Rapid Automated Analyses Performed in Parallel

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The analytical concept of monitoring chemical reactions simultaneously in a number of discrete samples is explored. A new class of analyzers based on this concept has been introduced. One such system uses centrifugal force to transfer and mix reagent and sample in a multiple-cuvet rotor. A second, completely new system (CHAMP) is introduced here. In both systems, data signals can be generated at 3.3-ms intervals. Thus, these devices are potentially very fast and can be interfaced directly with a computer, which facilitates their integration into computerized information systems of hospitals.

Clinical procedures can be automated to operate either sequentially or in parallel. That is to say, the same event can take place in one sample after another or in all samples at the same time.

Early attempts to automate analytical procedures were simple exercises in mechanizing the various steps performed by the chemist. The objective, then as now, was to treat standard and unknown samples identically. Consistency of repetitive operations presented the major problem.

Accordingly, the sequential pattern—continuous flow—was the basis of the first successful automation in the laboratory: the "AutoAnalyzer" (Technicon Corp., Tarrytown, N.Y. 10591).

This pattern is also used in several "discrete sample" systems, i.e., systems in which each sample's integrity is maintained throughout the analysis, the sample being in individual glass or plastic reaction vessels.2 The vessels are physically moved past stations where additions, reactions, or measurements occur in sequence.

The sequential pattern necessarily limits the maximum rate of analyses, an inherent shortcoming when various manipulations are performed consecutively on single samples. It was expedient to trade speed for the simplest set of technical problems in the infancy of automation. Time, however, has become an increasingly important factor with increasing workloads and the advent of computerized laboratories.

In the computerized laboratory of tomorrow, many analyses will be done in parallel with all additions, reactions, and other steps occurring in a number of samples at the same time. This batch approach is not new. Analytical chemists have used it in a variety of analyses for many years except for the final measurement, in which the absorbance, for instance, of only one sample is measured at a time.

Recently introduced was a means of measuring a number of samples simultaneously in the quantitation step with a multiple cuvet rotor that spins between a light source and a photodetector (1), opening the way to automating analytical procedures in a parallel pattern. Very fast analyzers based on this concept are commercially available (Figure 1).

A new system can be made that combines both the parallel and sequential modes of operation. Samples are prepared in the conventional sequential mode and measured speedily in the parallel mode. The system, designated CHAMP, is described here for the first time.

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GeMSAEC

A number of papers chronicle development of this type of rapid analyzer [2–3; (Hatcher, D. W., Clinical analyses in parallel: What impact will it have on automation? 5th National Meeting, Ass. Advan. Med. Technol., Boston, Mass., March 23, 1970.)]. It consists of a flat rotor, containing a number of peripheral cuvets, that rotates between a light source and a photodetector (Figure 2). Rapid and repetitive absorbance measurements are made as these cuvets pass continuously through the beam of light. The photodetector generates data signals at about 3-ms intervals, a rate more appropriate for real-time (on-line) data acquisition by a computer.

Interfacing the computer and the fast analyzer makes all of the potentials of electronic computing available to the laboratory. Concentrations of unknowns can be calculated, the quality of the results can be controlled, statistical evaluations can be made, and the results can be presented in the form requested. All of these electronic manipulations are performed during the few seconds required for the analysis. The analyst knows instantly if the results are accurate; fall within the norm for the patient’s sex, age, and race; or if any specimen should be re-examined.

Because of their versatility, parallel-type analyzers can revolutionize the application of (e.g.) kinetic methods, clinical profiles, and pediatric procedures in improving health care.

Kinetic Measurements

The impact in the area of kinetic measurements is best illustrated by considering data requirements. These requirements, although not identical in all enzymatic methods, fall into the general pattern of collection, calculation, presentation, evaluation, and transfer. Sometimes several of these processes are merged into a single step, usually via a strip-chart recorder. Adequate data are collected by this means; however, its biggest disadvantage is that it is too slow.

One seeks to determine the initial rate of the enzymatic reaction in kinetic procedures. Sufficient data for this purpose must be collected rapidly. This is easily done for a number of samples with the GeMSAEC system. It is necessary only to specify the desired time-interval between data points. These data are then reduced in real time.

The strip-chart recorder has been the most popular means of reducing data in the laboratory. Its popularity, however, is a stumbling block in

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Fig. 1. Commercially available parallel-type analyzers


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1 The acronym GeMSAEC, pronounced “gem sack,” is derived from the major sources of support, the National Institute of General Medical Sciences and the United States Atomic Energy Commission.
many attempts to harness the computer to laboratory problems. Once this fact is accepted, new means of data presentation may become acceptable.

A cathode-ray tube (crt), controlled by a computer, is a convenient means of presenting data in real time for evaluation, but it is not necessary to do this, since the computer can be programmed to select linear portions of the data and make the desired computations. The various situations that exist in kinetic methodologies—initial lag periods, departures from linearity, and the like—make one cautious about too much of the “magic box” type of analysis. The crt interface provides confidence that the results are based on adequate reliable data.

After the data are evaluated, they can be transferred (presented) by any of several routes: a simple teletype printout, punch cards, magnetic tape, paper tape, telephone line, or mainframe cables.

Clinical Profiles

Clinical “profiles” have become a fixed part of the laboratory output. A profile of twelve chemical determinations is commonly obtained on hospital admission. Physicians are becoming accustomed to multiphasic profiles as a means of assessing the physiological state of their patients, and they continue to seek new profiles for assessing the efficiency of individual organs. At present, this is the best that can be done until definitive tests are developed for each physiological function.

The clinical laboratory can enter a new and exciting era in clinical chemistry with versatile profiling. Soon each physician will be able to request the specific tests his patient needs and have a choice as to the degree to which any abnormality is investigated automatically. He will be able to decide which tests should be included in a patient’s profile, and which ones should be done repeatedly.

Today, three institutions are interfacing parallel-type analyzers to their computer systems. Recent developments in repetitive sampling of specimens will make these systems the most versatile available. The user will instruct the computer as to what tests are desired and insert the specimen sampler into the rotor. Automatic sample and reagent additions will be controlled by the computer. In effect, the samples will be held constant while the analyzer cycles through any desired combination of determinations. The complete report for each sample can then be compiled, stored, transferred, or reported electronically.

The list of determinations now adaptable to these analyzers already exceeds 30, although it is unlikely that all of these could be made a part of this Automated System within the next few months.

Fig. 2. Principles of operation of the GeMSAEC fast analyzer

Measured volumes of reagents and samples, held unmixed in individual depressions in the fluorocarbon transfer disk, move radially into individual cuvets, which continually pass through a beam of light, generating a series of signals at the photomultiplier. These signals are monitored via the oscilloscope and appropriate electronic circuitry

Pediatric Applications

The small volumes required in the new analyzers can be very important to pediatric patients. Many of the tests that have been adapted require less than 10-μl samples. Complete metabolic screening could become routine for the newborn, and require less than 0.5 ml of serum.

Pediatricians as a rule are not oriented toward clinical chemistry because the available micro-methods are so laborious, although a skilled analyst can provide results of good quality. Hitherto, automated instruments have been avoided because of the large volume of serum needed. Besides using small sample volumes, such analyzers are not expensive, and new methods of molding parts for these parallel analyzers should decrease their cost, ensuring ready availability of cheap laboratory tests.

Utility

The clinical laboratory staff must be oriented toward instrumentation if they are going to meet demands placed on them. Ideally, the performance of instruments in the laboratory should meet requirements that are set by the laboratory personnel. In practice, few instruments do this. The fast analyzers require little daily maintenance and no setup time between different analyses. Ideally, an automatic analyzer should possess the following characteristics:

- versatility
- no or minimum setup time
- speed in data acquisition and reduction
- accuracy and precision
- simplicity in design and operation
- compact with minimum laboratory space requirements
- minimum maintenance
- minimum capital investment and per assay expense
The savings in reagent cost is worth attention. A significant portion of the expense in automated procedures is reagents, especially for most enzymatic methods. The new analyzers are scaled for a total volume of 500 µl. Because of this and because they are discrete samples, reagent requirements are decreased. Even smaller systems are feasible, and the volume required may be decreased 50- to 100-fold in the future.

CHAMP

A new analytic system with great potential is one that incorporates the proven technology of the sequential pattern and the speed of the parallel pattern. The complete system has not been built, but the first prototype of the readout system has been constructed and preliminary feasibility studies made.

The readout system, called CHAMP for Continuous Heterochromatic Absorbance Measuring Probes, is simply constructed. It has a single light source and a single photodetector. A synchronous, dual-shaft motor is mounted centrally. The ends of both shafts are cut 45° from the vertical and polished to a mirror finish. Light from the one source is deflected to five bundles of fiber optics, mounted peripherally, by the mirrored end of the lower shaft as it rotates. The light traverses the fiber bundle of each probe and passes through the liquid. It re-enters the probe and is conducted to the top of the rotating shaft, where it is deflected to the photodetector (Figures 3-5).

Each probe is a separate entity and generates an independent electrical signal at the photodetector. The rapidity and periodicity of the signals from the photodetector depend on the rate at which the motor rotates. These signals occur at 10-ms intervals with a 1200 rpm motor and as few as five probes, which enables real-time computing.

Computerized facilities will find this type of readout system very versatile. The vessel holding the reaction mixture is not important; neither is the volume of the reaction mixture, except in calculating absolute amounts. The individual probes can be calibrated against a designated reference probe and the factors stored and used if required. The probes can easily monitor a number of flow cells, the migration of bands of protein molecules in a number of electrophoresis columns, and the reaction vessels in colorimeters, multi-channel titrators, and spectrophotometers.

Each of these and other applications would require some ingenuity in engineering. The true significance, however, will be in providing the output data in a form and at a rate ideally suited for electronic computations. The output from diverse applications will be remarkably similar and the desired computations need only be specified by code. The similarity in output data will simplify
the software required to put all of the various systems on line and in real time.

An inherent problem limiting the application of high-resolution chromatography, is the slow elution rate. A CHAMP detector that permits simultaneous monitoring of any number of columns by means of flow cells can reduce the time of analysis per sample to a factor equal to the number of columns being monitored. This is significant with elutions that normally require 40 h.

Quantitative gel electrophoresis offers unique possibilities in the clinical laboratory. It can provide a “fingerprint” of the proteins in the patient's body fluids and an isolated, biologically active sample of these proteins. Full exploitation of these possibilities has awaited two developments: A rapid means of monitoring the columns, to avoid denaturation of the proteins during staining procedures, and a rapid, sensitive means of measuring the enzymatic activity of the exceedingly small samples of protein recovered from a gel. Quartz columns mounted peripherally to a CHAMP detector system fulfills the monitoring needs; a fast analyzer can solve the analysis problems.

Round test-tube holders and a CHAMP detector head will provide a unique state of semiautomation in the laboratory. In this way, electronic data-processing capabilities are achieved for small numbers of specimens, emergency specimens, and unusual special analyses that must be run manually. Subroutines of the total software package of the laboratory could monitor the detector head. Coded reaction vessels embossed with machine-readable patient identification numbers could interface this system to the computerized information system, thus eliminating all manual transcription of data and patient-identification numbers, a major source of error in the laboratory.

The CHAMP detection system could also be the basis of any number of analytic instruments. It could be used in a high speed, continuous-flow analyzer. Discrete sample analytical trains can be made simply with fraction-collector type turntables and a CHAMP detector head. Multichannel titrators with individual, constant-rate burets can be designed with this type of detector. More...
sophisticated automated systems can be designed, and several systems now on the market could be upgraded significantly with this means of detection.

A very fast, discrete-sample analyzer could be fabricated with a network of conveyer belts to move the reaction vessels. Pneumatic pumps, proportioning pumps, or mechanically driven syringes could be used to dispense reagent and specimen samples in sequence or in parallel. Machine-readable coding affixed to coded reaction vessels could identify for the computer the specific reaction, the patient, and how the output data is to be processed. This would be a completely random access system in that all pertinent information is gathered at each probe.

The major technical advantages of such a readout system are: The single light source and single photomultiplier tube decrease electronic drifts. The short time intervals between reading (a few milliseconds) and the continuous periodicity of data output expand the general applicability of subroutines, simplify computer interfacing and software development. Machine-recognizable coded reaction vessels can be fabricated without regard to their optical properties. Lastly, the technical advantage of both the sequential and parallel modes are made compatible.

The major negative factor is the limitation of the fiber optics, which can be circumvented. It was expedient to use fiber optics in testing the concept with the prototype.

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References