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AUTOMATED ANALYTICAL TECHNIQUES

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Abstract:
Automation is the performance of operation without human intervention. Automation may involve operations like- Preparation of samples, Measurements of responses, Calculation of results. There are different automatic methods we are adopting now a days like chromatographic systems (GC, HPLC), automatic methods are also used for injecting samples accurately at nanogram and picogram level by using robotic technology. Main objective of automatic methods of analysis is to process large number of samples, determination of several components in the same sample, reduction of human participation, to avoid manual errors, lowering of consumption of sample or reagents. Mainly two different types of automated systems being used at present they are-Discrete analyzer, Continuous flow analyzer. This article mainly deals with the methods of automation their merits, demerits and procedure for operation of these automatic systems and main focus on continuous flow analyzer in which the large number of samples can be analyzed at a time. It can analyze up to 60 samples per hour which makes this instrument ideal and also increases its use in analyzing biological compounds like proteins, peptides and other biomolecules present in blood, plasma, urine. Automated gives rapid, accurate, precise, results without any kind of manual errors. By feature it seems like miniaturize which increases its use. By implementing automated methods one can easily reach the industrial expectation of analysing the sample at high speed, decreasing the manual errors and increase in accuracy of results.

Key words: *automated methods, GC, HPLC, discrete analyser, contioues analyser, robotic technology.*

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INTRODUCTION:

Automation is the performance of operation without human intervention. Automation may involve operations like- preparation of sample, Measurements of response, Calculation of results.

Automated analysis is a process with a view to clearly defining the aspects of technical approach to measuring techniques and human decision making.

NEED FOR AUTOMATION:

1. In all spectroscopic and chromatographic techniques the most crucial and important step is sample preparation because the sample consumption concentration of the system is decreased to microgram, Nano gram and picogram as its sensitivity of detection is increased.

So, to achieve the accurate sample concentration we need the automated methods like robotics.

2. Sequential injection system is one of the automated technique (in HPLC, GC, MS) is established to decrease the time of analysis and also increase sensitivity and accuracy of the results.

3. In research and analytical laboratories whenever the identical tests have to be performed on large number of samples automation of analytical process becomes a necessity.

4. Cost reduction.

OBJECTIVES OF AUTOMATION:

- Facilitate an analytical method or technique.
- Processing a large number of samples.
- Determination of several components in the same sample.
- Reduction of human participation.
- To avoid manual errors.
- Lowering of consumption of sample or reagents.
- Analytes, which are sometimes present in very low concentration in sample.
- Reagents some of which are rare or expensive even unstable can be used by this technique.
- Rapidity
- Economic in a personnel and material expenditure.
- It is closely related to definite and indefinite errors arising from human factors.
- Easy data generation.

Types of automatic systems:

Automatic analytical systems are two types: 1) Discrete analyser

2) Continuous analyser

DISCRETE ANALYSER:

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.

Discrete systems fall into two main groups and are shown diagrammatically in figure.. 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 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 of sample to the following specimen. In most systems the sample (usually from 20 to 200 ,l) is drawn into the tip of the pipette by the movement of a syringe and at the same time a diluent syringe is filled from a reservoir. The pipette tip is then brought above a receiving tube and the sample discharged and washed out with diluent by emptying the sample and diluting syringes. It has been shown that if the diluent-to sample ratio is at least 5:1, then the amount of sample remaining within the pipette tip will usually be less than 1 % (Widdowson, 1968). Thus carryover of sample on the inside of the pipette can be minimized.

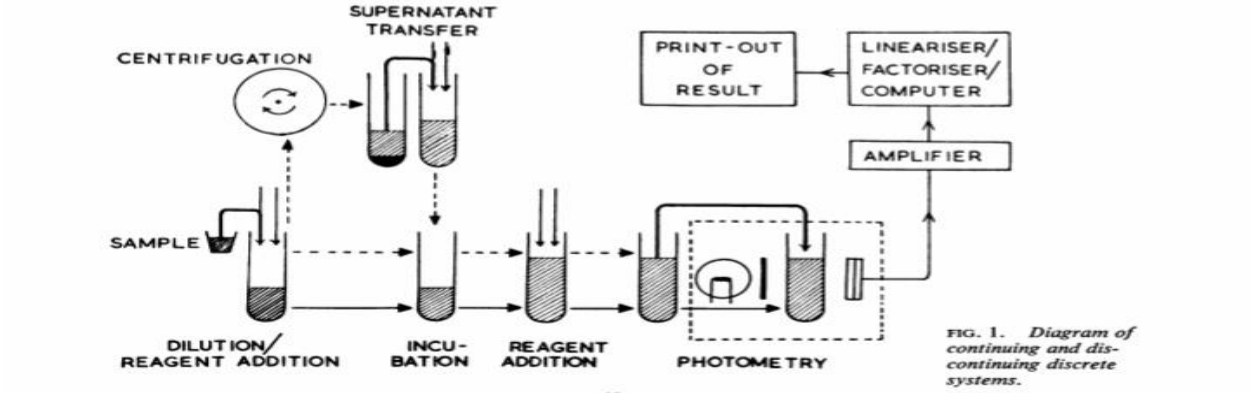

Figure 1: DISCRETE ANALYSER

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.

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, hemolysis, or jaundice.

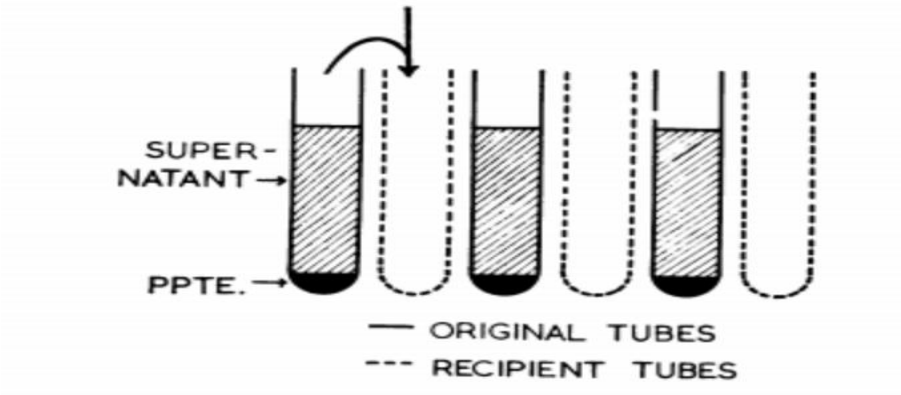

Figure2:PROTEIN SEPARATION

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.

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 . The temperature of the baths can be set between 250 and 750 and can be increased or decreased by 50° in a few minutes by circulating water from a large reservoir.

AUTOCHEMIST:

This is a continuous discrete multichannel analyser , 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.

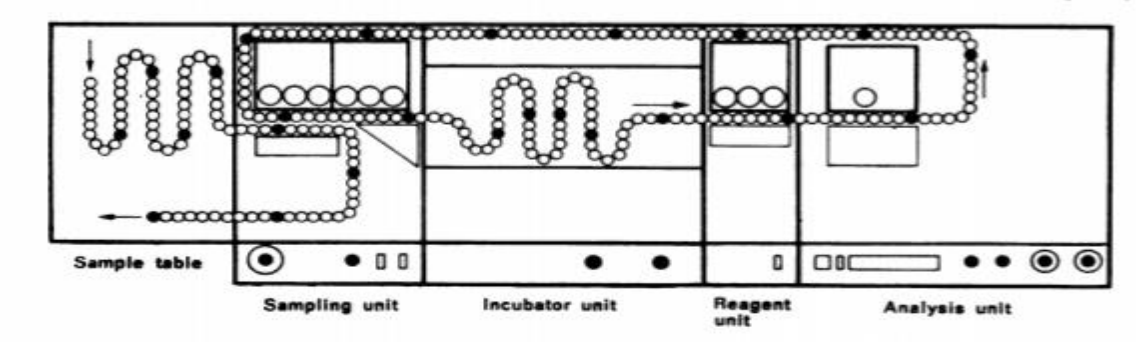

figure3:AUTOCHEMIST

CONTINOUES FLOW ANALYSIS:

CFA is also known as Segmented Flow Analysis (SFA), to outline against FIA technique. Existing ISO standards define: "Methods using flow analysis are automatizing wet chemical procedures and are therefore particularly suitable for processing large sample series.

How works CFA Analysers?

Each sample or calibrant, taken on the auto sampler, is splitted to flow into the different manifolds for simultaneous determination. The aspiration of the next following sample after 60 seconds gives an analysis frequency of 60 samples per hour. A sequence of 90 seconds will result in 40 samples per hour throughput. The aspiration of blank water in between is included in this interval. The number of samples/h multiplied by the number of simultaneous determinations gives the number of analysis per hour on the CFA analyser. The segmentation of the CFA assures best separation between successive samples, also for time consuming preparation steps on the analyser or after long delay for slow reactions. Consequently CFA provides better safety against any memory effect. Today's newcomers in the application of Segmented Flow .

Components:

The modular nature of CFA allows parts to be switched as needed. A general CFA contains the following parts (see figure 1): a sampler which selects the item to be injected into the flow stream; a pump to keep the flow stream in motion; a separation or mixing device; a heat source to speed up reactions; a detector to monitor the concentration of a particular component; and a data handling/presentation device to perform calculations and report results. The sampler is the device that holds the specimen bearing vials .

Today's newcomers in the application of Segmented Flow technique in most cases justify their decision upon the efficiency of integrated sample preparation procedures. The time savings from the omission of manual digestion of organic P or N compounds, of manual distillation for Cyanide, Phenolindex or Fluoride guarantees the profitability of investment. Well experienced users of CFA on the other hand purchase new analyzers to renew their instrumentation after a working life of 15 years or more. The PC based system control and data handling applies specific user-friendly software, comprising over 20 years of experience in CFA data handling, along several operating systems. Consider, that automatically documentation of original analysis data, integrated QM procedures and computerized calculation and reporting results in additional time savings against manual analysis procedures.

Figure4:CONTIOUES FLOW ANALYSER

Technical Data of a CFA Analyser:

• Segmented flow techniques Macroflow or Micro flow.

• Autosampler serial 52/104 positions or XY sampler 240 (300) positions

• Multichannel proportioning pump with 12/48 positions, segmentation by air or N2

• Individual method 'manifolds' for dilution, mixing, incubation, dialysis, distillation, UV digestion, hydrolysis, a.o.)

FLOW INJECTION ANALYSIS:

Flow-injection methods, in their present form, were first described in the mid 70s. Flow-injection methods are and outgrowth of segmented-flow procedures, which were widely used in clinical laboratories in the 1960s and 1970s for automatic routine determination of a variety of species in blood and urine samples for medical diagnostic purposes. The discoverers of flow-injection analysis found, however, that excess dispersion and cross-contamination are nearly completely avoided in a properly designed system without air bubbles and that mixing of samples and reagents could be easily realized. Sample and Reagent Transport System

Ordinarily, the solution in a flow-injection analysis is moved through the system by a peristaltic pump, a device in which a fluid (liquid or gas) is squeezed through plastic tubing by rollers.

Sample Injectors and Detectors:

The injectors and detectors employed in flow-injection analysis are similar in kind and performance requirements to those used in HPLC. For successful analysis, it is vital that the sample solution be infected rapidly as a pulse or plug of liquid; in addition, the injections must not disturb the flow of the carrier stream. Detection in flow-injection procedures has been carried out by atomic absorption and emission instruments, fluorometers, electrochemical systems, refractometers, spectrophotometers, and photometer.

Separations in FIA:

Separations by dialysis, by liquid/liquid extraction, and by gaseous diffusion are readily carried out automatically with flow-injection systems.

Dialysis and Gas Diffusion:

Dialysis is often used continuous-flow methods to separate inorganic ions, such as chloride or sodium or small organic molecules, such as glucose, from high-molecular-weight species such as proteins.

Extraction:

Another common separation technique readily adapted to continuous-flow methods is extraction. It is important to reiterate that none of the separation procedures in FIA methods is ever complete. The lack of completeness is of no consequence, however, because unknowns and standards are treated an identical way.

Dispersion D is defined by the equation

$$D = c_o/c$$

Where c_o is the analyte concentration of the injected sample

c is the peak concentration at the detector.

Dispresion is influenced by three interrelated and controllable variables: sample volume, tube length, and pumping rate.

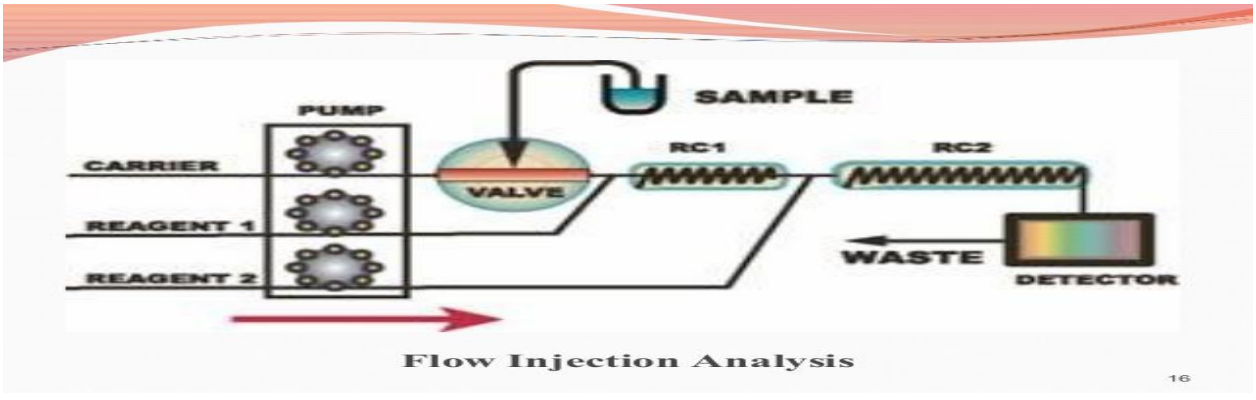

Figure5:FLOW INJECTION ANALYSIS

Applications of Flow-Injection Analysis:

In the flow-injection literature, the terms limited dispersion, medium dispersion, and large dispersion are frequently encountered where they refer to dispersions of 1 to 3, 3 to 10, and greater than 10, respectively.

Limited-Dispersion Applications:
Limited-dispersion flow-injection techniques have found considerable application for high-speed feeding of such detector systems as flame atomic absorption and emission as well as inductively coupled plasma. It is also used with electrochemical detectors such as specific-ion electrodes and voltammetric microelectrodes.

DISCRETE AUTOMATIC SYSTEMS:
A wide variety of discrete automatic systems are offered by numerous instrument manufacturers. Some of these devices are designed to perform one or more.

Automatic Sampling and Sample Definition of Liquids and Gases:
Several dozen automatic devices for sampling liquids and gases are currently available from instrument manufactures. This device consists of a movable probe, which is a syringe needle or a piece of fine plastic tubing supported by an arm that periodically lifts the tip of the needle or tube form the sample container and positions it over a second container in which the analysis is performed.

Robotics:
With solids, sample preparation, definition, and dissolution involves such unit operations as grinding, homogenizing, drying, weighing, igniting, fusing, and treating with solvents.
The robotic system is controlled by a microprocess The robotic system is controlled by a microprocessor that can be instructed to bring samples to the master laboratory station where they can be diluted, filtered, partitioned, ground, centrifuged, extracted, and treated with reagents. The device can also be instructed to heat and shake samples, dispense measured volumes of liquids, inject samples into a chromatographic column, and collect fractions from a column.

ANALYSES BASED UPON MULTILAYER FILMS:
During the past two decades, a technology has been developed for performing the various steps in a quantitative analysis automatically in discrete films arranged in multilayers supported on transparent, disposable The robotic system is controlled by a microprocessor that can be instructed to bring samples to the master laboratory station where they can be diluted, filtered, partitioned, ground, centrifuged, extracted, and treated with reagents. The device can also be instructed to heat and shake samples, dispense measured volumes of liquids, inject samples into a chromatographic column, and collect fractions from a column.

ADVANTAGES:
In the proper context, automated instruments offer a major economic advantage because of their savings in .labor costs.
A second major advantage of automated instruments is their speed, which is frequently significantly greater than that of manual devices.
The third advantage of automation is that a well-designed analyzer can usually produce more reproducible results over a long period of time than can an operator employing a manual instrument.
The reasons for this are machines do not suffer from fatigue, which has been demonstrated to adversely affect the result obtained manually particularly near the end of a working day.
The second reason is the high reproducibility of the timing sequences of automated instruments.

DISADVANTAGES:
Expensive
Design complicated.

CONCULSION:

- Automated methods gives rapid, accurate, precise, results without any kind of manual errors.
- Due to the on going demand for speed in industrial chemical analysis, contioues automation process are begining to replace discrete automation processes, in these areas where high number of assays or samples are expected.
- In the feature, it seems likely that automated systems will be miniaturzied and that this miniaturzied format will lend itself readily to increased use of automation.

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