Kirk-Othmer Encyclopedia of Chemical Technology.

AUTOMATED INSTRUMENTATION, CLINICAL CHEMISTRY

Clinical chemistry, initiated in the 1940s, involves the biochemical testing of body fluids to provide objective information on which to base clinical diagnosis. Between 1946 and 1975 clinical testing had a period of rapid growth in the United States, when the volume of tests increased by about 15% a year. The ever-increasing demand for high quality, routine clinical testing stimulated the development of automated techniques, and as early as the 1960s automation in the clinical laboratory was the rule rather than the exception.

Automation made the tests easier and less costly to perform, resulting in still greater demand. The growth rate, about 10% per year throughout the 1980s and into the 1990s, resulted not from a proliferation of laboratories as much as from increased productivity. Whereas the volume of tests more than quadrupled, the total number of United States hospital and private laboratories was reduced by half from 1975 to 1985 (1). Although growth was initially driven by automation, in the 1990s the growth in U.S. laboratory testing may be attributed to several factors. Among them are the discovery of new diseases and the introduction of new therapies as well as better understanding of body chemistry, and the aging of the U.S. population, which increases the risk of contracting age-related illnesses requiring clinical chemistry analyses (2).

1. Assay Automation

Clinical chemistry analyzers are automated instruments used for measuring concentrations of the various chemical constituents of blood or other body fluids. For a discussion of the related category of instruments used for the measurement of blood cell parameters, see Automated instruments, hematology.

Before the advent of automation, the steps a clinical technologist had to follow in performing a single clinical chemistry assay included: sample preparation, identification, centrifugation, and filtering; reagent preparation; manual metering and addition of sample and reagents into a reaction vessel; mixing and incubation in the reaction vessel; optical measurement of the mixture in a separate cuvette; and calculation and recording of the results. With the exception of the sample and reagent preparation procedure, which is sometimes done separately, these steps are all performed by an automated analyzer such as that shown in Figure 1.

Tubes or cups containing the liquid samples to be analyzed are placed by the operator into an area of the analyzer known as the sampler. The sampler, which in this case is a circular tray, moves each specimen to a position where it can be accessed by the sample aspirating probes. A sampling probe aspirates an amount of sample and dispenses it into a reaction cuvette, which is most often made of clear glass or plastic. Precise metering and transfer of the sample is controlled by the sample aspirating–dispensing system. The chemical reagents necessary for performing an assay on that sample are aspirated from reagent containers and dispensed into the same cuvette by the reagent handling system. Immediately after dispensing the reagents into the cuvette, sample and reagents are thoroughly mixed, eg, by a motor-driven mixing paddle, in order to obtain a uniform reaction rate within each cuvette. The reaction cuvettes, most often arranged at the periphery of a circular reaction tray, are held for several minutes at a constant temperature of 37°C or 30°C in the incubator,

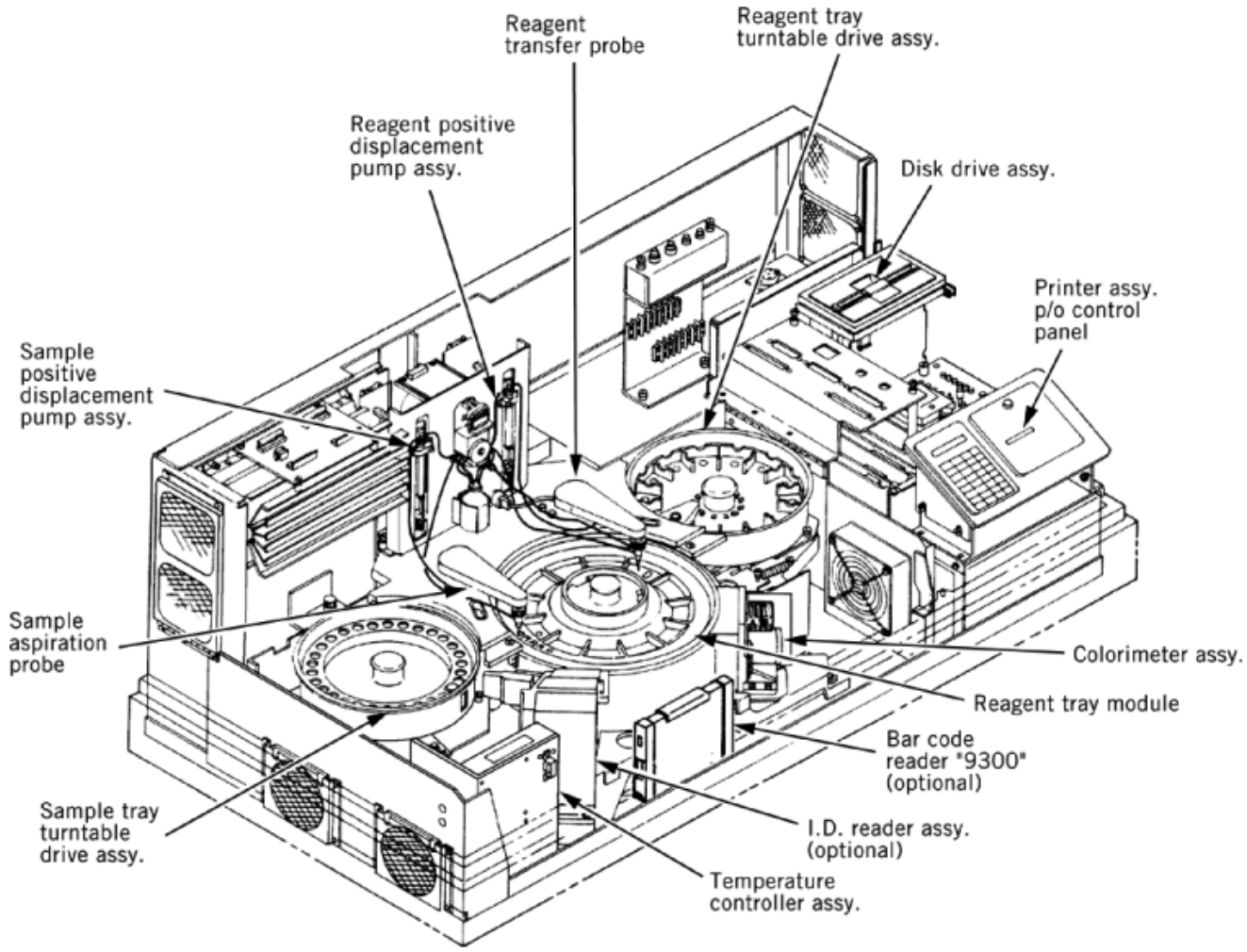

Fig. 1. Internal view of a clinical chemistry analyzer (Technicon RA-XT).(Courtesy of Miles Inc.)

or reaction chamber. During this time chemical reactions in the cuvettes are monitored by an optical detection system. At the end of the incubation period each cuvette is evacuated, washed, and dried at a washing station, to be ready for the next assay. The optical measurement results are converted into metabolite concentration units by the analytical processor computer. Collating the results of different assays into patient reports and storing the test results are usually done by a separate computer, known as results processor or data manager.

In some systems, known as continuous-flow analyzers, the reaction develops as the sample–reagent mixture flows through a conduit held at constant temperature. In such systems, the reaction cuvettes are replaced by optical reading stations called flow cells. In most analyzers, whether of discrete- or continuous-flow type, determination of electrolyte tests, eg, sodium and potassium levels, is done by a separate unit using the technique of ion-selective electrodes (ISE) rather than optical detection.

Automated methods are more reliable and much more precise than the average manual method: dependence on the technique of the individual technologist is eliminated. The relative precision, or repeatability, measured by the consistency of the results of repeated analyses performed on the same sample, ranges between

1% and 5% on automated analyzers. The accuracy of an assay, defined as the closeness of the result or of the mean of replicate measurements to the true or expected value (4), is also of importance in clinical medicine.

2. Technology

An automated system for clinical analysis consists of the instrument (hardware), the reagents, and the experimental conditions (time, temperature, etc) required for each determination. The reagents plus the experimental conditions are sometimes referred to as the chemistry of the system. The chemistry employed is generally similar to that used in manual assays because most automated assay methods have been adapted from the manual ones. However, automated analyzers rarely afford the flexibility of experimental procedure that is possible in manual analysis.

Table 1 lists several of the chemical determinations and the corresponding reactions utilized, which are available on automated clinical analyzers. With the exception of assays for various electrolytes, eg, Na^+, K^+, Cl^-, and CO_2, determination is normally done by photometric means at wavelengths in the ultraviolet and visible regions. Other means of assay include fluorescence, radioisotopic assay, electrochemistry, etc. However, such detection methods are normally required only for the more difficult assays, particularly those of serum or urine constituents at concentrations below μg/L. These latter assays are discussed more fully in the literature (3, 4).

Essential features of an automated method are the specificity, ie, the assay should be free from interference by other serum or urine constituents, and the sensitivity, ie, the detector response for typical sample concentration of the species measured should be large enough compared to the noise level to ensure assay precision. Also important are the speed, ie, the reaction should occur within a convenient time interval (for fast analysis rates), and adequate range, the result for most samples should fall within the allowable range of the assay.

The assay methods listed in Table 1 for the various biochemical species can be classified according to reaction rate behavior, eg, end point vs kinetic methods, blanking schemes, or reaction principle and type of reagents employed.

2.1. End Point vs Kinetic Methods

Samples may be assayed for enzymes, ie, biocatalysts, and for other substances, all of which are referred to as substrates. The assay reactions for substrates and enzymes differ in that substrates themselves are converted into some detectable product, whereas enzymes are detected indirectly through their conversion of a starting reagent A into a product B. The corresponding reaction curves, or plots of detector response vs time, differ for these two reaction systems, as shown in Figure 2. Figure 2**a** illustrates a typical substrate reaction curve; Figure 2**b** shows a typical enzyme reaction curve (see Enzyme applications).

In substrate assays, the reaction product is measured at time t_1 (Fig. 2**a**) when the reaction is complete. The concentration of the species to be assayed is usually proportional to the net detector response at t_1, and generally there is no problem in relating this measurement to substrate concentration. These so-called end point assays correspond to a constant value and are less subject to errors occurring as a result of variations in experimental conditions (temperature, reagent concentrations, etc) than measurements made under rapidly changing conditions. In some cases, t_1 can be as long as 5–10 min, which would severely limit maximum analysis rates if such assays were performed individually. Automated analyzers address this problem in various ways. For example, most analyzers allow a series of samples to pass through the system one after the other. In this way each sample may spend up to 5–10 min within the system, but if n samples are in the system at any given time, the net analysis rate, expressed in samples per minute, is n divided by 5–10. Alternatively, all samples might be loaded into the system for parallel processing in the same 5–10 min. Continuous-flow analyzers are an

Table 1. Clinical Tests Offered on Automated Analyzers[a]

Assay	Reaction
	Chemical
albumin	albumin + bromcresol green (BCG) $\longrightarrow$ albumin BCG[b]
calcium	calcium + cresolphthalein complexone $\longrightarrow$ complex[b]
chloride	$2Cl^- + Hg(SCN)_2 \longrightarrow HgCl_2 + 2\ SCN^-$ $3\ SCN^- + Fe^{3+} \longrightarrow Fe(SCN)_3$[b]
creatinine	creatinine + sodium picrate $\longrightarrow$ colored product[b]
iron	iron + Ferene$-$S $\longrightarrow$ colored complex
total bilirubin	bilirubin + sulfanilic acid + NO_2^- $\longrightarrow$ azobilirubin[b]
total protein	protein + $CuSO_4 \xrightarrow{OH^-}$ cuproproteinate complex
	Biochemical
alanine aminotransferase (ALT)	1$-$alanine + oxoglutarate $\xrightarrow{ALT}$ pyruvate + 1$-$glutamate pyruvate + NADH $\xrightarrow[\text{dehydrogenase}]{\text{lactate}}$ lactate + NAD
alkaline phosphatase	$p-$nitrophenyl monophosphate $\xrightarrow[\text{phosphatase}]{\text{alkaline}}$ $p-$nitrophenol[b] $+PO_4^{3-}$
aspartate transaminase (AST)	glutamate + $\alpha-$ketoglutarate $\xrightarrow{\text{aspartate transaminase}}$ oxaloacetate oxaloacetate + NADH $\xrightarrow[\text{dehydrogenase}]{\text{malate}}$ malate + NAD
carbon dioxide	phosphoenolpyruvate + HCO_3^- $\xrightarrow[\text{phosphoenolpyruvate carboxylase}]{}$ oxalacetate + PO_3^{3-} oxalacetate + NADH $\xrightarrow[\text{oxidase}]{\text{cholesterol}}$ malate + NAD
cholesterol	cholesterol esters $\xrightarrow[\text{kinase}]{\text{creatine}}$ free cholesterol + fatty acids free cholesterol + O_2 $\xrightarrow[\text{oxidase}]{\text{cholesterol}}$ cholest$-$4$-$en$-$3$-$one + H_2O_2 H_2O_2 + phenol + 4$-$aminoantipyrine $\xrightarrow{\text{peroxidase}}$ quinoneimine dye
creatine kinase (CK)	creatine phosphate + ADP $\xrightarrow[\text{kinase}]{\text{creatine}}$ creatine + ATP ATP + glucose $\xrightarrow{\text{hexokinase}}$ ADP + glucose$-$6$-$phosphate NAD + glucose$-$6$-$phosphate $\xrightarrow[\text{dehydrogenase}]{\text{glucose}-6-\text{phosphate}}$ NADH + 6$-$phosphogluconate
gamma glutamyl transferase (GGT)	glutamyl$-p-$nitroanalide + glycylglycine $\xrightarrow{GGT}$ glutamylglycylglycine + $p-$nitroaniline
glucose	ATP + glucose $\xrightarrow{\text{hexokinase}}$ ADP + glucose$-$6$-$phosphate glucose$-$6$-$phosphate + NAD $\xrightarrow[\text{dehydrogenase}]{\text{glucose}-6-\text{phosphate}}$ 6$-$phosphogluconate + NADH
lactate dehydrogenase (LD)	lactate + NAD $\xrightarrow[\text{dehydrogenase}]{\text{lactate}}$ pyruvate[b] NADH
triglycerides	triglycerides $\xrightarrow{\text{lipase}}$ glycerol + fatty acids glycerol + ATP $\xrightarrow[\text{kinase}]{\text{glycerol}}$ glycerol$-$1$-$phosphate + ADP ADP + phosphenolpyruvate $\xrightarrow[\text{kinase}]{\text{pyruvate}}$ ATP + pyruvate pyruvate + NADH[b] $\xrightarrow[\text{dehydrogenase}]{\text{lactate}}$ lactate + NAD
urea nitrogen	urea + H_2O $\xrightarrow{\text{urease}}$ 2 NH_3 + CO_2 NH_3 + NADH + oxoglutarate $\xrightarrow[\text{dehydrogenase}]{\text{glutamate}}$ NAD + glutamate

[a] Coenzymes such as adenosine diphosphate (ADP), adenosine 5′-triphosphate (ATP), nicotinamide adenine dinucleotide (NAD), and nicotinamide adenine dinucleotide, reduced (NADH), are involved in some reactions (4).
[b] Chromophore, optical detection used.

example of the first scheme, whereas cuvette-based analyzers may work in the latter manner. A more important constraint in end point assays by automated analyzers is the need in some analytical reactions for multiple addition of different reagents at different times. Cuvette-based analyzers, for example, do not normally allow for more than two reagent additions.

For enzyme determinations, kinetic assay procedures are generally favored. These refer to the determination of the slope of the reaction curve. However, not all enzyme reaction curves are as simple as that shown in Figure 2**b.** Some reactions show a so-called lag phase, or reduced reaction rate, at the beginning of the reaction, as shown in Figure 2**c.** Other reactions level off quickly, much before the average time t_4, shown in Figure 2**b.** As a result, many clinical chemists prefer enzyme assays that are based on monitoring the reaction curve over a wide range in time so that the linear zero-order region can be readily identified and the slope calculated in this region. However, this method of monitoring represents a serious constraint on many analyzers; ie, if there is one detector, then the maximum analysis rate is $1/t_4$. Because t_4 is commonly 5–10 min, this would

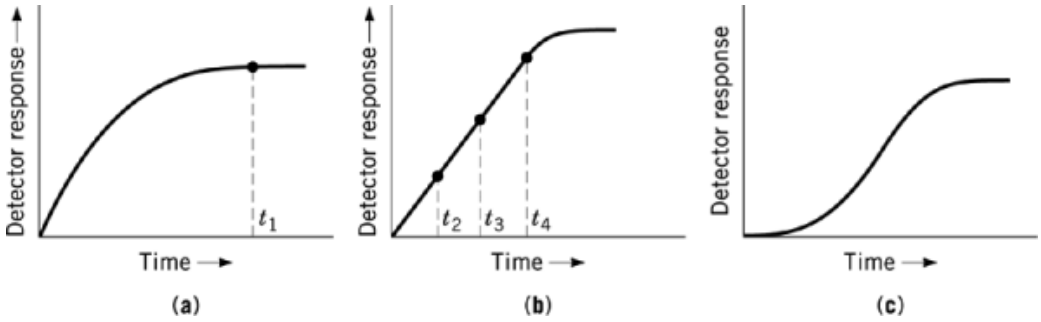

Fig. 2. Assay reaction curves for (**a**) substrates, (**b**) enzymes, and (**c**) enzymes exhibiting a lag phase or reduced reaction rate; where t_x is measurement time.

limit maximum analysis rates to only 6–12 samples per hour, which would be much too slow for large clinical laboratories.

Early instruments ignored the assay time problem, made a single measurement at some time, as in end point analysis, and calculated an assumed slope dividing the net detector response by the time after initiation of the reaction. For many assays, this was satisfactory. However, frequent interferences were encountered with these single-point procedures, as use of so-called 340 nm chemistries increased for various enzymes. The 340 nm chemistry is based on the production of the coenzyme reduced nicotinamide adenine dinucleotide (NADH), which absorbs light at a wavelength of 340 nm. One solution is the use of parallel blank reactions, where one of the reaction reagents is missing. Subtraction of the resulting blank reaction slope from the total reaction slope gives the desired enzyme reaction curve. However, this approach greatly increases the cost of both equipment and reagents. More importantly, it does not allow for detection of reagent depletion, where high enzyme concentrations cause a bending of the reaction curve in a very short time, as shown in Figure 2**b** after t_4. Newer cuvette-based analyzers, such as that shown in Figure 1, use multiple detector readings or multiple detectors. In these analyzers, a batch of samples for the same assay is rotated through a common detector station so that multiple points along the reaction curve are collected for each sample. This multiplexing of the detector results in an overall analysis rate of n/t_4, where n is the number of samples in the batch. Thus these analyzers achieve high throughput rates, including a fairly complete definition of the total reaction curve, plus reliable determination of the zero-order slopes for all samples.

2.2. Blanking Schemes

Whereas most clinical assays rely on the specificity of the assay reaction to avoid interferences that would require a blank correction, certain ubiquitous interferences, eg, lipemic turbidity, hemoglobin in hemolyzed sera, bilirubin [635-65-4], $C_{33}H_{36}N_4O_6$, in icteric specimens, present special problems in many otherwise specific assays. Sample interferences can be classified as follows: (*1*) fixed absorbance in the absence of reagents, eg, turbidity; (*2*) fixed absorbances as a result of reaction with reagents, eg, free-glycerol interference in assays for triglycerides; and (*3*) changing absorbance as the result of slow reaction with reagents.

Each interference type may require a different elimination strategy, the simplest of which is to remove the potential interference before beginning the assay. Removal is commonly done in manual analysis, usually by deproteinization, eg, by addition of some denaturing agent such as trichloroacetic acid. This technique also tends to remove other large molecules such as bilirubin and hemoglobin, as well as eliminating sample turbidity. However, such procedures are not easily adapted to automated analysis, and in fact are generally not used. Modern assay methods generally employ mild reaction conditions which not only do not cause sample

protein precipitation; these methods, because they frequently use highly specific enzymes as reagents, also have significantly reduced interference.

In cases where sample blank correction is required, a variety of techniques are employed. For interferences of type 1, a common technique is to use a separate determination in which an essential reagent for the primary reaction is eliminated; eg, the sodium nitrite, $NaNO_2$, in the usual reaction for bilirubin. The blank, or interference, response in the detector mimics that in the assay reaction, but the assay reaction itself does not occur. Subtraction of the blank from the assay result on a given sample then provides a correction for interferences of type 1. For interferences of type 2, a blank is run using the reagent that reacts with the interfering species. A modification of this technique uses two reagents. The sample is added to the first reagent, and the blank is measured. No assay reaction occurs. The second reagent, added later, contains the active component which produces the measurable assay response. A subtraction of the two measurements cancels out the unwanted contribution of the blank reaction to the desired response.

Separate sample blanking requires an additional analytical channel, and is therefore wasteful of both reagents and hardware. An alternative approach that is used on several automated systems, eg, Du Pont ACA, BM-Hitachi 704, Technicon RA-1000, is that of bichromatic analysis (5) where absorbance measurements are taken at two, rather than one, wavelength. When the spectral curves for the interference material and the chromogen of the species measured differ sufficiently, this can be an effective technique for reducing blank contributions to assay error. Bichromatic analysis is effective for blanks of both the first and second type.

Blanks of the third type are the most difficult to handle. Examples are found in the usual Jaffe reaction for creatinine, and in certain enzyme reactions. In the creatinine [57-00-1], $C_4H_9N_3O_2$, assay, if potential interferences are not removed by dialysis or some similar procedure, they exhibit both slower and faster reactions compared to those of creatinine. A two-point analysis can be utilized here with the measurement times selected to be later than the time required for fast-reacting interferences and before the time for significant reaction of slow-reacting interferences. This procedure is utilized in the creatinine assay on many automated chemistry analyzers.

2.3. Reactions and Reagents

The three general categories of reactions are chemical, biochemical, and immunological. Automated analyzers mainly utilize the assays listed in Table 1, which fall into one of the first two categories; trace-level assays are carried out by immunological methods (see Immunoassay). Chemical methods are based on reactions that do not normally take place in living systems, eg, the assay for calcium by the cresolphthalein complexone reaction. These assays are often relatively nonspecific, and may require removal of sample interferences or the use of blanking techniques. Chemical methods can be used by most types of automated clinical analyzers. Biochemical methods rely on reactions that play a role in the functioning of living systems; eg, the assay of glucose by the coupled enzyme system hexokinase plus glucose-6-phosphate dehydrogenase. Biochemical assays are often highly specific because the corresponding biochemical reactions in the body are specific. Automatic analyzers utilizing biochemical methods predominate in the marketplace because of greater specificity and reduction in cost through increased use.

Immunological methods suffer from many disadvantages, and automation has been attained only in the 1980s and 1990s. Immunological reactions utilize antibodies as reagents, and are often competitive binding procedures. Radioimmunoassay was the first technique employed for immunological methods. The use of nephelometric, turbidimetric, and fluorometric means of detection have gained ground; however, use of radioimmunoassay is decreasing in part because of the problems of radioactive waste disposal.

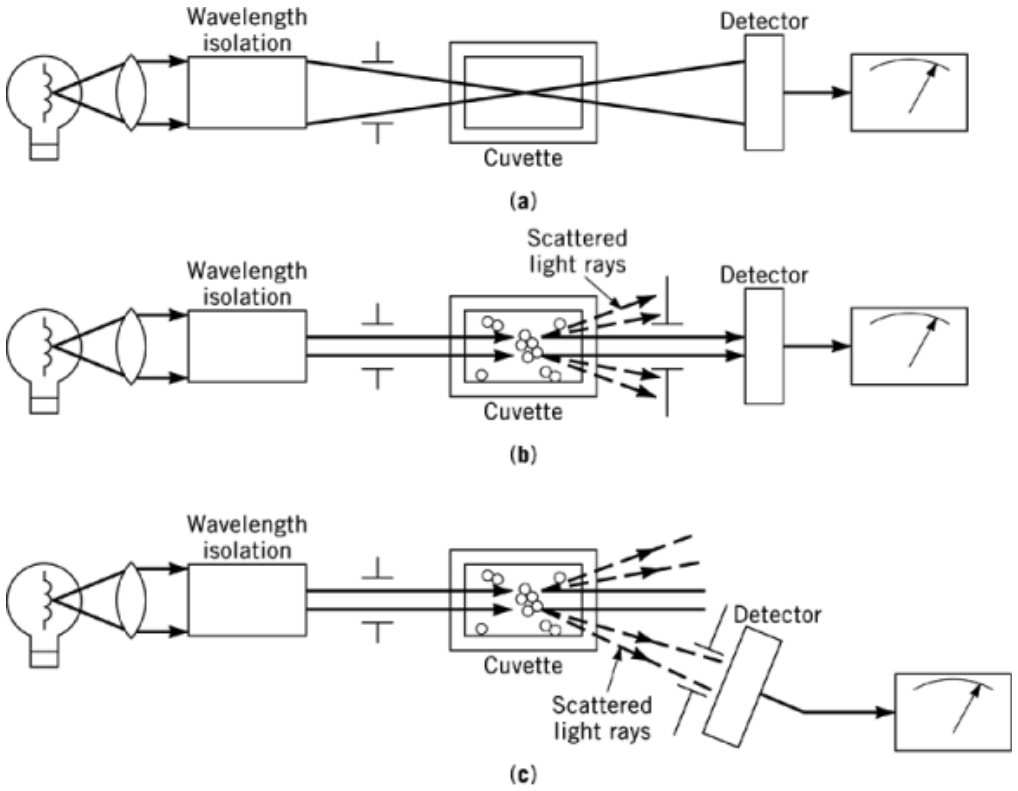

Fig. 3. Schematic of optical detection methods, (**a**) absorbance or colorimetric; (**b**) turbidimetric; and (**c**) nephelometric.

3. Measurement Methods

The majority of the various analyte measurements made in automated clinical chemistry analyzers involve optical techniques such as absorbance, reflectance, luminescence, and turbidimetric and nephelometric detection means. Some of these are illustrated in Figure 3. The measurement of electrolytes such as sodium and potassium have generally been accomplished by flame photometry or ion-selective electrode sensors (qv). However, the development of chromogenic ionophores permits these measurements to be done by absorbance photometry also.

3.1. Absorbance

Analyte measurements in clinical analyzers using liquid reagents are most commonly performed by transmission of light, ie, by absorbance photometry or colorimetry (Fig. 3**a**). The liquid to be analyzed is either held in a cuvette or passed through a flowcell having transparent walls. A light beam traverses the liquid compartment and then falls on an optical detector (see Photo detectors). Prior to striking the detector, a narrow wavelength band of radiant power centered at the wavelength of peak absorbance, the analytical wavelength, is isolated. Wavelength isolation, shown in Figure 3 as occurring before the light impinges upon the sample, may take place after the sample. In dilute solutions, analyte concentrations are proportional to the measured absorbance at the analytical wavelength in accordance with the Beer-Lambert law: $T = P/P_o = 10^{-\epsilon cl}$ where T is the relative transmittance, P_o is the beam power for a nul concentration sample, and P is the power for the sample being

measured, c is concentration in moles per liter (M), l is the path length in centimeters of the absorber, and ϵ is the molar extinction coefficient having units $M^{-1}\ \mathrm{cm}^{-1}$. The absorbance A is defined as $-\log_{10}T$, thus $A = \epsilon cl$ where l the path length, represents the length of the light path through the liquid chromophore in the flowcell or cuvette.

3.2. Reflectance

In dry chemistry analyzers, the diffuse reflectance of the chromophore is measured in the supporting solid matrix. The output response is approximated by the Kubelka-Munk function in which the diffuse reflectance of the sample depends only on the ratio of the absorption and scattering coefficients and not on their absolute values. The scattering coefficient is generally considered to be constant for a given matrix. Typically the liquid sample is deposited on one side of the reagent-impregnated strip or slide, and the optical measurement is made on the opposite side. In making diffuse reflectance measurements, it is important to minimize the specular component of reflection from erroneously affecting the reading.

3.3. Turbidimetry and Nephelometry

In contrast to classical absorbance methods, immunoassay reactions frequently involve agglutination in which the optical scatter signal of the agglutinated particles is measured by turbidimetric or nephelometric means. The principles of light scattering as it relates to analytical methods is discussed in reference 6.

Turbidimetry is considered a transmission type of measurement in which the detector is optically in line with the light source (Fig. 3**b**). Particles in the sample scatter the incoming beam away from the in-line detector, and consequently cause a loss in light power falling on the detector. This decrease in light intensity is measured and converted to optical density units relative to a solution without particles. In the absence of any absorbing species in the sample, the turbidity is proportional to the concentration of the particles, the scattering cross section, and the optical pathlength of the cuvette. The scattering cross section is a function of the relative particle size to wavelength ratio and to the refractive indexes of the particle and surrounding medium.

In nephelometry the detector is located at an angle to the light path and to the cuvette centerline. Essentially, only scattered light is collected by the detector (Fig. 3**c**). Although the detector signal level is considerably lower than in turbidimetric measurements, the signal is measured against an essentially zero background rather than a high signal level as in the turbidimetric technique. This results in an increase in the ratio of the maximum to minimum signals and generally is advantageous, providing the signal level is adequate, eg, where the electronic or shot noise is not significant, for the precision required.

In both turbidimetric and nephelometric measurements used for agglutination reactions, the relationship of the concentration of the analyte to the measured optical density or scatter signal is a complex one. Various optical and physical factors, such as the rate of formation of two- or three-particle agglutinates generated from the monomers, ie, unagglutinated particles, their orientation, and degree of multiple scattering, affect the signal to concentration relationship. Obviously the chemistry of the immunologic reaction is paramount to determining the kinetics and sensitivity of the reaction.

3.4. Fluorescence

The fluorescence detection technique is often used in clinical chemistry analyzers for analyte concentrations that are too low for the simpler absorbance method to be applied. Fluorescence measurements can be categorized into steady-state and dynamic techniques. Included in the former are the conventional simultaneous excitation-emission method and fluorescence polarization.

In time-resolved fluorescence, rare earths are frequently used as fluorescent labels. The fluorophores have large Stokes shifts, ie, shifts of the emitted light to a higher wavelength relative to the absorption

wavelength, and comparatively long decay times, approximately 0.5 ms. This simplifies the optical detection and minimizes serum background effects, as the background fluorescence has essentially decayed to zero when the longer lifetime signal is being read out. The more research-oriented instruments, based on fluorescence lifetime measurements, utilize the more sophisticated impulse–time decay and the frequency and phase domain techniques.

The vast majority of detectors, used in absorbance and fluorescence measurements, operate in the analogue mode, in which the average of a large number of time-overlapped electron pulses constitute the measured current. In this technique the amplified electron pulses, generated initially by the photons striking the photocathode, are counted individually as one single count per photon, irrespective of their amplitudes. For very low radiant power measurements, such as may be found in some fluorescence applications, a photon counting method may be used advantageously.

3.5. Ion Selective Electrodes Technique

Ion selective (ISE) methods, based on a direct potentiometric technique (7) (see Electroanalytical techniques), are routinely used in clinical chemistry to measure pH, sodium, potassium, carbon dioxide, calcium, lithium, and chloride levels in biological fluids.

In potentiometry, the potential difference in volts is measured between two electrodes, one a reference electrode having a stable potential under controlled conditions, and the other a working or measuring electrode. In the working ISE, an ion-selective membrane is used to separate two solutions of different ionic strength. As the sample containing the analyte, eg, sodium ions, to be measured flows past the ISE, changes in electrical potential take place between the ionically constant inner surface, saturated KCl solution, and the ionically variable outer surface, the sample. These changes in electrical potential are automatically measured against those of the reference electrode. The potential difference (voltage) between the two electrodes is related to the activity of the ions in solutions, and the activity is proportional to the logarithm of the respective analyte concentration of the sample, according to the Nernst (8) equation. The analogue signal generated is digitized and then converted to reportable concentration units by the instrument's computer processing unit (CPU).

4. Classification of Clinical Chemistry Analyzers

Automated clinical analyzers can be divided into discrete and continuous-flow systems. In discrete systems, the sample–reagent mixtures from different specimens are kept in individual reaction cuvettes during incubation and optical reading. A notable exception in this category are the dry chemistry analyzers, where the cuvettes were replaced by individual slides coated with dry reagent films. In continuous-flow analyzers, the sample–reagent mixtures form liquid segments flowing through a tube. Adjacent segments are separated by air bubbles. Optical readings are taken at different locations along the tube, which is kept at the incubation temperature.

Clinical analyzers can also be classified according to their degree of flexibility. Most of the modern systems are random access analyzers, for which the tests on various specimens are performed in any order programmed by the operator. Some analyzers operate in batch or profile mode, ie, they perform the same test or group of tests on every sample until the system is reset for another test or group of tests.

The throughput range, number of assays performed per hour, of clinical analyzers reflects the diversity of the market. Systems that perform less than 400 tests per hour are usually referred to as small analyzers; medium analyzers cover the range from about 500 tests per hour to 1,500 tests per hour, while high throughput analyzers can process up to 10,000 tests per hour. Table 2 lists a number of representative automated systems and their characteristics. Whereas small analyzers, eg, the Ames CLINISTAT, can easily fit on a desktop, high throughput analyzers, eg, the Technicon DAX-96, can occupy a good portion of a laboratory.

Table 2. Features of Automated Analyzers

Manufacturer and system	Throughput per hour: Samples	Throughput per hour: Tests	Number of resident methods	Amount of sample needed per test, μL	Incubator temperature, °C	Optical system[a]	Distinctive features
Roche COBAS MIRA	variable	125	30	2–95	37	AMW,P	disposable cuvettes
Technicon RA-1000	variable	240	12	2–30	30, 37	AMW,P	no probe wash
Ames CLINISTAT	up to 80	up to 80		10	37	R	dry chemistry
Abbott SPECTRUM	variable	400–600	23	1.25–25	25, 30, 37	AMW,P,T	polychromatic sample blanking
Beckman SYNCHRON CX3	up to 75	600	3 8	122/8 tests	37	AMW,P	STAT analyzer[b] Beckman liquid calibrators and controls
Kodak EKTACHEM 700	300 max	600	26	10, 11	37	R	dry chemistry slides, routine/STAT[b]
Baxter PARAMAX	240	720	32	2–50	37	AMW,P	closed container sampling option; unit dose dry tablet reagent
BM Hitachi 737	300	1,200	23	3–20	30, 37	AMW,P	
Technicon CHEM 1	variable	1,800	35	1	30, 37	AMW,P	continuous-flow capsule chemistry
BM Hitachi 736-50	300	8,100	27	10	30, 37	AMW,P	
Technicon DAX-96	300	10,200	34	3–67	37	AMW,P	

[a] AMW = absorption, multiple wavelength per test; N = nephelometry; T = turbidimetry; P = potential; and R = reflectance.
[b] A specimen that has to be processed with priority vs other specimens is known as STAT sample.

5. Functional Elements of Clinical Chemistry Analyzers

5.1. Sample Handling System

Venous or capillary blood, urine, and cerebrospinal fluid are specimens routinely used in medical diagnostic testing. Of these biological fluids, the use of venous blood is by far the most prevalent. Collection devices such as syringes and partial vacuum test tubes, eg, Vacutainer, are used to draw ten milliliters or less of venous blood. At collection time, the test tubes are carefully labeled for later identification.

The specimen, as drawn, contains cells, platelets, fibrin, and particulates. For most chemistry tests, eg, determination of glucose, cholesterol, etc, it is necessary to first separate the cellular blood fraction which, if present during the assay, would interfere with the determination and adversely affect the accuracy of the measurement. The cellular components are separated by centrifugation. The centrifuge is generally not part of the automated analyzer. If anticoagulants are added to the sample, the resultant supernatant layer is called plasma; if not, the sample must be allowed to stand for approximately ten minutes before centrifugation to allow completion of the clotting process. In the latter case, the supernatant is referred to as serum (see Fractionation, blood).

After centrifugation, the specimens are placed in a holder specifically designed to work with a particular instrument. Some clinical analyzers utilize sample carrier blocks; others use a rotary tray or a continuous flexible chain link. In some cases, an aliquot of the sample has to be poured into a secondary container, small cup, or tube which is then labeled and placed on the sample holder. Direct or primary tube sampling is preferable

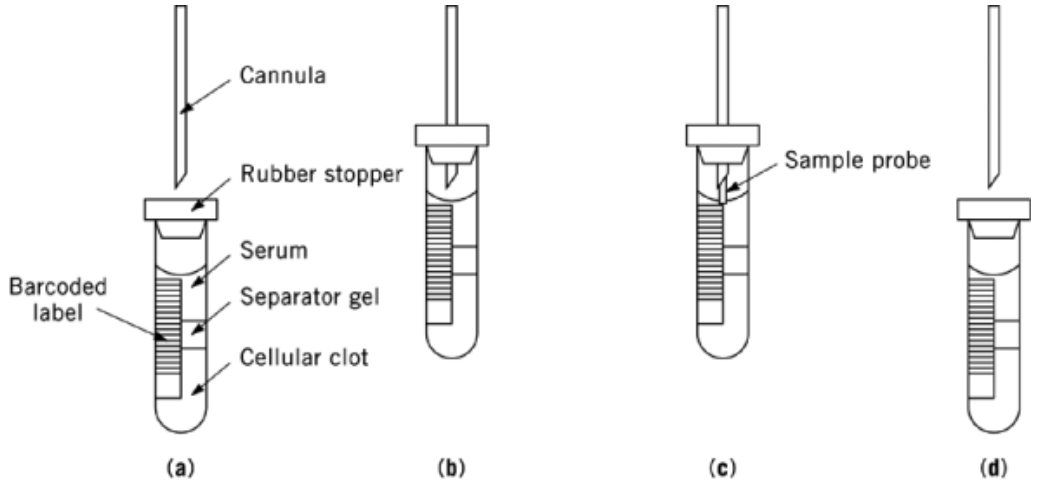

Fig. 4. Schematic of the Closed Container Sampling technique used in the Baxter PARAMAX analyzer showing (**a**) the collection tube with bar-coded label being brought into sampling position under the cannula; (**b**) the tube raised so that the cannula has penetrated the stopper; (**c**) the sample sensing probe coming through the cannula to aspirate the exact volume required for each assay; and (**d**) after sampling, where the tube is lowered away from the cannula. The stopper removes any sample from the cannula and reseals itself.

because it involves less sample handling and less risk of loss of patient identification. A trend in primary tube sampling is the introduction of closed tube sampling, which allows the proper amount of serum or plasma to be aspirated directly from the primary collection tube without having to first remove the stopper. One such technique is described schematically in Figure 4. The obvious advantage of such techniques is the significant reduction of risks associated with sample handling such as operator exposure to hepatitis B and HIV viruses.

Once loaded, the sample holder is placed onto the carrier transport mechanism of the system, which is referred to as the sampler. The function of the sampler is to bring each specimen into a position where a sample of it can be taken by the aspiration probe, then to transport it further to the unloading area. Most analyzers allow continuous feeding, or loading, and unloading of samples. Priority sampling, which allows the sampler to temporarily interrupt sampling of routine specimens in order to aspirate from emergency, or STAT, specimens, is also a common feature. The analyzer has to keep track of sample identity. Some systems use optical detection devices to read a bar-coded label on each test tube or sample cup before aspirating from it. This approach is known as positive sample identification. In other systems, the sample holders, eg, carrier blocks, are assigned unique numbers that can be read by an optical device, then the specimens are identified by their position on the carrier block.

When a test result for a particular specimen is found to have an elevated, out of method range value, some analyzers, eg, Beckman CX3, can automatically repeat the sampling from the same specimen. For elevated concentrations, the precision of the optical system is reduced so an automatic dilution of the sample, eg, by aspiration of a reduced amount of sample, is provided during the second sampling.

5.2. Sample Aspiration and Dispensing

Clinical chemistry analyzers perform tests mostly on blood serum or plasma, and less often on other body fluids. Precisely metered amounts of the sample have to be aspirated rapidly and without allowing intersample contamination, known as sample carry-over. Typical sample volume aspirated per test ranges between 1 μL

and 25 μL. Generally, pipetting is accomplished by motor-driven syringes connected through a fluid line to a thin aspiration probe (Fig. 1).

In most analyzers, sample carry-over is minimized by one or more of the following techniques: sensing the liquid level in the sample container, and limiting the probe immersion in the liquid to a few millimeters; or rinsing the aspiration probe inside and out after each immersion. Probe rinsing has been eliminated in a few analyzers by the use of an aspiration probe employing disposable tips. After aspiration of all the aliquots needed from a particular specimen, the tip is discarded, and a new one is loaded for the next sample. Yet another approach to carry-over prevention is where the aspirating probe is coated inside and out with a thin film of a chemically inert fluorocarbon oil. During immersion this coating prevents the sample from wetting the probe wall while allowing aspiration to take place.

5.3. Reagent Handling System

Many analyzers can be programmed to perform a wide variety of assays. However, reagents for only a limited number of tests, usually referred to as resident tests, are available on the instrument at any one time. The main reason for this limitation is that, for infrequently requested tests, the time period until reagent depletion may exceed the chemical stability time limit. Refrigerated compartments for the reagent containers, usually kept between about 4°C to 15°C, are generally provided on the medium to high throughput systems in order to extend the stability of the resident reagents. For most analyzers using liquid reagents, the reagent stability on the system ranges between 2 days to 30 days. In some systems, such as the Baxter Paramax, reagents are stored in tablet form and reconstituted as needed, thus extending on-board reagent stability to 60 days.

Precise metering of the amount of liquid reagent needed for a test is generally done using a motor-driven syringe. Other variants, such as peristaltic pumps or pneumatic syringes, are also employed. The amount of reagent used per test ranges between 0.1 mL and 0.7 mL. In some analyzers, a dedicated conduit links each reagent container to a dispensing nozzle above the cuvette. Other instruments use a single reagent probe to aspirate the reagents from the reagent containers and dispense them into the reaction cuvette. If the latter approach is used, reagent cross-contamination by the probe has to be avoided. This is usually achieved by a thorough rinsing of the probe after each reagent dispense. Reagent-to-reagent carry-over typically has to be kept to less than 1 part per 20,000. Coating the reagent probe with an inert liquid eliminates the need for the rinsing operation.

Two notable technological developments related to the reagent handling system occurred in the last 1980s. One of these was the introduction of the dry slide reagent technology where the reagents needed for a particular assay are deposited in thin layers on a slide (Fig. 5). The reaction starts when a drop of sample, deposited on the upper slide surface, dissolves the reagents. A top coating layer, covering the dry reagents, helps the uniform spread of the sample drop and filters out interfering substances. The dye formed in the reaction is measured by reflectance spectrophotometry from the lower side of the slide, the light being reflected by the top coating layer. The dry slide technology eliminates the preparation and handling of liquid reagents, as well as the need to meter the amount of reagents for each test. A slide cartridge is provided for each resident test. After opening the cartridge, the reagents are stable for up to seven days.

Another unique development, which significantly reduced the amount of reagent necessary for an assay, is an extension of the continuous-flow analyzer technology called capsule chemistry. The core of the system is a capillary Teflon tube coated on the inside with a thin, flowing film of fluorocarbon oil. Sample and liquid reagents, alternating with air bubbles, are aspirated into the tube forming a segmented flow. Each liquid segment surrounded by the film of immiscible fluorocarbon oil, forms a discrete capsule which does not contaminate the neighboring test capsules. Because of the small size of the capillary tube, the amount of liquid reagents per test is reduced to 14 μL, and the amount of sample to 1 μL per test. The small reagent volumes used allow on-board storage of reagent sufficient for up to 1200 tests for any of the resident methods. The reaction takes

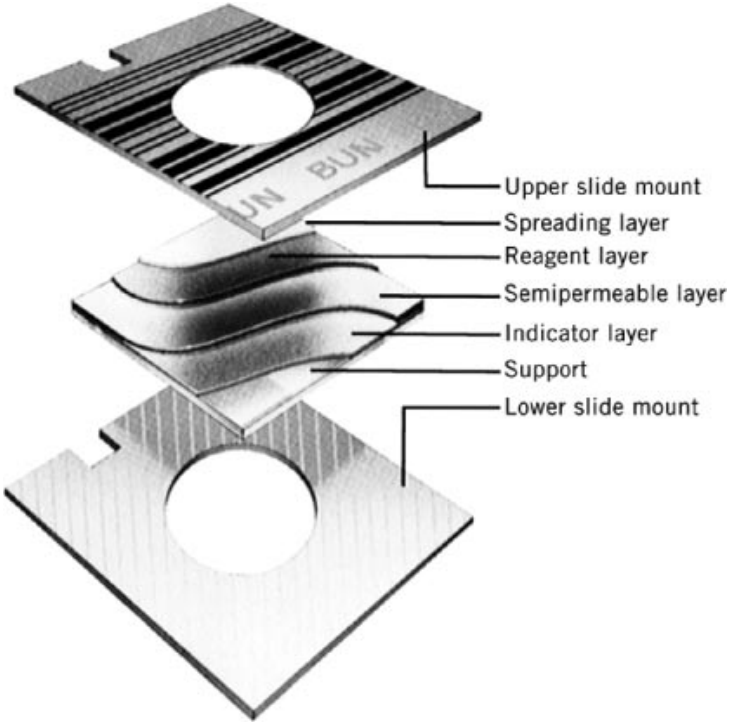

Fig. 5. Schematic of a dry reagent slide used in Kodak EKTACHEM analyzers. Courtesy of Kodak Instruments.

place while the liquid capsules are flowing through the capillary tube, and the photometric reading is taken through the tube wall, eliminating the need for reaction cuvettes (Fig. 6).

5.4. Optical Detection System

The optical detection system includes dedicated or time-multiplexed arrangements of the various elements such as the light source, cuvette or flowcell, wavelength isolation elements, and detector. A common layout may have a single light source with light distribution via fiber optics to many cuvettes or flowcell read stations. On the output side of the reaction cuvette or flowcell, fiber optics pipe the light signals to an optical chopper, through dedicated interference filters, and to a common multiplexed photomultiplier. A variation of this arrangement uses a rotating mirror device to multiplex the light output of many cuvettes into the input of a single grating-array photodiode.

5.4.1. Light Sources

The majority of clinical chemistry methodologies result in analyte reactant products which are analyzed using either ultraviolet or visible light. Light sources include the tungsten halogen lamp, lasers, light-emitting diodes, and arc lamps such as deuterium, xenon, and xenon-mercury of the continuous or the flash lamp type. The tungsten lamp generally has greater short- and long-term stability than the arc lamps, and consequently is the preferred source for wavelengths above 340 nm. Lasers (qv) and light-emitting diodes, which operate at a single wavelength, with the exception of tunable dye lasers, are special-purpose sources used in single wavelength applications (see Light generation).

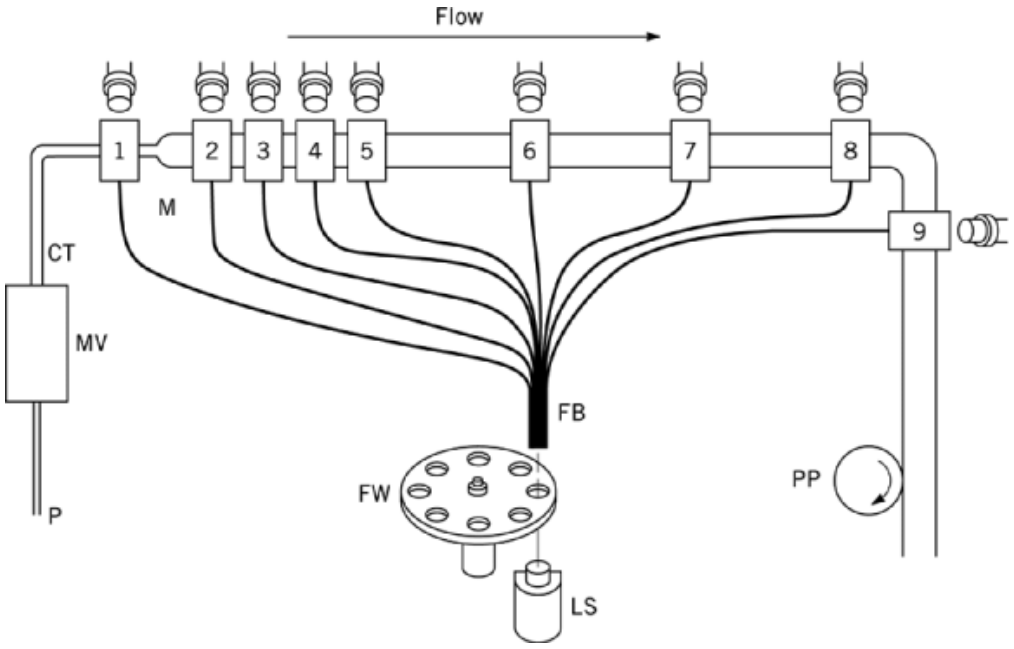

Fig. 6. Schematic of Technicon CHEM I analyzer where P=aspiration; MV=metering valves; CT=capillary Teflon tube; M=reagent mixing zone; FW=filter wheel; FB=fiber optic bundle; LS=light source; PP=peristaltic pump. The colorimetric reading stations are marked 1 through 9.

5.4.2. Reaction Cuvette/Flowcell

This critical element is often unique to the system, and differs to the greatest extent among various instruments. Wet chemical analyzers typically use a cuvette or flowcell to contain the reactant to be measured. The volume of this fluid may range from as little as 15 μL in flowcell analyzers to several hundred microliters in cuvette-based instruments. In absorbance measuring instruments, the light path lengths generally range from 1 to 10 mm. Because the path length of the cell affects the measured absorbance directly, the choice of this design parameter is carefully considered with regard to the system volume constraints.

5.4.3. Wavelength Isolation

There are several means of isolating the analytical wavelength needed for the measurement of the analyte. One of the most common methods is the use of a multilayer interference filter illustrated in Figure 7. When coupled with suitable blocking filters, this optical element has an extremely good rejection ratio of the out-of-band to in-band power, minimizing the stray light effects which can contribute to nonlinearity. The half-bandwidth of the filter, spectral bandwidth at half the peak transmission, is the fundamental filter specification, and this value should be small relative to the chromophore bandwidth. Bandwidths range from 2 to 20 nm. Where a large number of wavelengths are required for the system, a multiwavelength dispersive device such as a prism or grating is more suitable.

Prisms and gratings have historically been used for spectral band isolation in spectrophotometers. These optical elements provide a wide range of wavelengths and have very narrow bandwidth characteristics when coupled with suitable entrance and exit slits. The mechanically scanned prisms or gratings provide sequential wavelength information, whereas the more recent grating/multielement array detector combinations produce almost simultaneous (in the millisecond range) information. Acousto-optical tunable filters are newer devices

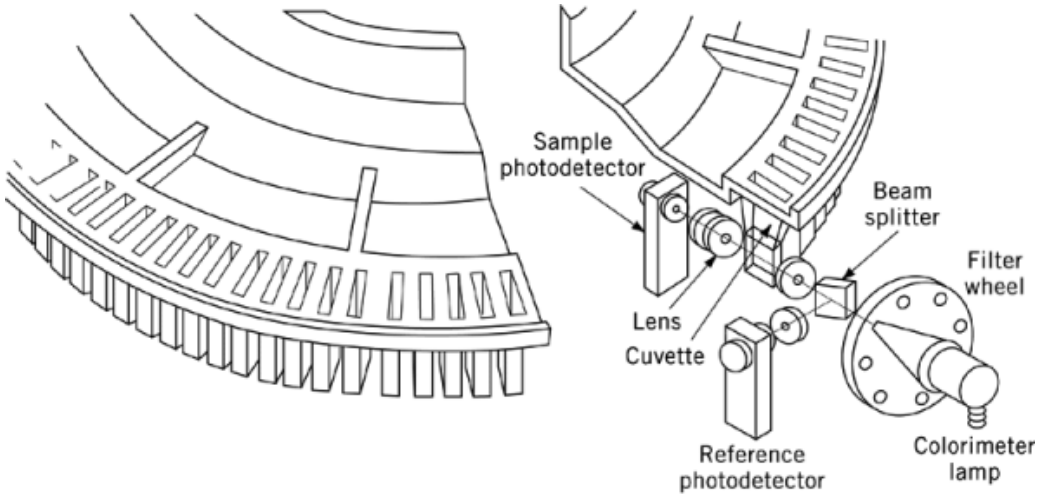

Fig. 7. Schematic of a reaction tray and colorimetric system.

which are used to isolate narrow spectral bands. They can operate at scanning speeds at least 100 times faster than the mechanically scanned devices.

5.4.4. Detectors

The solid-state silicon photodiode has become ubiquitous in clinical instruments and is the detector of choice for the near ultraviolet–visible spectral range. Among the detector's attributes are small size, high quantum efficiency (especially in the visible–near infrared region), low noise equivalent power, wide linear range, excellent response times, and simplicity of interfacing to the data acquisition hardware. Silicon photodiodes are available in single-element packages and in multielement arrays.

Another class of detectors consists of the vacuum phototube devices. Included in this group is the photomultiplier detector, which has high gain but requires a high voltage power supply. It has an advantage over the silicon detector in that it has extremely low noise, and thus is useful in applications requiring extremely low level light detection and/or extremely fast response times. Additionally, the ultraviolet quantum efficiency of multialkali photocathodes is generally superior to that of the silicon photodiode.

The photoconductive detector is primarily used in the visible-infrared region rather than the ultraviolet–visible range.

Silicon charge coupled devices (CCDs), commonly used in solid-state video cameras and in research applications, are being applied to low light level spectroscopy applications. The main advantage of area array CCDs over linear photodiode detectors is the two-dimensional format, which provides simultaneous measurements of spatial and spectral data.

5.5. Computer System

The brain of the modern clinical chemistry analyzer is its computer system. The part of the computer system that controls the functional aspects of the analyzer is known as the process control computer or analytical processor (AP); the test results are handled by the data management computer, also known as the results processor (RP).

The analytical processor stores the sample identification information, and, checking against the worklist input by the operator, determines which tests should be performed on the current sample. The AP controls the

aspiration of the right amount and type of reagents, dispenses the reagents together with the sample in the reaction cuvette, and collects the raw data from the detection system. Some tasks, eg, temperature control and motion profiles, are actually delegated to dedicated microprocessors reporting to the AP. These microprocessors are often referred to as embedded control processors. The analytical processor also monitors the status of various sensorsin the analyzer and warns the operator of abnormal conditions by displaying messages on the computer monitor, or by triggering audible alarms. Other functions controlled by the AP include start-up and shut-down procedures, calibration procedures, and, in some analyzers, self-diagnosis of failure modes.

The results processor computes the test results from the raw data furnished by the AP and collates these results together with the demographic patient data into test reports. Test results falling outside normal limits are flagged on the report to speed up the diagnosis process. These data managers can also store thousands of patient reports in their current memory. Some of the more sophisticated systems also store the actual reaction curves used to determine the test results.

Whereas the analytical unit's form and function is somewhat determined by a particular technology, the results processor's approach may be influenced by a larger system strategy. The RP might be part of a single analyzer, a stand alone entity, or it could act as a single results processor for two or more analytical units. In another configuration, the RP could act as an interface between one or more analytical units or between one or more analytical units and a larger, more centralized computer system.

6. Economic Aspects

It is estimated that the worldwide clinical chemistry diagnostics market is about $3 billion. This amount includes an estimated $700 million in instrument sales, and $2.3 billion in sales of reagents and consumables. Some of the principal instrument manufacturers are Hitachi (Japan), Miles Laboratories/Technicon Instruments (United States), E. I. du Pont de Nemours (United States), Beckman Instruments (United States), Eastman Kodak (United States), Abbott Laboratories (United States), Olympus (Japan), Toshiba (Japan), Hoffmann-La Roche (Switzerland), and Ciba Corning Diagnostics (United States).

The proliferation of automated analyzers in the 1980s enticed an increased number of manufacturers to enter the market, increasing competition and lowering selling prices. In 1991 list prices for automated clinical chemistry analyzers ranged from $30,000 to $80,000 for small (120–400 tests per hour) analyzers, $100,000 to $175,000 for medium (500–1500 tests per hour) throughput, and $200,000 to $350,000 for very high throughput systems capable of processing up to 10,000 tests per hour.

Automated clinical analyzers became affordable, not only to small hospital laboratories, but to doctors' group practices and to individual doctor's offices as well.

BIBLIOGRAPHY

Cited Publications

1. *Report #7076*, Biomedical Business International, 1987.
2. R. Gambino, *14th International Congress of Clinical Chemistry*, San Francisco, Calif., July 22–26, 1990.
3. N. W. Tietz, ed., *Textbook of Clinical Chemistry*, W. B. Saunders Co., Philadelphia, Pa., 1986.
4. L. J. Kaplan and A. J. Pesce, *Clinical Chemistry, Theory, Analysis, and Correlation*, The C. V. Mosby Co., 1989.
5. G. Kessler and L. R. Snyder, *Technicon International Congress*, Vol. **1**, Mediad Press, Tarrytown, N. Y., 1977, 28–35.

6. I. M. Kolthoff, P. J. Elving, and E. J. Meehan, *Optical Methods of Analysis*, Vol. **8**, *Treatise on Analytical Chemistry*, Part I, John Wiley & Sons, Inc. New York, 1986.
7. G. Eisenman and co-workers, *Science* **126**, 831 (1957).
8. G. Eisenman, *Glass Electrodes for Hydrogen and Other Cations, Principles and Practices*, Marcel Dekker, Inc., New York, 1967, p. 2.

PAUL GHERSON
HOWARD LANZA
MILTON PELAVIN
DAN VLASTELICA
Miles Inc.

Related Articles

Automated instrumentation, hematology; Enzyme applications; Electroanalytical techniques