Discrete analysis systems

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Discrete analysis systems are so called because the samples are treated and carried through the major part of the analytical process in separate containers. Such systems can be very simple involving only a diluting module and a colorimeter or be highly sophisticated multichannel machines. Up to the present time laboratory automation (or strictly 'mechanization') has been dominated by the continuous flow systems and discrete systems have not yet had time to establish themselves widely as serious competitors. In this paper I cannot attempt to give an evaluation of individual systems and I shall confine myself to a discussion of general principles and indicate how these systems may differ from one another.

GENERAL PRINCIPLES

Discrete systems fall into two main groups and are shown diagrammatically in Figure 1. In the continuous discrete system, where samples may be fed into the machine continuously and results obtained continuously, tube transport through the various analytical processes is fully automatic and in the multichannel analysers the tubes are washed automatically before receiving further aliquots of sample. In the discontinuous discrete system the samples are processed in batches with manual transfer of batches from one stage to another.

DILUTION OF SAMPLE The first stage in any analytical system is the addition of sample (blood, serum, or urine) to a diluent or reagent. In a mechanical system the same 'pipette' is used repeatedly and it is necessary to minimize carryover on the inside of the pipette. However, the following

[Diagram of continuing and discontinuing discrete systems]
sample may be contaminated by carryover of sample or diluent (or reagent) adhering to the outside of the pipette tip. This type of contamination is particularly serious where the same specimens are sampled repeatedly for different analyses. For example, for total protein determination, serum may be washed out with biuret reagent but if the biuret reagent contaminates the next serum specimen it may make it unsuitable for subsequent enzyme assay. Wiping the tip in a mechanical system is difficult but it may be helpful to cover the sample with a film of material which will not only prevent evaporation but will also wipe diluent from the tip as it pierces the film and wipe off serum as it withdraws. The use of a fine, clean, non-wettable tip will also be helpful. Where carryover is still significant it may be necessary to carry out the different determinations in a particular sequence.

In the AutoChemist the sample is not washed out with reagent or diluent but with an ‘air wash’ between serum samples. It is claimed that carryover of serum in the pipette tip is negligible. Since diluent or reagent is added from a separate pipette these solutions cannot contaminate the next sample. Also the simpler valve system required for this method of dilution reduces the danger of sticking or leaking valves.

Exact calibration of sample and reagent syringes is not generally required since standard and test specimens receive the same treatment.

MIXING OF SAMPLE AND REAGENTS The simplest way to achieve mixing is by discharging the sample and reagents forcibly into the reaction tubes. The success of this method depends upon many factors, notably the force of ejection, which in turn is dependent upon the diameter of the orifice of the pipette and the rate of discharge; also the shape of the bottom of the reaction tube and the ratio of its height to diameter is important. While the force of ejection may be sufficient to cause mixing of serum and diluent, mixing on addition of further reagents may be more difficult or impossible if the volume of additional reagent is much less than that of the solution already in the tube and other methods of mixing will be needed. Any type of paddle or stirrer in the solution may give rise to carryover problems but mixing by a jet of air might be feasible. Mixing by convection may be effective where the tubes are maintained at a higher temperature than the reagents. In the LKB reaction rate analyser each tube is spun rapidly clockwise and then anticlockwise to achieve very effective mixing after adding only 20 μl reagent to 2 ml of liquid. Another possible method would be to vibrate each tube or each rack of tubes using the principle of a vortex mixer.

ARRANGEMENT OF SAMPLE CONTAINERS AND REACTION TUBES Figures 2a and 2b show a compact 10 × 10 arrangement of sample cups and reaction tubes as used in the Analmatic. The diluter moves along the first row from left to right picking up the samples and discharging them with diluent or reagent into the adjacent reaction tubes, returns to the left

![Fig. 2a.](http://jcp.bmj.com/)

![Fig. 2b.](http://jcp.bmj.com/)

![Fig. 2c.](http://jcp.bmj.com/)

**FIG. 2.** Arrangement of sample cups and reaction tubes as used in the Analmatic (Figs. 2a and 2b) and a turntable arrangement (Fig. 2c) used in other systems.

\(^1\)LKB Instruments Ltd, 232 Addington Road, S. Croydon, Surrey.
while the rack is indexed backwards by one row, and then moves along the second row. If required other reagents may be added simultaneously or sequentially from dispensers. The rectangular pattern allows flexibility in setting up simultaneous blanks and where temperature control is important the rectangular rack fits readily into a water bath.

Other systems, such as the Mecolab, Clino-Mak, and Quickfit analyser use a turntable arrangement (Fig. 2c) which, although less compact for a given number of samples, is much simpler mechanically, and stations for dilution, reagent addition, and colorimetry can be placed at suitable positions around the turntable. The moving belt system of the Bioanalyst is similar in principle to the turntable arrangement.

It is time consuming to reload large numbers of reaction tubes into their racks for each analytical run and facilities for automatic washing and drying the tubes, as in the Clino-Mak system, are useful.

**PROTEIN SEPARATION** For the determination of many serum constituents methods are available which do not require the removal of protein. Many of the discrete systems therefore do not make provision for deproteinization although it will then often be necessary to make blank corrections for turbidity, haemolysis, or jaundice. However, discrete systems which have facilities for protein separation can compete more fully with the continuous flow system (which uses dialysis to remove protein). In the Mecolab system protein precipitant may be added to 15 tubes in a centrifuge head and in the Analmatic up to 100 tubes from the rectangular rack may be transferred quickly to a large centrifuge head. The tubes can then be returned to the rectangular rack without loss of identity, and, using a second diluter, the supernatants may be picked up and discharged with a volume of reagent into recipient tubes (Fig. 3). Alternatively, the precipitate can be analysed after inverting the tubes to decant the supernatant (protein-bound iodine, protein-bound hexose, etc.). The Quickfit analyser includes an automatic centrifuge for continuous sequential separation of the supernatant from the precipitated proteins at a rate of approximately 100 samples per hour.

**INCUBATION** Most systems have facilities for constant-temperature incubation of the reaction tubes, using water (Analmatic), warm air (Bioanalyst), or solid state (Vickers multichannel 300), to give temperatures up to about 70°. In the Analmatic the temperature of the water bath fitted to the preparation unit can be increased or decreased by 50° in a few minutes by circulation of water from a large reservoir.

**PHOTOMETRY** In most discrete systems the final coloured solution is drawn through a flow cell. Carryover between solutions is reduced (usually to less than 1%) by using the first part of the solution to wash out the cell. An air wash between solutions (as in the EEL automatic colorimeter) may also be helpful in reducing carryover.

In the Clino-Mak system the precalibrated glass reaction cuvettes pass directly into the light path of the photometer. The LKB calculating absorption meter uses disposable polystyrene cuvettes (less than 2d each) with a throughput of 1,500 cuvettes per hour. In tests on a demonstration model reproducibility was only slightly poorer than that given by the Gilford microspectrophotometer 300 and the method has the advantage that there is no carryover between solutions at this stage.

With the double-beam colorimeters used in the Analmatic and Vickers multichannel 300 blank and test solutions may be aspirated simultaneously into blank and test flow-through cells and the result obtained directly from the difference in optical density.

Flame photometry for sodium and potassium determination presents no special problems and deproteinization is not necessary if the samples are adequately diluted.

**PRINTOUT OF RESULT** The 'peak-picking' problems of the continuous flow system do not arise, since readings can be made in the steady state at fixed time intervals. In discrete systems most determinations follow Beer's law and after log/linear conversion of the signal and the introduction of a scale factor, a printout of the results can be obtained.
in concentration units (Vitatron digital colorimeter, EEL automatic colorimeter). If cadmium sulphoselenide photocells are used the output is directly proportional to optical density thus simplifying printout of concentration (Analmatic, Vickers multichannel 300). Small deviations from linearity can be corrected by linearizers using diode function generators (Analmatic). It can be anticipated that drift will not be a prominent feature in discrete systems but where this is found to occur it is possible to incorporate simple mechanisms to give automatic blank and standard drift corrections during the run (EEL automatic colorimeter linked to Griffin Bioanalyst or Quickfit analyser).

**DESCRIPTION OF SOME DISCRETE ANALYSIS SYSTEMS**

**ANALMATIC CLINICAL ANALYSIS SYSTEM**

This is a discontinuous discrete analyser. The preparation unit holds 100 samples and 100 reaction tubes in a thermostatically controlled water bath (Figs. 2a and 2b). The temperature of the baths can be set between 25° and 75° and can be increased or decreased by 50° in a few minutes by circulating water from a large reservoir. When deproteinization is required 100 reaction tubes can be spun in a large centrifuge head. The double-beam colorimeter has cadmium sulphoselenide photocells and uses interference filters. A linearizer is available where Beer's law does not hold.

not apply. There is a twin-channel flame photometer for simultaneous sodium and potassium determination (no internal standard). A printer gives results in concentration. The rate of analysis is 300 samples per hour. There are facilities for setting up simultaneous reference blanks. Price £3,560, excluding centrifuge.

**AUTOCHEMIST**

This is a continuous discrete multi-channel analyser (Fig. 4), in which 3 to 6 ml serum is distributed in racks of six tubes which can be linked together to form a train of, say, 100 samples. These pass into the machine along the outer loading belt to feed, in sequence, three long conveyor belts each having six analytical channels. The serum is dispensed together with reagents into the reaction tubes attached to these conveyor belts. The samples take 50 minutes for processing, including a maximum of 30 minutes' incubation at 50°C. The colorimeters are fitted with narrow band interference filters. After colorimetry the reaction tubes are automatically washed and drained to receive further sample aliquots. The inner loading belt feeds six short analytical channels (30 minutes at room temperature) with urine or deproteinized solutions. In the basic version of the AutoChemist only the first sample in each train of samples has automatic identification; the remainder must be in the same order as indicated on the sample list. If automatic identification of individual samples is required additional electronic equipment must be included. An on-line PDP-8 computer collects and processes the data from the analytical procedures and the results are printed out on a teletypewriter in concentration units against patient information. The rate of analysis is approximately 135 samples per hour for a maximum of 24 channels. Price £165,000. Minicube two-channel analysers may be built into the central processor for methods requiring corrosive reagents or temperatures up to 110°C. Manual determinations may be integrated with those coming from the AutoChemist through 'satellite' colorimeters. These are extras.

**AUTOLAB AUTOMATIC SYSTEM FOR CHEMICAL ANALYSIS**

This is a single-channel continuous discrete system (Fig. 5). Chains of samples pass into a sampling unit and are transferred with reagents to a continuous chain of reaction tubes. These can pass directly to the colorimeter module or additional chain sections can be inserted to give a longer path length for incubation and reagent modules. The time of incubation in the water bath can be varied from two minutes to 30 minutes by changing the number of chain sections. The colorimeter is fitted with narrow-band interference filters. If Beer's law applies the results are printed out in concentration units. Small stirrers are used to mix solutions after reagents have been added. There are no facilities for protein precipitation. The rate of analysis is 240 samples per hour. Price approximately £2,500.

**CLINO-MAK MARK II**

The turntable carries 90 samples in an inner ring. The diluter transfers 20 to 100 µl samples with diluent or reagent into an outer ring of glass reaction cuvettes (Fig. 2c). Four more reagents may be added around the turntable. The volume of reagent added can be varied by changing the time for which an electromagnetic valve controlling its flow remains open. The matched cuvettes pass directly into the light path of the colorimeter (fitted with a continuous interference filter) and the readings are displayed as peaks on a chart recorder. The Clino-Mak can be used as a continuous discrete analyser for simple determinations at room temperature but the turntable may be set aside for heating or prolonged incubation as in a discontinuous system. A separate Lavo-Mak module washes reaction cuvettes automatically. The cuvettes are dried by placing the turntable in an oven. There are no facilities for protein removal or direct printout of concentration. The rate of analysis is 300 samples per hour. Price approximately £2,500 (excluding import duty), recorder extra.

**GRIFFIN BIOANALYST AUTOMATED CHEMISTRY MODULE**

This is a single-channel continuous discrete analyser. A conveyor belt, following a rectangular path, carries a double row of 120 containers (sample cups on the outside and reaction tubes on the inside). Samples can be transferred, diluted, and reagents added at selected points using pneumatically actuated glass syringes. A small paddle can be used to mix the reagents. The reaction

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**Fig. 5.**

Diagram of the Autolab, a continuous discrete system.
tubes travel in a thermostatically controlled incubator trough heated by warm air up to 60°C. There are no facilities for deproteinization. The rate of analysis is 120 samples per hour. Price approximately £800.

The colorimeter module is extra. When linked to the EEL automatic colorimeter it is known as the EEL Griffin Bioanalyst. The colorimeter gives a printout of concentrations where Beer's law applies and has automatic zero and automatic standardization facilities. Price of colorimeter approximately £1,200.

The Bioanalyst module may also be linked to the Unicam range of spectrophotometers when it is referred to as the AC Chemical Processing Unit. The spectrophotometer readings are displayed on a chart recorder. The price will depend upon the type of spectrophotometer.

**MECOLAB LABORATORY ANALYSIS**
This is a discontinuous discrete system consisting of a series of modules for processing batches of 15 samples. Transfer between modules is done manually. The inner ring of the 'sampling unit' fits into a centrifuge for deproteinization if required. Solutions are presented to a double-beam recording colorimeter. Where Beer's law applies printout of concentration may be obtained by introducing a scale factor. Where there are deviations from Beer's law facilities are available for linearization. There are no facilities for temperature control. The maximum rate of analysis is 240 samples per hour. Price approximately £3,000.

**REAGENT CHARACTERISTICS**
Reagents, and the Joyce, Loebi autocolorimeter may be used.

**ROBOT CHEMIST**
This is not available in the UK at the present time.

**VICKERS MULTICHANNEL ‘300’**
This is a multichannel continuous discrete analyser at present in course of development.

Blood samples (5 ml) are collected into special disposable polystyrene vials containing anticoagulant.

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1. Evans Electroselenium Ltd, Halstead, Essex.
4. Quickfit & Quartz, Stone, Staffordshire.
5. Warner-Chilcott Laboratories Instruments Division, Morris Plains, New Jersey, USA.
The specimen is identified by depressing digits on the vial label and the vial is sealed. After centrifuging in batches, the vials are placed in the sample magazine (Fig. 6) and fed to a photoelectric 'serum check' position. Samples that are insufficient, inadequately separated, haemolysed, or lipaemic are automatically rejected but can be reentered later. Satisfactory samples pass to the primary transfer diluter which takes up a quantity of serum and dispenses it with diluent into an open tube on a distributor system for transfer to each of the reaction consoles in turn. A secondary transfer dilution is made from this tube into the cavities of the reaction console rotor. The rotor contains 60 reaction cavities or 120 cavities if simultaneous blank determinations are required. The temperature can be maintained at preset values. Reagents are added from dispensers placed around the periphery of the rotor. A suction probe transfers the final solution to the measuring head (colorimeter or flame photometer) and the probes are so positioned that aliquots of the same initial specimen are measured at each reaction console at the same time. At the same moment the sample vial will have reached the address reader for identification of the sample. After taking the final solution the reaction cavities are automatically cleaned and dried. Also, after passing all the reaction consoles the primary dilution tubes are inverted and washed before passing to the primary transfer diluter again.

The colorimeters are double-beam instruments using interference filters. Cadmium sulphoselenide photocells give an output which is linear with respect to optical density, simplifying direct calculation of concentration. There are facilities for compensating for deviations from Beer's law. The flame photometer measures sodium, potassium, and calcium using lithium or strontium as an internal standard.

It is proposed that the system will include a PDP-8 computer which will act as a process controller and also collect and analyse the signals from the measuring heads. Results will be printed out on a teletypewriter in concentration units against patient identification taken from the address reader. There are no facilities for deproteinization.

The system will be available as a four, six, or 12-channel machine operating at a rate of 300 samples per hour. It may also be possible to use individual reaction consoles fitted with a sampler plate. No prices are available.

**DISCUSSION**

**CAPITAL COST AND ANALYTICAL SPEED** In 1965 the Association of Clinical Biochemists Study Group on Automation in Clinical Chemistry submitted a memorandum to the Scientific Instrument Manufacturers Association (SIMA) entitled 'The development of a semi-automatic system of analysis in clinical chemistry'. (The principles of the system have been described by Northam, 1966.) This memorandum suggested that there was a need for a faster and cheaper system than the Auto-Analyzer and that in theory a discontinuous discrete system could be expected to meet these requirements. In attempting to assess the potential of discrete analysis systems comparison with the Technicon AutoAnalyzer as the only well tried automatic system in the hospital laboratory is inevitable. Interaction in the AutoAnalyzer flow-line is likely to limit its maximum rate of analysis to about 60 samples per hour at a capital cost of approximately £1,500 per channel. 'Peak-picking' equipment to print out results could increase this to £2,500 per channel. Similarly, the Technicon Sequential Multiple AutoAnalyzer (SMA-12/60), which presents the results in graphical form at 60 specimens per hour, costs about £1,500 per channel (12 test and four blank channels). Sample identification and printout of results bring the cost to about £2,000 per channel. In discrete systems the dilution step is the most complex but it should be possible to complete each dilution cycle in about 12 seconds, that is, at a rate of 300 samples per hour. In practice this has been shown to be feasible and in the Analmatic it is claimed that test and blank determinations can be carried out simultaneously each at 300 samples per hour. However, rate of analysis (ie, number of cycles per hour) should not be confused with throughput of specimens. For example, in the simple determination of albumin by a dye-binding method, for a batch of 100 specimens it would be necessary to use the preparation unit of the Analmatic twice (once for dilution and adding reagents and once to present the solutions for colorimetry) and the throughput (without duplication of the preparation unit) would be 150 samples per hour (discounting preparation and washing-up time). Since the capital cost of the apparatus is approximately £3,000 this is equivalent to a cost of £1,200 per channel at 60 samples per hour. Alternatively, if there is sufficient colour development in, say six minutes, a batch of 70 specimens could be analysed completely in one passage through the preparation unit by placing the colorimeter probe 30 samples behind the dilution and reagent addition probes. In this mode 70 specimens would be analysed in 20 minutes, equivalent to £850 per channel at 60 samples per hour. In the third mode, where simultaneous measurement of test v blank is necessary, and provided that no more than six pipettes are required for the two channels (as in total protein determination), 50 specimens may be analysed in two passages through the preparation unit. This is equivalent to £600 per channel at 60 samples per hour. Since this includes printout of results it follows that for simple determinations the capital cost per sample in the discontinuous discrete system is less than for the
AutoAnalyzer. However, it should be appreciated that when Technicon patents expire the cost of equipment for continuous flow analysis is likely to fall sharply. Capital cost in the fully automated discrete systems will be higher and for the AutoChemist (including computer) is equivalent to more than £3,000 per channel at 60 samples per hour. However, full evaluation of comparative capital, labour, and running costs will only be possible when discrete systems are in routine use.

CONTROL OF VARIABLES In the continuous flow system standard and test specimens are subjected to the same treatment with regard to time, temperature, dialysis, etc. In this situation it is not essential for any stage to go to completion. In fact, in the limited flow-through time it is usually impossible for all processes to go to completion and this may lead to some loss of sensitivity.

Similar advantages and limitations apply to continuous discrete systems. In some discontinuous discrete systems it is not necessary for reactions to go to completion since standards and tests can be maintained at the same temperature for the same length of time by carrying out the diluting, dispensing, and colorimetry sequence at exactly the same rate with the reaction tubes immersed in a constant-temperature bath. However, where prolonged incubation is required, for example, to achieve greater sensitivity, this system is more flexible, since the batch of tubes can be set aside in another bath leaving the treatment unit free for other determinations.

MECHANICAL RELIABILITY The discrete systems are more complex mechanically than continuous flow systems and it follows that the individual components must be of high quality if they are to function reliably through thousands of cycles. In the continuous flow system the recorder trace can be used to monitor performance of the system as a whole. For example, if insufficient sample has been taken into the flow line this will show as a spiky peak; irregular pumping of reagent lines may be shown by base-line shifts or irregularly shaped peaks; surging in the flow-line will be indicated by varying peak widths, poor mixing by a ‘noisy’ trace. In the SMA-6/60 and -12/60 models the performance of each channel is continuously monitored and displayed on a cathode ray oscilloscope.

In discrete systems, monitoring the colorimeter signal will give no indication of the performance of the other modules. For example, if the sample syringe only picks up half the correct volume of serum on one cycle and then functions correctly for subsequent cycles there will be no indication that the result is only reading half its true value. The possibility of undetected random errors of this type is in many ways a more serious problem than outright failure of a component which prevents the system from working at all.

Various steps may be taken either to reduce or reveal random errors. The former could be achieved by designing ‘fail-safe’ syringes with positive mechanical drive in both directions, sufficiently powerful to overcome sticking or cause complete failure of the unit. The extra cost of fitting high-precision pistons and barrels would be fully justified. A preliminary test run each day with a series of standards and control specimens may be necessary to establish that all components are functioning normally.

Random errors would be revealed with a high degree of probability by duplicating each test run, either in parallel or in sequence. This may appear to be extravagant but would be feasible in a high-speed system taking only small volumes of sample and would give a bonus of increased precision for each determination. Computer analysis would be almost essential to calculate the mean values for each pair and to detect differences between duplicates greater than would be expected from the analytical error.

Vickers propose to equip their multichannel 300 with an on-line PDP-8 computer which will not only collect the data and analyse it statistically but will also act as a process controller. The computer will be programmed to monitor vital components and display an error signal in the event of malfunction during any cycle.

FUTURE DEVELOPMENTS We are still only at the beginning of the development of automated systems in chemical analysis and the discrete systems available are all based on mechanization of manual procedures. Some of these machines are very large: the 12 reaction consoles of the Vickers multichannel 300 are each as large as washing machines and the AutoChemist weighing four tons is 6 ft wide, 13 ft long, and 9 ft high. Clearly miniaturization, that is, a scaling down of the whole system, would be advantageous if this can be made compatible with good precision and reliability.

Other systems based on novel concepts (the AutoAnalyzer is in this category) may be developed. Dr G. M. Widdowson, at the Presbyterian Hospital, San Francisco, is examining the use of specific electrodes (linked directly to a computer) to give a wide range of analyses on samples of body fluids. LKB Instruments are advanced in the development of micro-colorimeters and coulometers and these may well have applications in automatic analysis.
with the advantage that optically clear solutions are not required. Guigan in Paris is investigating the use of a long plastic film containing plastic compartments identified by magnetic strip. After injection of samples and reagents into the compartments the film can be drawn through heating modules to a colorimeter rather like ciné film. The coloured solutions are measured directly through the transparent walls of the compartments and it is claimed that readings can be made at a rate of 7,000 samples per hour.

CONCLUSIONS

It seems probable that there will be a need for a range of automatic systems of analysis in the laboratory and that the discrete system will be complementary to the continuous flow system each being used for those determinations for which it is best suited. The large continuous discrete analysers will only be economic in a very large laboratory or in a centralized laboratory serving a group of hospitals.

It must be made quite clear that at present there is little objective information on the performance of discrete systems and intending users are strongly advised to satisfy themselves that a machine can meet their requirements efficiently. It has been found already that instruments that have not been extensively ‘field tested’ during development may require considerable modification as production models. Manufacturers with confidence in their product might be willing to rent the machine for one year with an option to buy if it proves satisfactory in the users’ laboratory (this has been done for the Auto-Chemist). The Laboratory Equipment and Methods Advisory Group (LEMAC) of the Department of Health and Social Security in Britain will be publishing a schedule for testing automated equipment. There will also be rigorous testing of mechanical and electrical safety and reliability and trials in one or more laboratories.

SUMMARY

The general principles of discrete analysis systems are discussed. Descriptions of discrete analysers have been taken mainly from the manufacturers’ literature and it is stressed that there is little objective information available at present on their performance in the hospital laboratory. The potential advantages and disadvantages of discrete analysis systems have been compared with those of continuous flow systems. Future developments are considered briefly.

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