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Chapter

Advancement in Analytical and Bioanalytical Techniques as a Boon to Medical Sciences

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Abstract

The most important objectives frequently found in analytical and bioanalytical chemistry involve advancement of analytical techniques and its application to relevant medical/clinical problems. Keeping in view to these aspects, the present chapter is primarily focused on the development of advanced analytical techniques applied in the medical field. For example, N-acetyl-beta-D-glucosaminidase (NAG) enzyme is a specific biomarker of acute kidney injury. A biomarker is an entity that is purposely measured and estimated as an indicator of normal biological process, pathogenic process, or pharmacological responses to a therapeutic intervention. Hence, successive measurements of urinary NAG may enhance its clinical use as an indicator of ongoing tubular injury. Hence, in order to obtain information for selective monitoring of biomarker, the development of a practical and valid analytical method is important. Experimentation is driven by the need to know more about the medical effects and safety features of the biologically active analyte. It is therefore more important to evaluate the information that is already available for that particular analyte and to quantify the level of uncertainty for the proposed technique.

Keywords: analytical, bioanalytical, biomarker, clinical, medical, N-acetyl-beta-D-glucosaminidase

1. Introduction

This chapter represents a collective attempt to present a wide range of analytical techniques applied for the clinical development process. Analytical chemistry is concerned with the chemical characterization of matter and refining the qualitative and quantitative problem about that matter. It plays a vital role in almost all the aspects of scientific research and development, for example, clinical, forensic, environmental, and pharmaceutical sciences. In medicine, analytical chemistry is the key for clinical laboratory tests which imparts basis of disease diagnosis and chart progress for recovery to the physicians [1]. **Figure 1** describes the scheme through which physicians ruled out or analyze the disease prognosis and therapeutic drug monitoring in patients. In accord with this, an analytical chemist also explores the idea of developing advanced technique for betterment of human healthcare and in sorting out the problems related to the disease diagnosis. Implementation of an analytical technique mainly depends on the varying degree of selectivity, sensitivity, accuracy, precision, cost, and rapidity of that particular technique. The techniques

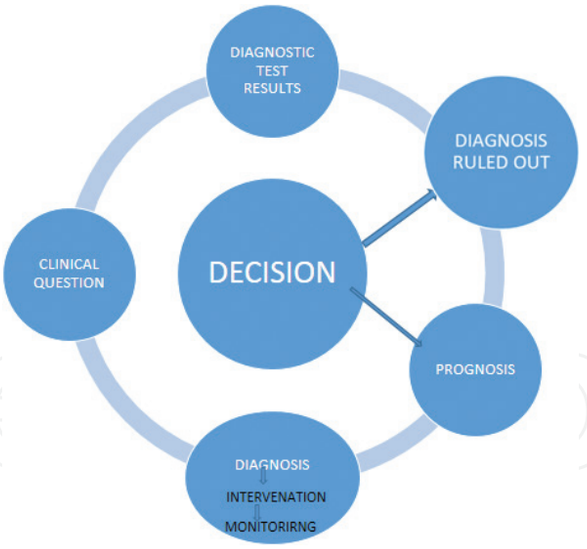

Figure 1.
Schematic representation of four common decision making steps in which the result of an investigation is involved.

employed may be based either on physical property or chemical property of an analyte. An analyte is defined as a constituent which has to be determined in a given sample type. The classical analytical techniques include gravimetric, volumetric, and titrimetric methods; on the other hand, instrumental techniques involve ultraviolet-visible (UV-Vis), infrared (IR), and near-infrared (NIR) spectrophotometry fluorimetry, atomic spectroscopy (absorption/emission), electroanalytical chromatography, and radioimmunoassay. Instrumental techniques are usually more sensitive and selective than classical techniques but are less precise. Precision of techniques means the repeatability of a result and is expressed as standard deviation. Selectivity of an analytical method defines the measurement of a particular analyte from sample solution to a certain degree, in the presence of other analytes, without any interference. However, sensitivity of a method describes the ability to recognize two different concentrations.

However, medical and clinical analyses are undergoing the greatest extension of instrumental methods [2]. Interest in identifying biologically active compounds is growing rapidly and providing new challenges for the analytical chemists. These challenges have been resolved by the introduction of bioanalytical technique as a modern approach to disease diagnosis and therapy. A bioanalytical method is a combination of different procedures which are (i) collection, (ii) processing, (iii) storage, and (iv) analysis of a biological sample (blood-cerebrospinal fluid (CSF), serum, plasma, or urine, tissue, and skin). This method is also useful for quantitative determination of drugs and metabolites in biological samples. For that reason, technologies used to perform bioanalytical methods vary according to the analyte's nature. Hence, to find out the appropriate technologies involved in a bioanalytical method for the purpose of quantification of an analyte, the method validation is important. This procedure is termed as bioanalytical method validation (BMV). Few techniques commonly applied in bioanalytical studies include hyphenated (combination of two techniques) techniques like liquid chromatography (LC), gas chromatography (GC), capillary electrophoresis (CE) coupled with mass spectrometry (MS), and advanced automated chromatographic techniques, for example, high-performance liquid chromatography (HPLC) [3].

During the past decades, the analytes that have been targeted in bioanalytical studies include amino acid, peptides, proteins, serum enzyme, tumor and cancer genes, carbohydrates, vitamins, catecholamines, cardiac risk factors, etc. [4].

With the recurrent analysis of biomolecules, numerous analytical techniques and instrumentation have been evolved and applied in the field of medical sciences which are as follows:

- Sensors
- Electrophoresis
- Chromatography
- Mass spectrometry
- Optical techniques (microscopy)
- Radio- and immunochemical techniques
- Hyphenated techniques
- Point-of-care instrumentation

Biological samples have the potential to deliver important biomarkers in the clinic due to accessibility of these biological materials. In clinical development, the most important benefit offered by biomarkers is to limit investigational drugs to critical care patients who would gain the therapies to observe the effectiveness of those drugs [5]. The role of a biomarker is to give information about the biological mechanism involved within a disease or treatment of disease having the capability to correlate with the clinical findings. One of the most tangible problems that research scientists are facing in recent years is finding disease biomarkers that are translated well from animal or computer simulation to humans. For example, increase in enzyme activity in computer or animal model may have a significant impact in theoretical computer or animal model, whereas same enzyme activity enhancement may have a very limited or no clinical impact.

There is no denying that "analytical and bioanalytical technique" is a broad topic, incorporating technologies from classical chromatography to point-of-care instrumentation. But unifying and doing them as quickly, accurately, and inexpensively as possible are drives to make chemical or biochemical measurements. Over the preceding sections, we would study the technological improvements along those lines across the broad field of analytical methods. Every subsection of analytical techniques applied in medical field has experienced improvement and advancement as well.

Researchers are interested in mapping the neural connectivity of the brain through scanning electron microscopy (SEM). This could be now employed with more powerful microscopes, such as focused ion beam and multi-beam SEM, to collect serial images of ultrathin brain slices [6]. They can now build surface plasmon resonance substrates out of silver rather than the more typical gold and an SPR microscope to image and quantify 1296 binding events in parallel [7]. Those scientists who are interested in surface properties can now scan those surfaces faster than ever, thanks to high-speed atomic force microscopy [8].

2. Sample collection and storage

We have to keep in mind that biological samples, collected from the patients, must be transported to the initial assessment center as soon as possible.

The type of preservatives should be known to protect the samples from degradation prior to cryopreservation at a reasonable cost. Cryopreservation is a process to store biological samples at very low temperature for prevention of damage. The purpose is to find readily accessible and data-rich biological samples. The stability of a wide range of bioanalytes and cells as a component of whole blood should be estimated, taking into account different anticoagulant (inhibition of coagulation of blood) media, at different temperatures and under varying transport conditions. Bioanalytes can be known biochemicals, such as DNA, defined proteins, and specific metabolites, or unknown analytes, such as the constituent plasma/serum proteome and metabonome [9].

Design and testing of the sample handling protocol considered as key factors that affect the stability of biological samples, including anticoagulants, stabilizing agents, and temperature, elapsed time from collection to initial processing and endogenous degrading properties (enzymes, cell death). We also aim for cost-efficiency by avoiding collecting multiple sources of material for the same analyte. The samples undergo minimal processing locally in the assessment centers before being shipped to the main laboratory for processing with the aim of cryopreservation within 24 h of collection. Samples are protected against degradation during shipping by being chilled at 4°C (only peripheral blood lymphocytes, at 18°C). Once the samples get processed in the laboratory, they are placed in cabinets maintained at −80°C for the working archive or in nitrogen vapor at −180°C or below for the backup archive.

3. Sample preparation

Biological samples involve plasma, serum, CSF, bile, urine, tissue homogenates, saliva, seminal fluid, and frequently whole blood. Quantitative analysis of drugs

Sample preparation techniques	Advantages
Liquid phase extraction (LLE)	LLE is one of the first methods used for extraction. It depends on the partitioning of analytes between two immiscible liquids. The resulting extract may be directly analyzed or further purified and concentrated by subsequent LLE and evaporations.
Solid phase extraction (SPE)	SPE is a method for the isolation and concentration of selected analytes from a fluid sample by their transfer on a solid phase. The analytes are recovered by elution or thermal desorption. This method has high recovery, uses less organic solvent than LLE.
Affinity separation: molecularly imprinted polymers (MIPs)/ antibodies	The affinity sorbent may consist of an immobilized antibody or a molecularly imprinted polymer. This technique is highly specific and very sensitive, but the sorbent is difficult to prepare; it suffers from cross-reactivity and leaking of template.
Solid phase micro extraction (SPME)	SPME, a solvent free extraction method, consists of a single extraction step, but the experimental variables must be well controlled. It reduces solvent and sample volume needs and sample preparation time. Improves detection limits i.e., parts per trillion level detection.
Ultrafiltration and microdialysis (MD)	Ultrafiltration consists of filtering the sample through a special size-excluding filter, either by applying pressure (10–100 psi) or by centrifugation. The method is widely used, simple, efficient, but suffers from ligand binding to the filter and shift of equilibrium. Dialysis and MD can be used to separate an analyte by diffusion through a semi-permeable membrane.

Table 1.
Sample preparation techniques used for biological samples [32].

and metabolites containing huge amounts of proteins and large numbers of endogenous compounds within these samples is very complicated. Direct injection of drug containing biological sample into a chromatographic column results in the precipitation or absorption of proteins on the column packing material, resulting in an immediate loss of column performance. A number of advances have made to convert sample preparation techniques, used for the cleanup of drugs in biological samples into formats that are acceptable for high-volume processing with or without automation. The most widely used cleanup methods for separation of biomolecules from biological samples are summarized in **Table 1**.

Because of this, sample preparation became a prominent step in the analysis of biological samples. In recent years, the necessity of new developed method is largely required. Frequently, it was earlier considered as a separate procedure prior to the analysis, while it nowadays has become a more or less integrated part of the analytical procedure. It is necessary to lay the foundation of their development on a systematic and scientific approach. Thus, fundamental understanding of the different processes involved in a sample preparation method is served as a basis for its optimization [10].

We should select the appropriate sample preparation method on the basis of requirements of the assay and time allowed to run sample preparation method.

4. Spectrophotometry

The spectrophotometric technique is used to study interactions between electromagnetic radiations and analyte (**Figure 2**). The concentration of an analyte is determined by using a graph which is called standard analytical curve. An example is determination of iron in blood serum. The iron content of blood serum is determined after deprotonation (by precipitating protein) with trichloroacetic acid and reduction with hydroxyl ammonium sulfate. Iron (II) ions are reacted in the medium buffered with ammonium acetate and with diphenyl-1,10-phenanthroline-disulfonic acid disodium salt (bathophenanthroline disulfonate–Na), and the absorbance of the complex formed is measured at 535 nanometer. The concentration belonging to the absorbance data of the test solution is read from the standard analytical curve and multiplied by three for threefold dilution [11].

4.1 Optical spectroscopic techniques of human models

Optical spectroscopy for biomedical applications covers up the plethora of medical technological and fundamental research areas. This includes screening and early detection of diseases which remain clinically silent over long periods. It is a noninvasive, fast spectroscopic technique. The technique is also capable of observations from femtosecond time scale at nanometer spatial resolution, so it can be applied in all areas of life sciences. This technique is to make an early, noninvasive, and patient-specific diagnosis near the source of the disease and then to treat the

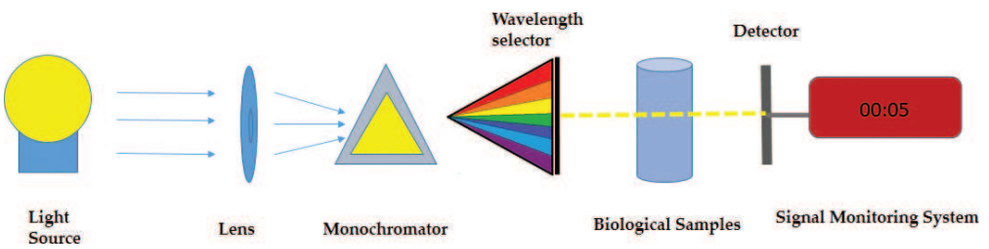

Figure 2.
Schematic representation of biological sample determination using spectrophotometric technique.

disease at primary stage, for example, Alzheimer's disease and coronary disease. Thus, optics offers a wide variety of diagnostic methods and products of biomedical spectroscopy [12].

4.2 Absorptions and reflectance spectroscopy

This method involves investigations of brain dysfunction and mental health problems like depression, epilepsy, and Alzheimer's disease [13]. The direct absorption like near-infrared (NIR) techniques and instrumentation are particularly suitable in routine neonatal care applications.

However, diffused reflectance spectroscopy, in the UV-Vis-NIR region, can be used for biomedical applications, like studies on skin condition (vitiligo, psoriasis, skin cancer) and glucose concentration measurement [14].

4.3 Photoacoustic spectroscopy (PAS)

This includes measurement of concentration of biomolecular species. Examples are glucose determination, characterization of tissue status (biopsy tissue), and imaging application. PAS can provide information on three-dimensional distribution of specific molecular species in a specimen, by appropriate choice of excitation wavelength [15].

4.4 Raman and infrared spectroscopy

The "fingerprint" molecular specific technique will be of great advantage in understanding the biochemical interactions involved in induction, progression, therapeutic invention, and regression. This technique is suitable for biomedical applications such as breath analysis, drug-cell interaction, microscopy, and imaging of biopsy sample (tissue, fine needle aspiration) [16–18].

4.5 Fluorescence spectroscopy

Depending on excitation wavelength, the fluorescence peak has been observed in blood and urine spectra. A few examples are spectra observed from epithelial tissues, proteins, NAH, FAD, and hemoglobin [19].

4.6 Mass spectrometry

Mass spectroscopy (MS) measures masses within the sample. In mass spectroscopy, chemical species get ionized and ions get sorted on the basis of their mass-to-charge ratio (**Figure 3**). Major application of MS includes confirmation of immunoassay-positive drug screens, identification of inborn errors of metabolism, and analysis of steroid hormones [20]. Conclusive identification of molecules that range in size from tens of daltons (small molecules) to hundreds of thousands of daltons (biomolecules) is based on different principles.

Discovery of highly sensitive polymerase chain reaction (PCR) was a major step forward in the biomedical research and diagnostics. This technique is used for analysis of small quantities of short sequences of DNA and RNA without cloning. PCR can detect the presence of pathogens earlier than the culture tests. The miniaturization of MS systems allows a transportable device that minimizes the need of highly skilled operators and allows for rapid and accurate MS analysis in a point-of-care format (near the physician's clinic) [21].

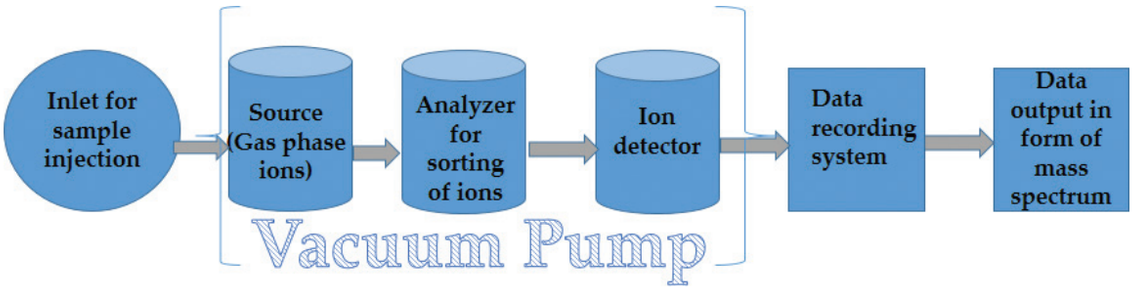

Figure 3.
Schematic representation of mass spectrometry detection of sample.

4.7 Imaging techniques

Advances in medical imaging present a great opportunity in drug development. A number of different imaging technologies are available. These include computed tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron-emission tomography (PET), and single photon emission computed tomography (SPECT). If adequately qualified, imaging biomarkers can be very helpful in the early stages of clinical development [22].

5. Sensors

An electrochemical sensor consists of a diffusion barrier, a sensing electrode (working electrode, measuring electrode, or anode), a counter electrode (cathode), and an electrolyte. Their fabrication includes various types of systems such as conductometry, voltammetry, potentiometry, and capacitance, and it is an important tool to detect various analytes in environmental, clinical, and biological fields due to their high sensitivity, cheapness, and miniaturization. These sensors have the potential to achieve sensitive, specific, and low-cost detection of biomolecules which is relevant to the diagnosis and monitored treatment of disease [23]. A few are listed below:

5.1 Potentiometric sensors

Techniques based on measurement of potential sensor are termed as potentiometry, for example, determination of potassium in blood serum by direct potentiometry with an ion-selective electrode. The determination of urea is a frequent task of clinical laboratories. The basis of enzyme electrode function is the selective recognition of urea by urease enzyme. The following reaction takes place:

$$\text{Urea} + 3\,H_2O \xrightarrow{\text{Urease}} 2\,NH_4^+ + HCO_3^- + OH^- \qquad (1)$$

This reaction can be followed using different potentiometric electrodes [24].

5.2 Molecularly imprinted polymer (MIP) sensors

MIP could be one of the important tailor-made systems for targeted analyte recognition exclusively even their presence in complex real biological samples in parts per million to parts per billion levels. The general idea is to create the cavity in the presence of the guest. The guest organizes and promotes energy-minimized interactions with polymer forming around it. Thus, after washing the guest out, the polymers retain

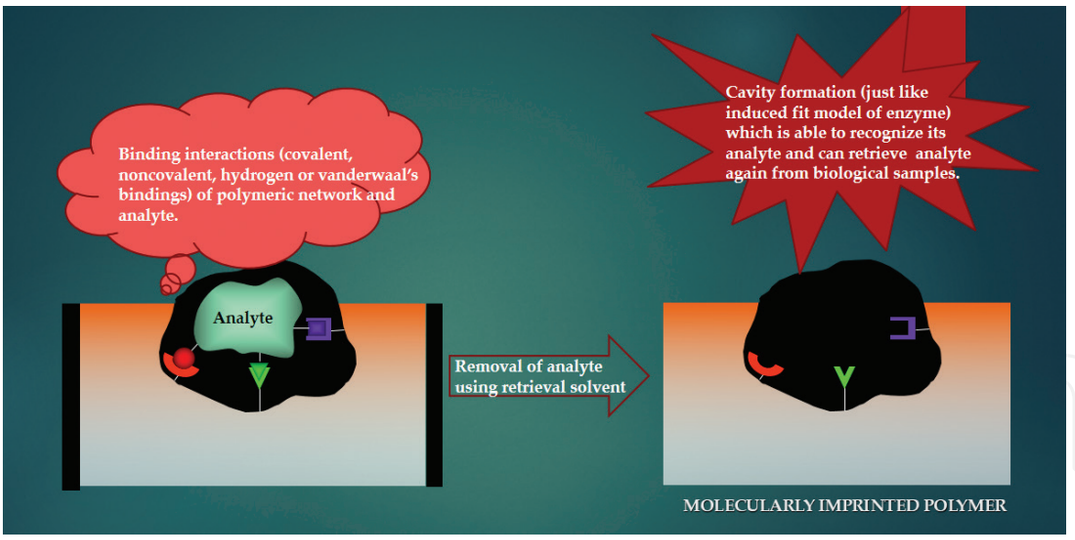

Figure 4.
Synthesis of molecularly imprinted polymer.

a template cavity of the guest's size and shape which subsequently display binding selectivity toward the guest just like induced-fit model of enzyme (**Figure 4**). The MIP-modified sensors can be used for biological and pharmaceutical analyte determination from biological samples. An example is the development of a polyscopoletin-based MIP nanofilm for the electrochemical determination of elevated human serum albumin (HSA) in urine. The results suggest that MIP-based sensors may be applicable for quantifying high-abundance proteins in a clinical setting [25].

5.3 Biosensors

The need for rapid, simple handheld testing devices in medicine paves the way for introduction of biosensor. Biological sensors are optical, electrical, and piezoelectrical devices that have the ability to detect biological compounds, such as nucleic acids and proteins [26, 27]. Early diagnosis of inherited disease is important for effective treatment and is sometimes lifesaving. Methods, like enzyme-linked immunosorbent assay and PCR, can require highly skilled professionals and expensive chemicals and can be time-consuming. In this area, many biosensor schemes had developed as an alternative to classical methods.

The latest advancements in nanotechnology result in its application for cancer biomarker recognition [28]. Several other biosensors include nanomaterial-based biosensors [29], peptide nucleic acid-based biosensors, biosensors for medical mycology, optical DNA biosensors, and last but not least, the biosensors for the diagnosis of heart disease [30].

6. Chromatographic separation techniques

In chromatographic separation technique, various constituents of the mixture in the given sample travel at different speeds, causing them to separate. This technique involves two phases: a mobile phase and a stationary phase. The separation mainly depends on the differential partitioning between these two phases. A bonded phase is a stationary phase that is covalently bonded to the support particles or to the inside wall of the column tubing. The stationary phase is the substance fixed in place for the chromatography procedure, and the mobile phase is moving in a definite direction [31]. Examples include the silica layer in thin

layer chromatography (TLC). Archer John Porter Martin and Richard Laurence Millington Synge won a Nobel Prize in chemistry for chromatography invention [32]. Their work encouraged the rapid development of several advanced chromatographic methods such as paper chromatography, gas chromatography, and HPLC. The differences in a compound's partition coefficient bring about differential retention on the stationary phase and thus affect the separation process. Chromatography may be preparative or analytical. The preparative chromatography separates the components of a mixture for later use and is thus a form of purification. Analytical chromatography is done normally with smaller amounts of material and is for establishing the presence or measuring the relative proportions of analytes in a mixture. **Figure 5** describes a chromatogram for a biological system where the signal is proportional to the concentration of the specific analyte separated.

Depending upon the shape of stationary phase, chromatography may be (i) planar chromatography, having one-dimensional bed support such as paper or TLC, or (ii) column chromatography with three-dimensional bed support. TLC is useful for separating mixtures of organic compounds and is often used to monitor the progress of organic reactions and to check the purity of products.

On the basis of physical state of mobile phase, chromatographic technique may be GC or LC. GC can be used to separate mixtures of volatile organic compounds. A GC consists of a flowing mobile phase, an injection port, a separation column containing the stationary phase, a detector, and a data recording system. LC is useful for separating mixtures of ions or molecules that are dissolved in a solvent. If the matrix support, or stationary phase, is polar (e.g., paper, silica, etc.), it is normal-phase chromatography; and if it is nonpolar (C-18), it is reversed-phase chromatography.

In short, chromatography is a method of separating the constituents of a solution, based on one or more of its chemical or physical properties. This could be charge, polarity, or a combination of these traits and pH balance. The solution is passed through a medium which will hinder the movement of some particles more than others. These principles are used to isolate and analyze enzymes, pigments, amino acids, constituents of DNA, and almost any other molecule you can imagine. A wide variety of chromatography techniques had developed to allow mixed substances to be separated [33].

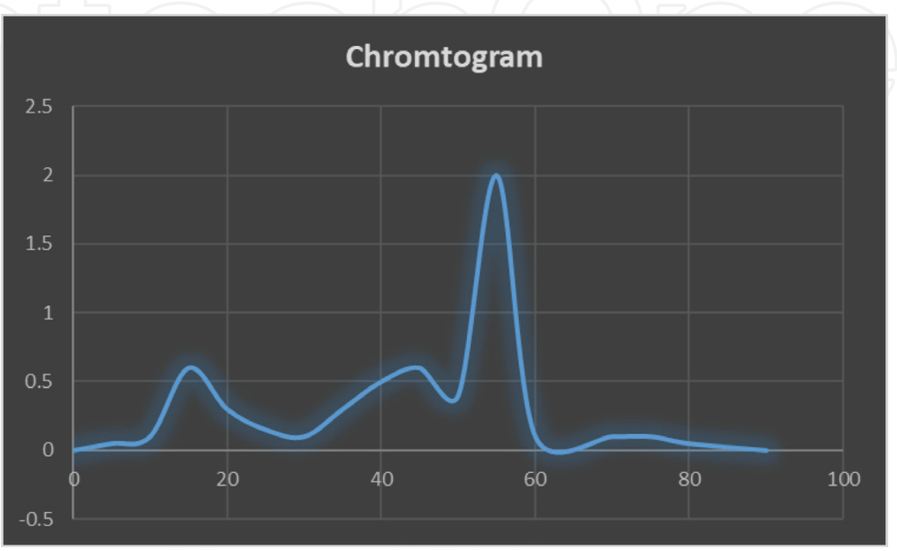

Figure 5.
Chromatogram response of a biological sample. The retention time is plotted on X-axis and signal on Y-axis obtained from detector corresponding to the response created by the analytes exiting the system.

7. Capillary electrophoresis (CE)

Jorgenson and Lukacs in 1981 invent capillary electrophoresis (CE) most often termed as capillary zone electrophoresis (CZE) [34]. It is a type of electrophoresis in which analytes are separated by applying an electric field across buffer solution-filled capillary tubes. The proposed instrumental technique was later implemented in a number of applications such as bioanalytical [35] and forensic drug analysis [36]. This technique is an alternative to the gel electrophoresis or LC (**Figure 6**).

CZE method correlated well with an automated kinetic fluorescent assay. An example is analysis of NAG by CE after incubation of urine samples using synthetic substrate, methylumbelliferyl-β-D-glucosaminide [37].

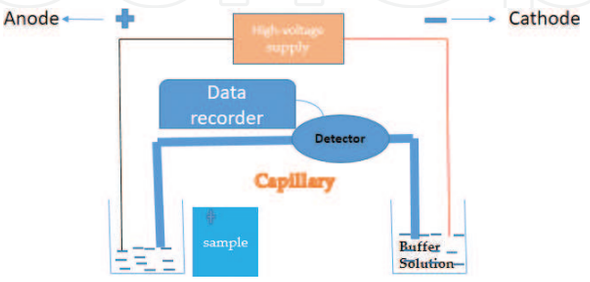

Figure 6.
Capillary electrophoresis separation method.

8. Microscopy

In the simplest microscopic methods, a specimen is illuminated by visible light and observed either against a bright background (bright-field microscopy) or a dark background (dark-field microscopy). The presence of cells that are not expected in the healthy person may be an indicator of disease. For example, a simple microscopic analysis of blood sample identifies the sickle cell anemia, and analysis of urine quantifies the presence of pus cells, which is an indicator of infection.

Light microscopy uses light as the illumination radiation. This is used to identify the microorganisms based on their morphology. An application of microscopy is to count the number of different cells per unit volume of blood or any other sample using a hemocytometer. Fluorescence microscopy has emerged as a very powerful tool for studying molecular processes owing largely to the advancement in optics and discovery of the green fluorescent protein and development of its analogs with different spectral properties [38]. Several advancements in the field of fluorescence microscopy have been achieved that includes the following techniques:

- Confocal laser scanning microscopy (CLSM)

CLSM is a type of fluorescence microscopy that allows imaging of the samples at different focal planes that light emitting from below or above the desired focal plane is eliminated. This results in very high lateral resolution and allows determining the spatial localization of the molecules [39].

- Total internal reflection fluorescence (TIRF) microscopy

TIFR is another type of fluorescence microscopy wherein the optics allows imaging of the molecules that are almost like microscopic slide. The resolution of light microscopes depends on the wavelength of the light used. The smaller the

wavelength of the light used, the better the resolution obtained. Wavelength of the visible light imposes a resolution limit of ~0.2 μm on the light microscopes. Hemocytometer (Neubauer chamber) is a glass slide which has a counting chamber at the center. A glass cover is placed on the hemocytometer, and the sample is gently introduced into the chamber. The sample chamber has a grid which allows counting of cells in a defined region using a microscope [40].

9. Gene therapy protocol

Gene therapies are considerable improvements over the existing therapy because of the advantage in dosing schedule, patient compliance, toxicity, immunogenicity, and cost. Owing to this, gene therapy provides novel approaches for the treatment of inherited and acquired diseases. The development of a nonviral gene delivery vehicle capable of efficient, cell-specific delivery will be a valuable addition to the clinical armamentarium [41]. For example, the liver places a central role in the metabolism and production of serum proteins; it is an important target organ for gene therapy. Hepatic metabolic diseases as well as acquired diseases may also serve as targets for hepatic gene therapy [42]. Most recent gene therapy protocols describe delivery of foreign genes by means of injecting lentiviral particles [43].

10. Immunological and radioisotope techniques

Immunoassays are the quantitation of bioanalyte that depends on the reaction of an antigen (analyte) and an antibody. These methods are based on a competitive binding reaction between a fixed amount of labeled form of an analyte and a variable amount of unlabeled sample analyte for a limited amount of binding sites on a highly specific anti-analyte antibody. When immunoanalytical reagents (analyte or antibody) are mixed and incubated, the analyte is bound to the antibody forming an immune complex. This complex is separated from the unbound reagent fraction by physical or chemical separation technique. Analysis is achieved by measuring the label activity (e.g., radiation, fluorescence, or enzyme) in either of the bound or free fraction [44].

Immunoassay methods have been widely used in many important areas such as diagnosis of diseases, therapeutic drug monitoring, clinical pharmacokinetic and bioequivalence studies in drug discovery, and pharmaceutical industries. A few immunoassays based on different labels are as follows:

- Radioimmunoassay (RIA) methods have been used successfully for the determination of a limitless number of pharmaceutically important compounds in biological fluids. RIA is used to analyze thyroid hormone testing in patients after iodine-131 therapy.

- Enzyme immunoassay (EIA) is analogous to RIA except that the label is an enzyme rather than a radioisotope.

- Fluoroimmunoassay (FIA) is analogous to RIA except that the label is a fluorophore rather than a radioisotope.

- Chemiluminescence immunoassay (CLIA) involves a chemiluminescent substance as a label.

- Liposome immunoassay (LIA) is the assay involving a liposome-encapsulating marker.

- Cloned enzyme donor immunoassay (CEDIA) methodology is a novel approach which uses the DNA technology to produce homogenous enzyme immunoassays for drugs.

- Flow injection immunoassay (FIIA) methods were recently introduced to enhance the efficiency of immunochemical reaction, as well as to increase the performance of the analysis.

- Capillary electrophoresis immunoassay (CEIA) has been recently introduced as a sensitive analytical technique, particularly when combined with a sensitive detection method.

11. Hyphenated separation techniques and its application in clinical chemistry

The hyphenated techniques improved detection limits, sample identification capability, and miniaturization potential; hence, about 60% of the application of electrochemical detection (ED) has been found in the field of bioanalysis. The principle of ED used in biomedical analysis is a transfer of charge between substances in a column effluent and a working electrode; mainly, two types of ED either coulometric detection or amperometric detection are frequently used. The main advantages of using ED are the selectivity and sensitivity over UV-Vis detection. In HPLC, most of the application has been carried out by using UV-Vis detector, and ED is only used in small portion. The development of HPLC with ED facilitated highly sensitive and selective determination of homovanillic acid (HVA) and vanillylmandelic acid (VMA) in urine for the differential diagnosis of neuroblastoma pheochromocytoma and related tumors [45]. HPLC, coupled with UV-Vis using photodiode array as a detector, is widely used for determination of different drugs in serum. Other applications include determination of vitamins, antioxidants, and other components in biological samples.

CE coupled with MS provides an advantage of the sensitivity (parts per million range) and selectivity of these detection systems [46]. A detector that is becoming more frequently attached to CE is inductively coupled plasma mass spectrometry (ICP-MS). To date, CE-ICP-MS has been performed using a quadrupole detector within the MS allowing a small number of elements to be analyzed at any one time [47].

12. Lab on a chip (LoC)

Lab on a chip is defined as a microform of analytical devices that assimilate numerous laboratory operations such as PCR and DNA sequencing into a single chip on a very small scale. Miniaturized version of LoC provides cost-efficiency, use of low-volume reagents, high parallelization, high diagnostic speed, high sensitivity, and high expandability [48].

On the other hand, chronic disease (CD) healthcare is experiencing few limitations owing to lengthy and costly diagnosis procedures. Rapid, reliable, and low-cost diagnostic tools at point-of-care (PoC) instrumentation are therefore on high demand. LoC technology has a high potential to enable improved biomedical applications [49, 50]. In this regard, research toward developing new LoC-based PoC systems for CD diagnosis is fast growing into a nascent area such as chronic respiratory diseases (CRD), diabetes, and chronic kidney diseases (CKD) [51].

13. Conclusions

This chapter summarizes various non-separation and separation methods used for biomedical analysis. Although routine clinical methods indicate normal/abnormal levels of bioanalytes in urine/blood, still they often lack specificity due to severe complex biological sample interferences. In this regard, sample cleanup and highly sensitive techniques have proven to be helpful for early-stage disease diagnosis [52, 53] and detecting medical abnormalities [54]. To date, an array of hyphenated techniques plays an important role in the determination of bioanalytes, with improved selectivity and sensitivity.

Acknowledgements

I would like to sincerely thank Prof. R.S. Dubey and Prof. S.S. Pandey for their extensive support and guidance in the completion of this book chapter.

Conflict of interest

The author has declared no conflict of interest.

Thanks

I would also like to thank the staffs of the central library, Banaras Hindu University, Varanasi, India, for providing valuable books and journals.

Abbreviations

NAG	N-acetyl-beta-D-glucosaminidase
UV-Vis	ultraviolet-visible
IR	infrared
NIR	near infrared
BMV	bioanalytical method validation
LC	liquid chromatography
GC	gas chromatography
CE	capillary electrophoresis
MS	mass spectrometry
HPLC	high-performance liquid chromatography
SEM	scanning electron microscopy
CSF	cerebrospinal fluid
SPE	solid-phase extraction
SPME	solid-phase microextraction
MIP	molecularly imprinted polymer
CLSM	confocal laser scanning microscopy
TIRF	total internal reflection fluorescence
RIA	radioimmunoassay
FIA	fluoroimmunoassay
EIA	enzyme immunoassay
CLIA	chemiluminescence immunoassay
LIA	liposome immunoassay

CEDIA	cloned enzyme donor immunoassay
CEIA	capillary electrophoresis immunoassay
PCR	polymerase chain reaction
LoC	lab on a chip
PoC	point of care

Appendices

The *analyte* is the substance to be separated and to be found during chromatography from the mixture.

A *chromatograph* is an equipment that enables a sophisticated separation, for example, gas chromatographic or liquid chromatographic separation.

The output of the chromatograph is termed as *chromatogram*. In the case of an ideal separation, different peaks on the chromatogram correspond to different components of the separated mixture.

The *effluent* is the mobile phase leaving the column.

The *eluent* is the solvent that carries the analyte.

The *retention time* is the characteristic time it takes for a particular analyte to pass through the system (from the column inlet to the detector) under a set of conditions.

The *solute* is termed as the sample component in partition chromatography.

The *solvent* means any substance capable of solubilizing another substance, such as liquid mobile phase in LC.

The *detector* refers to an instrument used for quantitative and qualitative detection of analytes.

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References

[1] Caroli S, Gyula Z. Analytical Techniques for Clinical Chemistry: Methods and Applications. Hoboken, N.J.: John Wiley & Sons, Inc.; 2012

[2] Beale DJ, Jones OAH, Karpe AV, Dayalan S, Oh DY, Kouremenos KA, Ahmed W, Palombo EA. A review of analytical techniques and their application in disease diagnosis in breathomics and salivaomics research. International Journal of Molecular Science. 2017;**18**(1):24

[3] Begum SS, Sushmaa BS, Vijayaraja S. Bioanalytical techniques—An overview. PharmaTutor. 2015;**3**(9):14-24

[4] Joseph L, Burtis CA, Bruns DE. Tietz Fundamentals of Clinical Chemistry and Molecular Diagnostics. 7th ed. 2008. Indian Journal of Clinical Biochemistry. 2015;**30**. DOI: 10.1007/s12291-014-0474-9

[5] Gad SC. Clinical Trials Handbook. Hoboken, N.J.: Wiley & Sons, Inc.; 2009

[6] Morgan JL, Lichtman JW. Digital tissue and what it may reveal about the brain. Biomed Central Biology. 2017;**15**:101-110

[7] Jia P. Plasmonic optical sensors: performance analysis and engineering towards biosensing [PhD theses]. The University of Western Ontario; Electronic Thesis and Dissertation Repository 2014;**2157**. Available from: https://ir.lib.uwo.ca/etd/2157

[8] Eghiaian F, Rico F, Colom A, Casuso I, Scheuring S. High-speed atomic force microscopy: Imaging and force spectroscopy. FEBS Letters. 2014;**588**(19):3631-3638

[9] Holland NT, Smith MT, Eskenazi B, Bastaki M. Biological sample collection and processing for molecular epidemiological studies. Mutation Research. 2003;**543**:217-234

[10] Pawliszyn J, Lord HL. Handbook of Sample Preparation. Hoboken N.J.: John Wiley & Sons Inc.; 2011. DOI: 10.1002/9780813823621.ch16

[11] Pungor E. A Practical Guide to Instrumental Analysis. Boca Raton: CRC Press LLC; 1995

[12] Sevick-Muraca E, Benaron D. OSA Trends in Optics and Photonics on Biomedical Optical Spectroscopy and Diagnostics. Washington, DC: Optical Society of America; 1916. pp. 271-272

[13] Xie J, Qian Z, Yang T, Li W, Hu G. Near-infrared spectroscopy technique to evaluate the effects of drugs in treating traumatic brain edema. Journal of Physics; Conference Series. 2011;**277**:012041

[14] Rao J, Butler DF, Taylor CR. Voorhees Van AS, Bissonnete R. Photodynamic Therapy for Dermatologists. https://emedicine.medscape.com/article/112157-overview

[15] Kartha VB, Santosh C. Biomedical Spectroscopy. India: Manipal University Press; 2014

[16] Severcan F, Haris PI. Vibrational Spectroscopy in Diagnosis and Screening. Amsterdam, Netherlands: IOS Press; 2012

[17] Rahman IU, Movasaghi Z, Rehman S. Vibrational Spectroscopy for Tissue Analysis (Series in Medical Physics and Biomedical Engineering). Boca Raton: CRC Press; 2013

[18] Driskell JD, Zhu Y, Kirkwood CD, Zhao Y, Dluhy RA, Tripp RA. Rapid and sensitive detection of rotavirus molecular signatures using surface enhanced Raman spectroscopy. PLoS One. 2010;**5**(4):e10222

[19] Magidson V, Khodjakov A, Digital Microscopy Methods in Cell Biology, 2013

[20] Jannetto PJ, Fitzgerald RL. Effective use of mass spectrometry in the clinical laboratory. Clinical Chemistry. 2016;**62**(1):1-7

[21] Xu W, Manicke NE, Cooks GR, Ouyang Z. Miniaturization of mass spectrometry analysis systems. JALA Charlottesv Va. 2010;15:433-439

[22] Keereweer S, Kerrebijn JD, Van Driel PB, Xie B, Kaijzel EL, Snocks TJ, et al. Optical image guided surgery—Where do we stand? Molecular Imaging and Biology. 2011;**13**(2):199-207

[23] Stradiotto NR, Yamanaka H, Zanoni Maria Valnice B. Electrochemical sensors: A powerful tool in analytical chemistry. Journal of Brazilian Chemical Society. 2003;**14**(2):159-173

[24] Ho WO, Krause S, McNeil CJ, Pritchard JA, Armstrong RD, Athey D, et al. Electrochemical sensor for measurement of urea and creatinine in serum based on AC impedance measurement of enzyme-catalyzed polymer transformation. Analytical Chemistry. 1999;**71**(10):1940-1946

[25] Stojanovic Z, Erdőssy J, Keltai K, Scheller FW, Gyurcsányi RE. Electrosynthesized molecularly imprinted polyscopoletin nanofilms for human serum albumin detection. Analytica Chimica Acta. 2017;**977**:1-9

[26] Serra PA. Biosensors. 1st ed. Vukovar, Croatia: InTech; 2010

[27] Gouvea. In: Serra PA, editor. Biosensors for Health Applications, Biosensors for Health, Environment and Biosecurity. London, U.K.: IntechOpen; 2011. pp. 71-86. DOI: 10.5772/16983

[28] Zhang L, Lv D, Su W, Liu Y, Chen Y, Xiang R. Detection of cancer biomarkers with nanotechnology. American Journal of Biochemistry and Biotechnology. 2013;**9**(1):71-89

[29] Atta NF, Galal A, Ali S. In: Serra PA, editor. Nanobiosensors for Health Care, Biosensors for Health, Environment and Biosecurity. London, U.K.: IntechOpen; 2011. DOI: 10.5772/17996 Available from: https://www.intechopen.com/books/biosensors-for-health-environment-and-biosecurity/nanobiosensors-for-health-care

[30] Ozkan-Ariksoysal D. Biosensors and their Applications in Healthcare. London U.K.: Future Science Group; 2013. Published Online. DOI: 10.4155/9781909453647

[31] Christian GD. Analytical Chemistry. 6th ed. Hoboken N.J.: John Wiley & Sons, Inc; 2016

[32] MLA style: Richard L.M. Synge – Facts. NobelPrize.org. <https://www.nobelprize.org/prizes/chemistry/1952/synge/facts/>

[33] Pandey K, Dubey RS, Prasad BB. A critical review on clinical application of separation techniques for selective recognition of uracil and 5-fluorouracil. Indian Journal of Clinical Biochemistry. 2016;**31**(1):3-12

[34] Jorgenson JW, Lukacs K, Arman D. Zone electrophoresis in open-tubular glass capillaries. Analytical Chemistry. 1981;**53**(8):1298-1302

[35] Zhong X, Zhang Z, Jiang S, Li L. Recent advances in coupling capillary electrophoresis-based separation techniques to ESI and MALDI-MS. Electrophoresis. 2013;**35**(9):1214-1225

[36] Anastos N, Barnett NW, Lewis SW. Capillary electrophoresis for forensic

drug analysis: A review. Talanta. 2005;**67**:269-279

[37] Friedberg M, Shihabi ZK. Analysis of urinary N-acetyl-β-glucosaminidase by capillary zone electrophoresis. Journal of Chromatography B, Biomedical Sciences and Applications. 1997;**695**(1):187-191

[38] Voura EB, Jaiswal JK, Mattoussi H, Simon SM. Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy. Nature Medicine. 2004;**10**(9):993-998

[39] Castellani C, Fedrigo M, Frigo AC, Barbera MD, Thiene G, Valente M, et al. Application of confocal laser scanning microscopy for the diagnosis of amyloidosis. Virchows Archiv. 2017;**470**(4):455-463

[40] Poulter NS, Pitkeathly WT, Smith PJ, Rappopor JZ. The physical basis of total internal reflection fluorescence (TIRF) microscopy and its cellular applications methods. Journal of Molecular Biology. 2015;**1251**:1-23

[41] Morgan JR. Gene Therapy Protocols: Reference Methods in Molecular Medicine. IInd ed. Vol. 69. New Jersey: Human Press Totowa; 2002

[42] Wu CH, Walton CM, Wu GY. Targeted gene transfer to liver using protein DNA complex. Methods in Molecular Medicine. 2002;**69**:15-23

[43] Buchschacher GL, Wong Stoul F. Bio artificial muscles. Development of lentiviral vectors for gene therapy for human diseases. Blood. 2000;**95**:2499-2504

[44] Darwish IA. Immunoassay methods and their applications in pharmaceutical analysis: Basic methodology and recent advances. International Journal of Biomedical Sciences. 2006;**2**(3):217-235

[45] Frattini P, Santagostino G, Schinelli S, Cucchi LM, Corona GL. Assay of urinary vanillylmandelic, homovanillic, and 5-hydroxyindole acetic acids by liquid chromatography with electrochemical detection. Journal of Pharmacological Methods. 1983;**10**(3):193-198

[46] Ramos-Payan M, Juan A, Ocana-Gonzalez RM, Llobera FA, Bello-López MA. Recent trends in capillary electrophoresis for complex samples analysis: A review. Electrophoresis. 2018;**39**:111-125

[47] Chetwynd AJ, Guggenheim EJ, Briffa SM, Thorn JA, Lynch I, Valsami-Jones E. Current application of capillary electrophoresis in nanomaterial characterisation and its potential to characterise the protein and small molecule corona. Nanomaterials. 2018;**8**(99):1-29

[48] Yılmaz B. Omics Technologies and Bio-Engineering Towards Improving Quality of Life. Elsevier B.V.; 2018. DOI: 10.1016/C2015-0-01634-3

[49] Herold KE, Rassoly A. Lab-on a Chip Technology: Fabrication and Microfluidics. London U.K.: Horizon Scientific Press; 2009

[50] Herold KE, Rassoly A. Lab-on a Chip Technology: Biomolecular Separation and Analysis. London U.K.: Horizon Scientific Press; 2009

[51] Wu J, Dong M, Rigatto LY, Lin F. Lab-on-chip technology for chronic disease diagnosis. npj Digital Medicine. 2018;**1**:7

[52] Prasad BB, Tiwari K, Singh M, Sharma PS, Patel AK, Srivastava S. Ultratrace analysis of dopamine using a combination of imprinted

polymer-brush coated solid-phase microextraction and imprinted polymer-sensor techniques. Chromatographia. 2009;**69**: 949-957

[53] Mishra OP, Rai AK, Srivastava P, Pandey K, Abhinay A, Prasad R, et al. Predictive ability of urinary biomarkers for outcome in children with acute kidney injury. Pediatric Nephrology. 2017;**32**(3):521-527

[54] Prasad BB, Tiwari K, Singh M, Sharma PS, Patel AK, Srivastava S. Zwitterionic molecularly imprinted polymer-based solid-phase microextraction coupled with molecularly imprinted polymer-sensor for ultratrace sensing of L-histidine. Journal of Separation Science. 2009;**32**:1096-1105