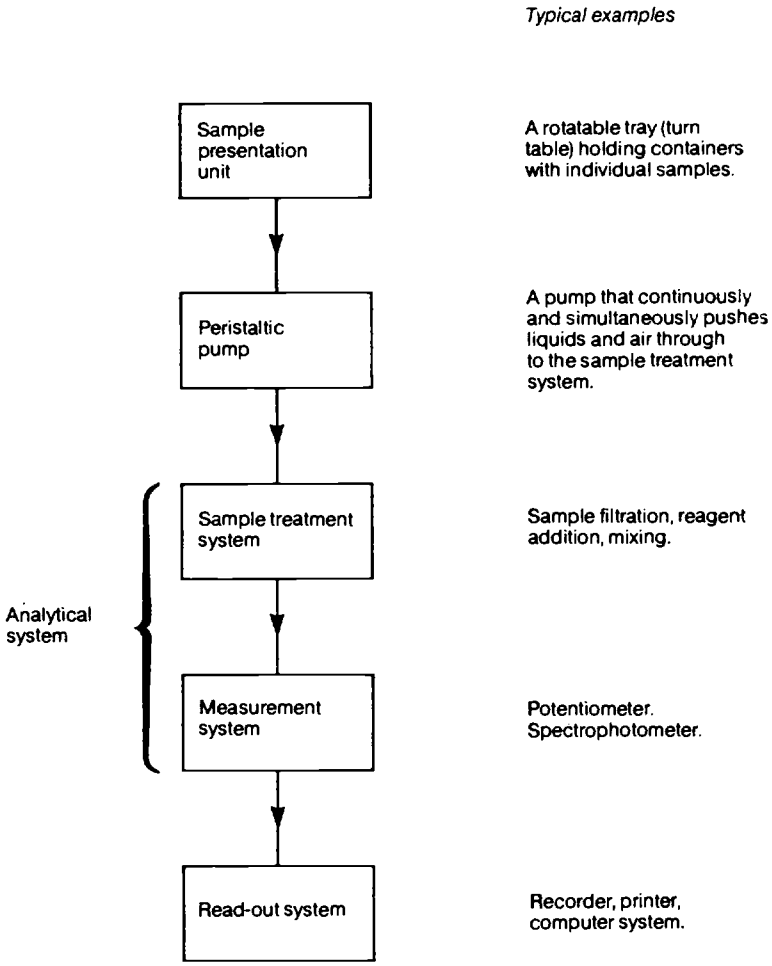

Figure 2 Basic equipment for continuous flow analysis

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 peristaltic 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 3), or a number of sample

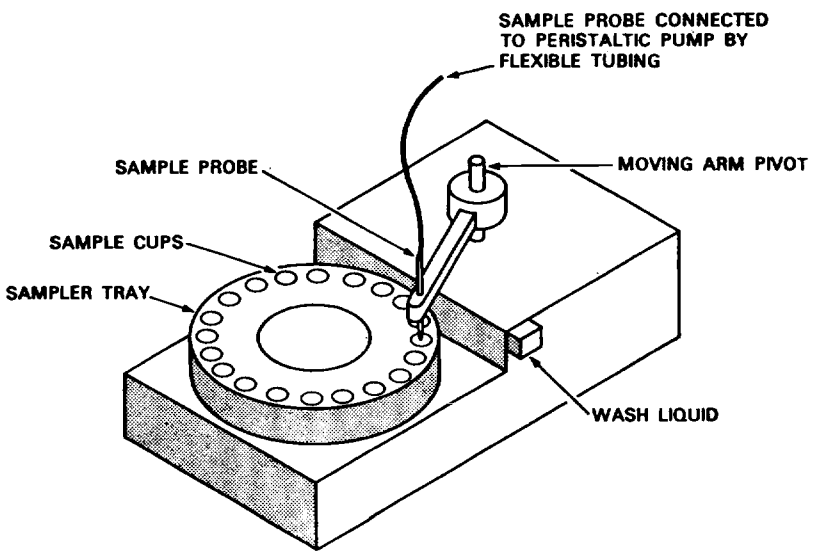

Figure 3 A sample cup presentation unit

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

tubes snapped together and wound into spools moving by a conveyor belt principle (Fig 4). 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

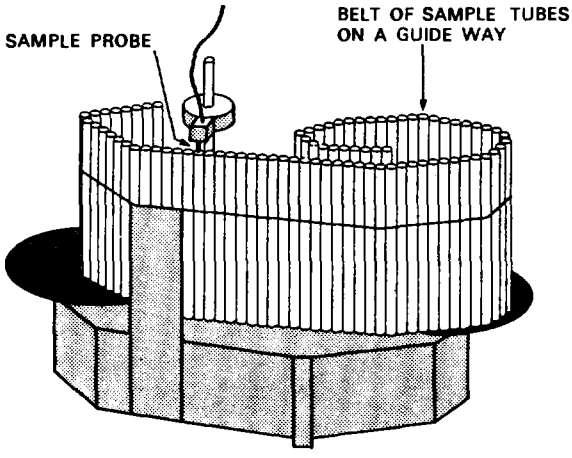

Figure 4 A sample tube presentation unit

withdrawing the sample. 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 on 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 ratio, 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 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 5 (a). 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 5 (b). If the

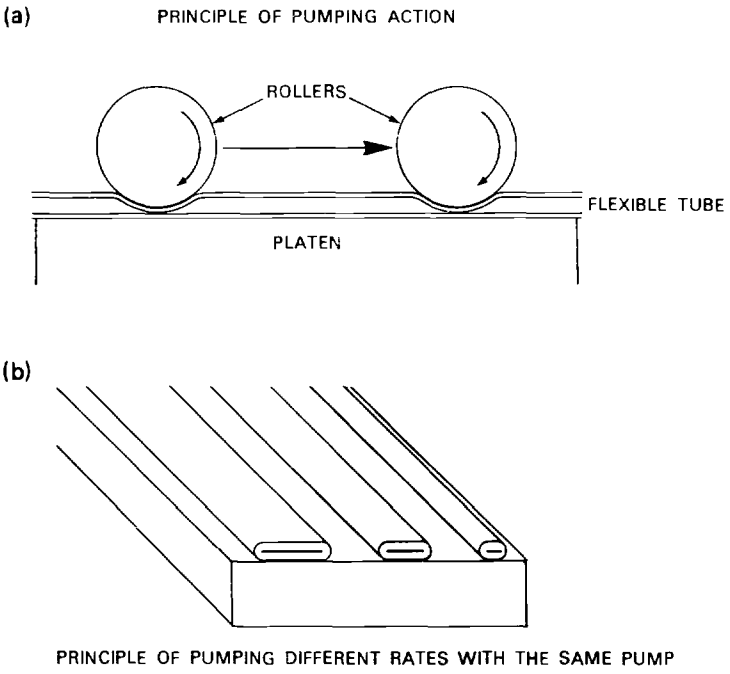

Figure 5 Principle of the peristaltic pump

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 6. For instance, water has been used to displace chloroform and other organic solvents.

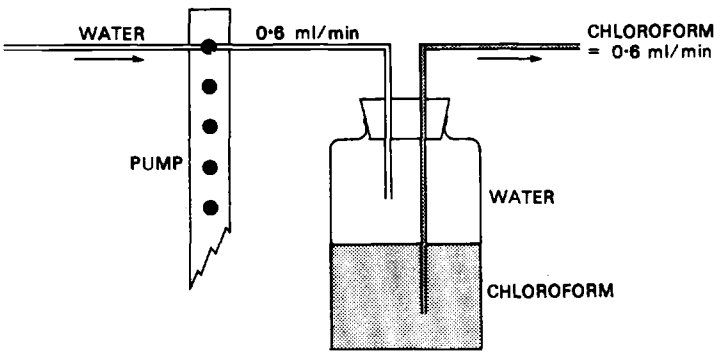

Figure 6 Displacement Bottle technique

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 7. With this type of pump the flow rate can be altered by changing either the motor speed or the internal diameter of the tube.

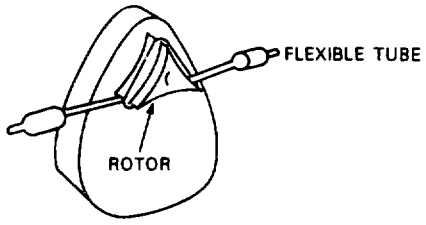

Figure 7 Alternative design of the peristaltic pump

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.

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 8) 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.

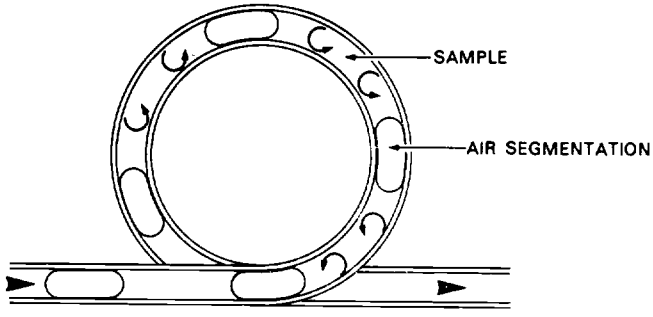

Figure 8 Function of a typical mixing coil

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 reagents 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.

3.4 **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 transferred to the recipient stream by osmosis, leaving most interfering ions or molecules in the donor stream (Fig 9). 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;

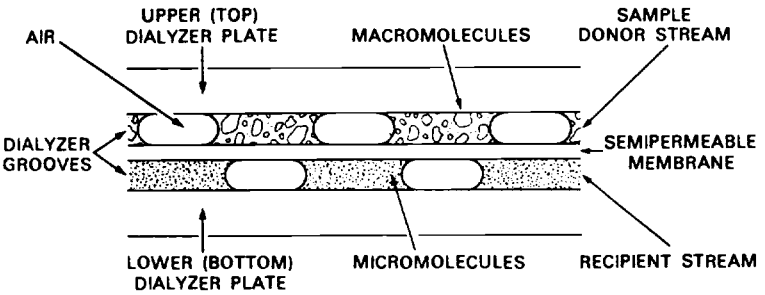

Figure 9 Dialysis unit

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 and 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.

3.4.2 *Reagent addition and mixing*

The sample and reagent(s) merge in appropriate stages through T-connections (Fig 10). Normally after each addition of a reagent the two dissimilar merging streams are

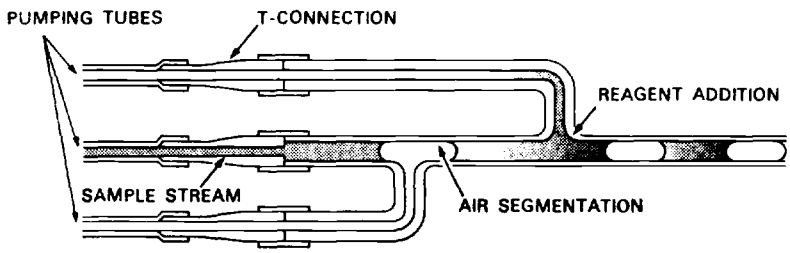

Figure 10 T-connections reagent addition and mixing

thoroughly mixed before the next reagent is added. This mixing is achieved by pumping both streams through a helix of glass tubing whose axis is mounted horizontally (Fig 8) 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 homogeneous mixture. The degree of 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. The use of these coils 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 afford time for the reaction products to develop sufficiently for measurement. Delay coils are of varied lengths of glass-tubing which are normally coiled for convenience. They are selected according to the need of the method and they are usually mounted with their axis vertical.

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 of a single test.

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 of 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 11. The coil in which the sample is to be heated is kept in the bath, H (in Fig 11), which is maintained

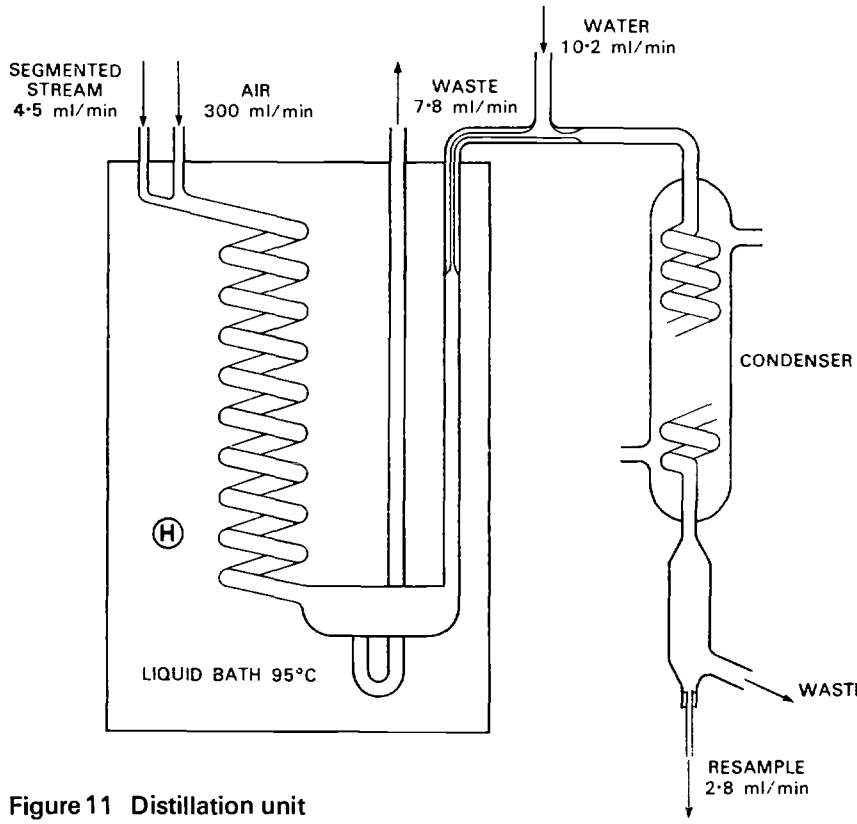

Figure 11 Distillation unit

at the required temperature. The sample and reagents are pumped into the coil and the emerging stream has its involatile components trapped and the vapour containing the determinand is condensed. The condensate is then resampled by the sample presentation system for analysis.

3.4.7 *Solvent extraction and phase separation*

Two immiscible liquids eg the sample and the solvent, are 'mixed' as described previously (Section 3.4.2) or in a beaded coil (Fig 12). The resultant stream enters a

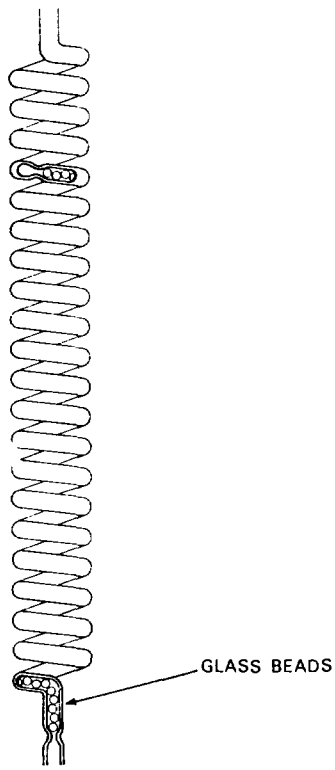

Figure 12 Mixing in a beaded coil

separator via a 'side-arm' positioned in its middle. The two liquids separate relative to their density characteristic and the required liquid pumped to the next treatment stage, the other liquid going to waste (Figs 13(a), 13(b)). This separator can normally be of a comparable size to the other components used in this system.

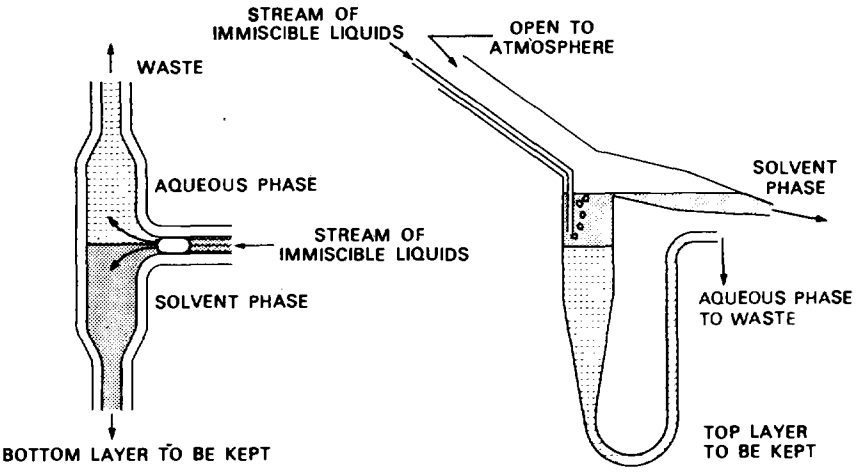

Figure 13 Phase separation

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 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.

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.

3.5.2 *Electrochemical methods*

The field of electrochemical analysis embraces a wide range of techniques including potentiometry, polarography, amperometry, conductimetry, coulometry, chronopotentiometry 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 absorption 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 determinand 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 response 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 may be detected by responses of the calibration check standards differing significantly from their initial responses. The following procedure is suggested for these occasions, providing the rectilinear relationship previously mentioned is maintained; the responses for the calibration check standards should be used to construct a series of calibration curves. A separate calibration curve is then used for each group of samples. Each of these calibration curves is produced by plotting the blank-corrected response for the check standards against concentration and the blank-corrected responses for each sample are converted to concentrations using the appropriate calibration curve.

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 ration 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 ration 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 14). 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 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, dictate 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.

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

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 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 determinand 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

Calibration stable

Test No.	Solution
1	Blank
2	Calibration Std 0·2 C_m*
3	Calibration Std 0·4 C_m
4	Calibration Std 0·6 C_m
5	Calibration Std 0·8 C_m
6	Calibration Std 1·0 C_m
7	Blank
8–20	Samples 1–13
21	Sample 1**
22	Control Std†
23	Blank
24–37	Samples 14–27
38	Sample 14**
39	Control Std†
40	Blank
41	Repeat cycle 8–23

Calibration unstable

Test No.	Solution
1	Blank
2	Calibration Std 0·2 C_m*
3	Calibration Std 0·4 C_m
4	Calibration Std 0·6 C_m
5	Calibration Std 0·8 C_m
6	Calibration Std 1·0 C_m
7	Blank
8–20	Samples 1–13
21	Sample 1**
22	Control Std†
23	Calibration Std††
24	Blank
25–37	Samples 14–26
38	Sample 14**
39	Control Std†
40	Calibration Std††
41	Repeat cycle 7–23

Carry-over Present

Calibration stable

Test No.	Solution
1	Blank
2	Blank
3	Calibration Std 0·4 C_m*
4	Calibration Std 0·2 C_m
5	Calibration Std 0·8 C_m
6	Calibration Std 0·6 C_m
7	Calibration Std 1·0 C_m
8	Blank
9	Blank
10–22	Samples 1–13
23	Sample 1**
24	Control Std†
25	Blank
26	Blank
27–39	Samples 14–26
40	Sample 14**
41	Control Std†
42	Repeat cycle 8–24

Calibration unstable

Test No.	Solution
1	Blank
2	Blank
3	Calibration Std 0·4 C_m*
4	Calibration Std 0·2 C_m
5	Calibration Std 0·8 C_m
6	Calibration Std 0·6 C_m
7	Calibration Std 1·0 C_m
8	Blank
9	Blank
10–22	Samples 1–13
23	Sample 1**
24	Control Std
25	Calibration Std††
26	Blank
27	Blank
28–40	Samples 14–26
41	Sample 14**
42	Control Std†
43	Calibration Std††
44	Repeat cycle 8–25

* Where C_m 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 C_m (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 C_m 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 $0 \cdot 0\ C_m$ standard (blank) to zero response and the $1 \cdot 0\ C_m$ 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 above zero and the $1 \cdot 0\ C_m$ 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 of 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(f)). 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 coworkers[8] remains the basis of quantitative considerations 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 (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 response with changes in sample concentration

In the development of a continuous flow procedure, a linear relationship between the response and 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 ($W_{\frac{1}{2}}$)[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 response 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 $W_{\frac{1}{2}}$ is calculated directly from the slope of the linear portion of a plot of log C_t against time. The initial non-exponential part of the 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 mainly due to air-segmentation[16, 17], precedes the exponential part.

Sample interaction (Fig 14) can be quantitatively expressed by using $W_{\frac{1}{2}}$ and L. If the between sample time is t_b, the value of the expression $(t_b - L)/W_{\frac{1}{2}}$ gives a measure of the interaction of a sample with the following one. For values of $(t_b - L)/W_{\frac{1}{2}}$ 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 of L and $W_{\frac{1}{2}}$ the lower the degree of interaction; or conversely, for a given acceptable percentage interaction the lower the values of L and $W_{\frac{1}{2}}$, the faster the available sampling rate.

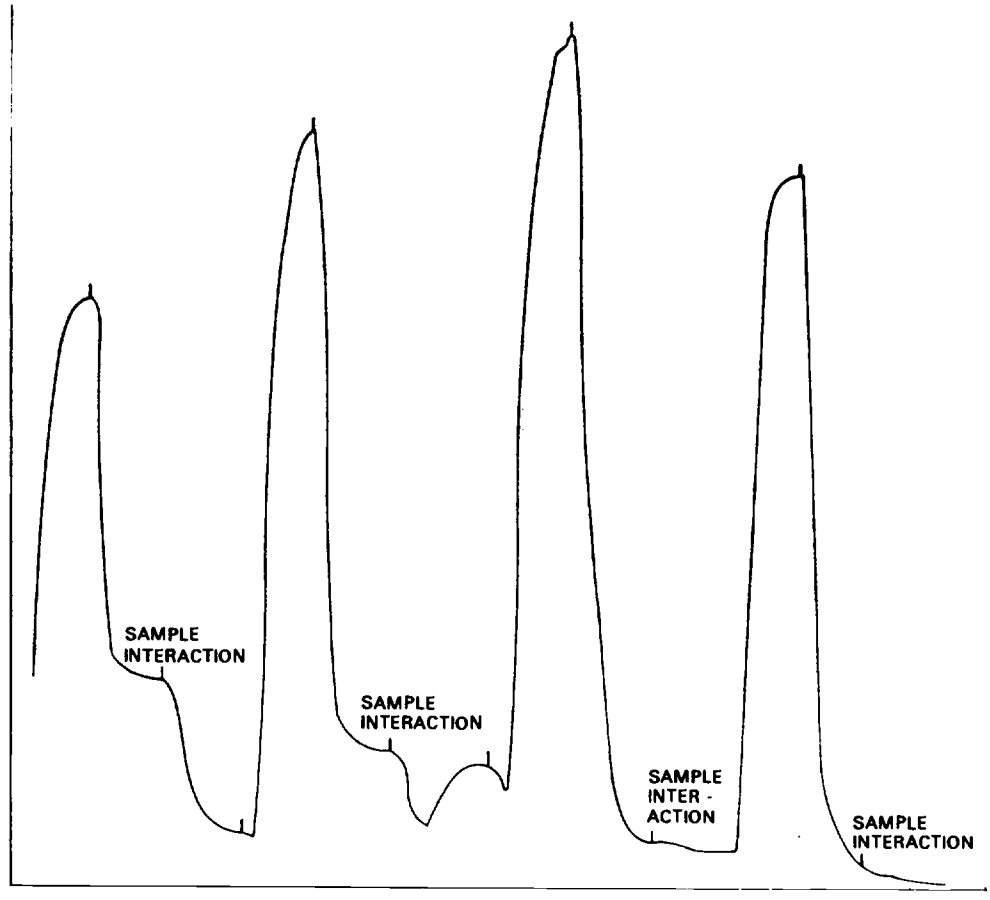

Figure 14 Sample interaction 50 samples per hour with 12 sec wash

The effect of carry-over can be measured as the following considerations indicate: consider the typical absorption curve in Fig 15. 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 absorption 'r' due to peak 1. For any particular manifold and pumping rate we have

$$r = k\,h$$

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

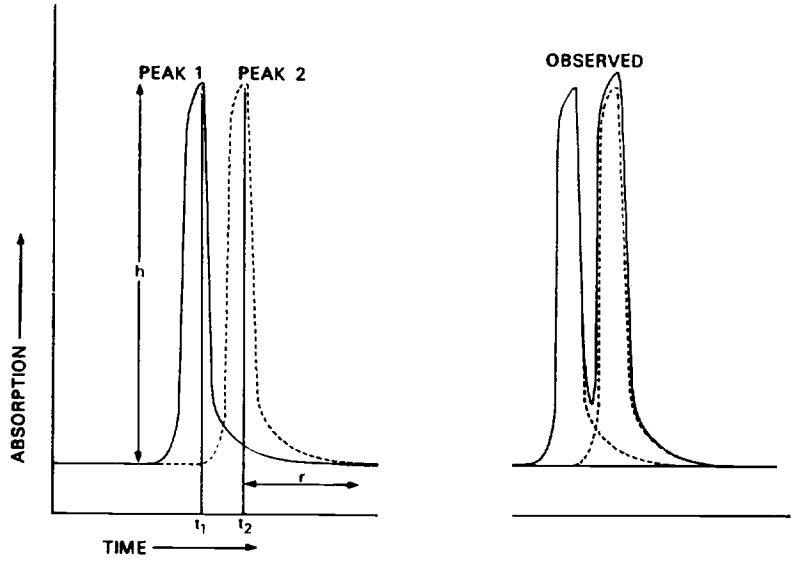

Figure 15 A typical absorption curve illustrating the effect carry over will have on a subsequent curve (see also Figure 14)

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 15 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 corrected 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. 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.

System malfunctions normally fall into three categories:

1. Electrical/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. Regular maintenance is advisable of 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 continual 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 tube failure; check that 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.

(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 particulate 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 response 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 a sharp deflection on the readout. However, if this air bubble interference is infrequent it can easily be removed, by pinching the pull-through tube for ca 2 s but the sample treatment system should be redesigned if the problem is consistently present.

7.3 Chemical Malfunction

Problems with the system chemistry arise as a rule from using unstable 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 interfere with the determination of the particular determinand eg do not use nitric acid if the determinand 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 algae 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 when a single determinand is determined.

(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 allows lower detection limits than manual analysis using a similar procedure.

(c) If a laboratory reaches the position where the majority 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. Instruments increasingly have the facility of automatic calibration so that the results can be provided by them 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 micro-processors 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. Reference 19 explains in detail the principle and use of control charts. One standard synthetic solution of known concentration corresponding to about $0{\cdot}8\ C_m$ (where C_m 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 than that used to prepare the calibration standards but stored under the same conditions, is analysed per block of samples 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 $\mu \pm 3\ \sigma$ 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 at limits $\mu \pm 2\ \sigma$ 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 parts 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 peristaltic 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 pressure.

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, 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.

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Address for Correspondence

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