

APPLICATIONS OF SPECTROSCOPY AS CLINICAL DIAGNOSTIC TOOL

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ABSTRACT

The purpose of this article is to underline the possibility of efficiently using spectroscopical methods as diagnostic tools. Spectroscopy involves the study of the interrelationships between the spectral characteristics of objects and their biophysical attributes. It provides a tool for the development, refinement and testing of models relating biophysical attributes to remotely-sensed data. The disciplines evaluating spectroscopy in the context of existing modalities are required. The ability of spectroscopy to furnish non-invasive information about tissue chemistry in patients bodies well for its impact on clinical research and diagnostic screening tools in detecting various diseases. In addition, several key applications are also reported.

Keywords: *Spectroscopical methods; diagnostic Applications; Instrumental uses; Clinical research.*

INTRODUCTION

Spectroscopy is the study of the interaction between matter and radiated energy¹. Historically, spectroscopy originated through the study of visible light dispersed into its wavelength components by a prism. Later, the concept was expanded greatly to comprise any interaction with radiative energy as a function of its wavelength or frequency. Spectroscopic data is often represented by a spectrum, a plot of the response of interest as a function of wavelength or frequency. Spectral measurement devices are referred to as spectrometers, spectrophotometers, spectrographs or spectral analyzers.

Classification of methods

Spectroscopy is a sufficiently extensive field hence many sub-disciplines exist, each with numerous implementations of specific spectroscopic techniques. The various implementations and techniques can be classified in several ways:-

A. Type of radiative energy.

Spectroscopy is differentiated by the type of radiative energy involved in the interaction. In many cases, the spectrum is determined by measuring the changes in the intensity or frequency of this radiative energy. The types of radiative energy studied include:

- i. Electromagnetic Radiation (Microwaves, UV radiations, X-rays and gamma radiations)
- ii. Particles as a source of energy (electrons and neutrons).

- iii. Radiating pressure waves (Acoustic Spectroscopy)

- iv. Mechanical methods which can employ to radiate energy².

B. Nature of Interaction.

It is established upon the nature of the interaction between the energy and the material. These interactions include:-

- i. Absorption Spectroscopy- measures the energy, absorbed by the material from the radiative source.

- ii. Emission Spectroscopy- measures the radiative energy which is released by the material.

- iii. Elastic scattering and reflections spectroscopy- measures the reflected or scattered incident radiation by a material.

- iv. Crystallography- measures the scattering of high energy radiation, such as X-rays and electrons, to examine the arrangement of atoms in proteins and solid crystals.

- v. Impedance spectroscopy- studies the ability of a medium to impede or slow the transmittance of energy.

- vi. Inelastic scattering- measures the exchange of energy between the radiation and the matter that shifts the wavelength of the scattered radiation.

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APPLICATIONS

Infrared Spectroscopy and Raman spectroscopy applications

a) In oral cancers diagnosis

Tobacco is one of the main etiological factors for oral cancer. Since oral cancer is not easily detected this is the reason for high mortality associated with this cancer. Optical spectroscopic methods including Raman spectroscopy (RS) are being projected as alternate for diagnosis. Ex vivo studies have shown that pathological conditions can be distinguished from normal tissues. Good quality in vivo Raman spectra can be recorded from oral cavity. Using multivariate analysis methods e.g. PCA (Principle Component Analysis) and LDA (Linear Discriminant Analysis), classification between oral normal, premalignant and tumor conditions with efficiency of ~88% can be achieved. Spectral features from normal, healthy controls are dominated by:-

- Lipid features indicated by **C=O band of esters, strong αCH_2 bend, two sharp features in amide III region and a sharp peak in amide I region.**

Tumor spectra are dominated by:-

- Protein bands which are indicated by **broad amide III, broad and shifted αCH_2 and broad amide I.**

(Amide II is the most intense absorption band in proteins. It is primarily governed by the stretching vibrations of the C=O (70-85%) and C-N groups (10-20%). Its frequency is found in the range between 1600 and 1700 cm^{-1} . The exact band position is determined by the backbone conformation and the hydrogen bonding pattern, **Amide III** is a very complex band dependent on the nature of side chains and hydrogen bonding. Therefore, these bands are only of limited use for the extraction of structural information.) This study shows that spectra from habitual tobacco users are dominated by protein bands but exhibit some differences, as compared to healthy controls, such as:

- **Minor shift in amide III and CH_2 bend as well as broadening of amide I region which could suggest changes in protein secondary structures. Spectra from premalignant patches show similarities like broadening of amide III, amide I and CH_2 region** with spectra from tumors.

These differences are explored for classification with supervised linear discriminant analysis (LDA) method. Findings of the study indicate the feasibility of discriminating premalignant from contra lateral normal and tumour sites of subjects with oral cancer as well from healthy controls with and without tobacco habits³⁻⁴.

b) In Characterization of Mineralised tissue (Bone and Teeth)

i. Bone

IR and Raman spectroscopy have been used for several years to characterise mineralised structure in

living organism especially bone and teeth. The parameters monitored are **mineral to protein ratio** (the extent of mineral formation in tissue), size and perfection of crystal and ratio of carbonate ions and phosphate ions in hydroxyapatite lattice. The spectral properties of bone by Raman imaging are generally examined at 785 nm. Recently Raman images from bone are also collected at 532nm. Due to high quantum efficiency of detectors at shorter wavelength more intense Raman signals are obtained at lower wavelength. Most of the bands of Bone Raman Spectrum can be assigned to mineral phosphate, calcium, carbonate and matrix collagen. In Raman spectroscopy most of the studies reported the relative peak intensity or peak area; because of this reason absolute band intensity is seldom used in Raman spectroscopy as they are affected by Raman Scattering efficiency and other optical effects like grain size, refractive index and surface roughness of spectrum (Fig. 1).

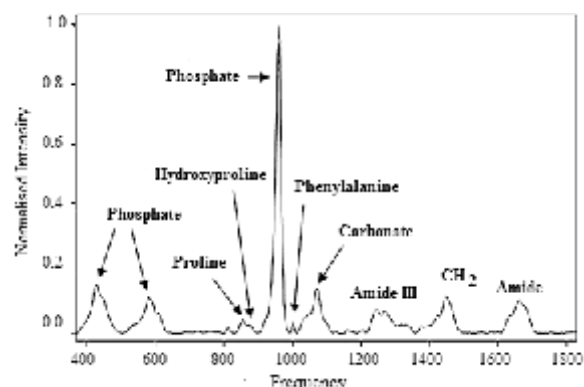


Fig. 1: Raman spectrum taken from the tibia of 6-week-old mouse bone showing the assignments for bone mineral and matrix-specific Raman bands. The spectrum was acquired using 785nm excitation, baseline-corrected, and then normalized to the intensity.

If the primary phosphate band (approximately 959 cm^{-1}) is divided by Amide I (1616-1720 cm^{-1}) this will give mineral to matrix ratio which indicates the amount of mineralisation. These mineral to matrix ratio can be used to access age related mineralisation trends. Methylene side chain (CH_2 at 1450 cm^{-1}), amide III (1243-1320 cm^{-1}), phenylalanine (approximately 1002 cm^{-1}) and CH Stretch (2940 cm^{-1}) depicts the matrix band. The choice of matrix band depends upon the embedding conditions, instrument and its orientational sensitivity towards mineralised collagen fibrils. Raman measure of carbonate to phosphate (959 cm^{-1}) provides the valuable information regarding the chemical composition of human bones as it varies with age, bone architecture and mineral crystallinity. Another carbonate related parameter includes carbonate to amide I ratio (at 1665 cm^{-1}) which indicates bone remodelling. The width of primary phosphate band near 959 cm^{-1} gives information about mineral crystallinity⁵⁻⁷.

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ii. Teeth

Raman spectroscopy provides information about biochemical composition and molecular structure of teeth. As compared to healthy dentin, differences which are noted include depth of demineralization, adhesive infiltration and degree of conversion at the interface with caries-affected dentin. Dentin in teeth is also a useful model for the study of mineral maturation during biomineralization. Raman spectroscopy has an advantage of being sensitive to both the mineral and the organic component of dental tissue, so matrix mineral interaction thus allowing for the study of mineral-matrix interactions as well as the properties of each individual component. Raman spectroscopy also exhibits peak characteristics of inorganic (hydroapatite) and organic (collagen) of teeth.

Typical Raman Spectrum of normal dentine contains two characteristic parts:

- Vibrational modes of phosphate ranges from 400 to 1100 cm^{-1}
- Organic group Vibrational mode in 1200 to 3000 cm^{-1}

Peak due to inorganic mineral component are exhibited at wave number shift of 429 cm^{-1} , 589 cm^{-1} , 961 cm^{-1} and 1076 cm^{-1} . Peak at 961 cm^{-1} , 589 cm^{-1} and 429 is of phosphate group (PO_4^{3-}), peak at 1076 is of carbonate group/carbonate vibrations (CO_2^{3-}), representing the inorganic component of Raman Spectra and while peak at 1668 cm^{-1} and 2942 cm^{-1} is of amide I and CH vibrations, representing the organic component of Raman Spectra. Peak of phosphate at 961 cm^{-1} is the strongest peak.

In case of demineralised dentine, no new peak is found as compare to the spectra of normal dentine. No change in FWHM (Full width at Half Maximum) of various peaks, as compare to the normal spectra. But it is notable that in demineralised dentine, peak intensity of organic constituent increases and that of inorganic compound decreases⁸⁻⁹.

c) In Characterization of Blood

Blood is a specialized body fluid that delivers necessary substances to the cells in the body— such as nutrients and oxygen; and transports waste products away from those same cells. It is composed of blood cells suspended in a liquid called blood plasma. Plasma, which constitutes 55% of blood fluid, is mostly water (90% by volume) and contains dissolved proteins, glucose, mineral ions, hormones, and carbon dioxide. The blood cells are mainly red blood cells (erythrocytes) and white blood cells, including leukocytes and platelets. The red blood cells contain hemoglobin, an iron-containing protein.

The spectrum of whole blood is almost the same as the blood platelets. This suggests that the spectrum mainly comes from red blood cells. The major Raman

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peaks are located around 742, 778, 991, 1074, 1120, 1160, 1210, 1335, 1383, 1442, 1542, 1614, 2159, 2914 cm^{-1} . A report about the measurement of hemoglobin oxygen saturation shows that peaks of 1375, 1590, 1640 cm^{-1} would increase with high O_2 saturation, these peaks do not appear if the O_2 saturation of blood is low, so it is a mode of detection of hypoxia. The small sharp peak located near 2330 cm^{-1} is probably that of atmospheric N_2 which occurs because of a high excitation power density and large collection solid angle near the surface of fibers at the distal end of the catheter.

Serum is the supernatant fluid when coagulated blood has been centrifuged; it is devoid of all coagulation factors comparing with plasma. The spectrum of serum without blood cells shows a different behaviour from that of whole blood in the low frequency range. The major Raman peaks are located around 820, 1044, 1335, 1383, 1442, 1542, 1614, 1653, 2159, 2646, 2914 cm^{-1} .

Peaks at 1335, 1383, 1442, and 1614 cm^{-1} mainly comes from CH_2 , CH_3 stretching (lipids, carbohydrates and proteins). In high frequency, the dominant peak is at 2914 cm^{-1} , 2646 cm^{-1} which mainly comes from the protein and carbohydrate component so Raman spectroscopy can also be used for the detection of level of sugar in the blood¹⁰⁻¹¹.

UV Visible Spectroscopy Applications

Ultraviolet/visible (UV/VIS) absorption spectroscopy has been used in the clinical laboratory for many years.

a) Enzymatic Study

In clinical chemistry UV-visible spectroscopy is used extensively in the study of enzyme kinetics. Enzymes cannot be studied directly but their activity can be studied by analysing the speed of the reactions which they catalyse. Reagents or labels can also be attached to molecules to permit indirect detection and measurement of enzyme activity. The widest use in the field of clinical diagnostics is as an indicator of tissue damage. When cells are damaged by disease, enzymes leak into the bloodstream and the amount present indicates the severity of the tissue damage. The relative proportions of different enzymes can be used to diagnose disease, say of the liver, pancreas or other organs which otherwise exhibit similar symptoms¹².

b) Enzyme Rate Assay

Enzymes are biocatalysts, which are extremely efficient in converting their chosen substrates into product. Apart from their efficiency, they are also highly specific and often do not work with a slightly different substrate. Often it is useful to study the rate of this catalysis by measuring either the rate of depletion of substrate or

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the formation of products. This may be a matter of simply measuring the absorbance of one of the reaction components directly or by forming an absorbing conjugate with another molecule. Enzyme kinetics is usually zero order. This means that, after an initial lag phase, there should be a linear relationship between substrate (or product) concentration with time until one of these components shall be completed. It is therefore useful if the instrument software is able to allow the user to choose where this linear portion is and to use this portion to calculate the rate. The activity is normally expressed in International Units (IU) by applying a simple factor to the measured slope (absorbance/time). Enzyme rate assays are nearly always performed at a single wavelength (340 and 405nm are commonly used) and require a temperature-controlled environment. Most enzyme reactions are fairly slow, taking place for 5min or more. In order to increase productivity, most UV/VIS instruments offer a cell changer as an accessory. This is a shuttle device, which can hold six or more cuvettes at once. The instrument then cycles through each of the cell positions taking a reading on each cell every 30 s during the course of the reaction. The collected data can be analyzed either using the instrument's own kinetic software or externally, using either a computer or manual calculation.

An example of a clinical rate assay is the determination of butyrylcholinesterase (BchE). Certain individuals express a mutant form of the BchE gene. This then encodes for a defective form of the enzyme, which lacks the ability to hydrolyze succinylcholine. In some rare cases, the complete BchE gene is missing. A defective or missing gene will not, normally, be of any consequence. If, however, succinylcholine is used during tracheal intubation in the administration of inhalation anesthetics, this will then cause the patient to undergo complete paralysis. The test for this enzyme is commonly performed using a UV/VIS spectrophotometer with a temperature-controlled cell holder (most tests will be performed at 37 °C)¹³⁻¹⁵.

c) Porphyrin Analysis

Porphyria is the name given to the condition, which result from an over production of porphyrins. Porphyrins are the precursors in the production of haemoglobin. Sufferers are prone to bouts of severe abdominal pain, vomiting, severe personality changes and sensitivity to light. Some types of porphyria produce characteristic dark-colored ("port wine") urine. Dark coloured urine is the symptom that porphyria is present. One of the main types of porphyria (a generic term for porphyrin-related disease) is variegating porphyria. In this case a mitochondrial enzyme called protoporphyrinogen oxidase is defective (owing to incorrect genetic coding) and as a result excess protoporphyrin (one of the porphyrin types) is produced. The protoporphyrinogen reacts with oxygen to produce protoporphyrin in an

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uncontrolled reaction. Other porphyria types include acute intermittent porphyria. Accurate diagnosis of the exact type of porphyria is vital as incorrect treatment could have very serious consequences.

Porphyrins have a characteristic UV/VIS absorption peak (Soret peak) in the region 400–410nm depending on the type of porphyrin present (coproporphyrin has a peak between 402 and 403nm whereas the uroporphyrin peak lies between 406 and 407 nm). The urine sample is filtered, diluted with distilled water and acidified with hydrochloric acid. The sample is scanned between 300 and 500nm and the spectrum peak positions are noted. The measurement is taken by first constructing a baseline at points at either side of the main peak (usually around 380 and 430 nm) and then measuring the height of the peak down to this baseline¹⁶.

d) Haemoglobin Analysis

Haemoglobin (Hb) is metalloprotein molecule which is present in red blood cells. It consists of a non protein part with an iron atom surrounded by heme groups. It has remarkable oxygen transportation properties where it can change its conformation to accept oxygen (oxyhemoglobin). This process can be inhibited by carbon monoxide, which has a 200 times stronger affinity for hemoglobin (carboxyhemoglobin) than oxygen, resulting in severe respiratory problems and death in cases of carbonmonoxide poisoning. Hemoglobin possesses an iron atom core in its ferrous (Fe²⁺) state. If the iron is oxidized to its ferric (Fe³⁺) state, its oxygen transport capabilities are diminished and the molecule is called methemoglobin (metHb). Total Hb can be measured by performing a reaction of total Hb present with potassium cyanide to form Hb-CN complex. In this method the haemoglobin reacts with KCN and Sodium hexacyanoferrate (III) to produce Hb-CN complex. This complex is analysed by UV visible spectroscopy, this complex has a peak around 546 nm, which can be measured and quantified¹⁷.

e) Molecular Biology

The use of UV/VIS spectroscopy for molecular biology strictly falls outside the scope of clinical analysis. But some routine clinical laboratories are using molecular biology techniques (such as the PCR (Polymerase Chain Reaction) and automated dideoxy sequencing) as these give more direct diagnosis for genetically based disorders. UV/VIS spectroscopy is useful for assessing the purity of the starting template in either a sequencing or PCR reaction and, as a result, saves time in optimizing the reaction and helps to reduce reagent costs. Pure DNA and RNA absorb at 260nm. Protein (which is the main source of contamination) absorbs at around 280nm. This method measures the absorbance at 260 and 280nm and the ratio (A₂₆₀=A₂₈₀) is calculated. Optionally, a third reference point can be taken at 320nm (to assess the amount of

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turbidity and scatter) and this absorbance value can be subtracted from either of the two absorbance values prior to calculating the ratio. If the ratio of the two absorbances is between 1.7 and 2.0, then the DNA preparation is considered to be pure. If it is >2, then there is probably a high RNA content. A lower ratio would indicate a high protein or phenol content (a reagent commonly used in DNA extraction).

Mass Spectrometry Applications

a) In Endocrinology

Continuous optimisation of antibody mediated detection methods has reduced but unfortunately not fully removed the internal faults of immunoassay for measurement of small molecules and proteins. Cross platform variability, inadequate specificity, antireagent antibodies are the factors that have negative effects on the usefulness of immunoassay. The increase in the testing of vitamin D has resulted in the dramatic increment and implementation of clinical mass spectrometry. LC-MS/MS method for vitamin D are able to separate vitamin D₂ from D₃ and provide the information on the epimeric form of vitamin D; neither of which is currently possible with immunoassay. The rate of development of steroidal hormone assay for diagnostic and forensic testing is continuous increase. A lack of immunoassay specificity and accuracy at low concentration in steroidal assay is a drawback that leads to the recommendation of mass spectroscopy as a preferred method of analysis. MS requires a high degree of technical competence, skill and experience to provide needed improvement for measurement of endocrine function¹⁸.

b) In Monitoring of Diabetic Neuropathy

Diabetic Neuropathy is associated with the increase in the level of glucose in the blood, which may leads to cardio vascular disease, which is the important factor for mortality and morbidity.

Two ways of onset of diabetes

- i. Genetic
- ii. Environmental (smoking, bad eating habits, hyperlipidemia, etc)

c) Mass spectral approach

Onset of diabetes is reflected in the urine protein profile. Albuminuria is widely used as a test for the onset of neuropathy. MS is a different and extremely interesting approach to describe human urine profile. Mass spectral approaches are widely used to study urinary proteome

- i. Protein mixture is first treated by electrophoresis to separate various mixtures present in the sample. This separation of protein is called as Partial Separation. The protein band on electrophoresis plate is generally separated by enzymatic digestion and then detected by using specific MS procedure. Electrospray ionisation technique generates protonated molecules

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which are identified by using Tandem Mass Spectroscopy.

- ii. An approach called SELDI (surface-enhanced laser desorption /ionization) which is specifically used for protein containing analytes. It involves deposition of the sample and matrix on a surface modified to have specific chemical properties before proceeding with laser ionization. Surfaces commonly used in this context include weakly positive ion exchange, hydrophobic surfaces, metal-binding surfaces, and strong anion exchangers. Only the analytes with a good affinity with a given treated surface will remain fixed on the surface, while all the other compounds are removed by washing¹⁹.

d) In Detection and Quantification of lipids and other metabolites

An ultra performance liquid chromatography-time of flight-mass spectrometry (UPLC-TOF-MS) system is employed for quantification of lipids. Earlier, principal component analysis (PCA) was used for group differentiation and marker selection. Several compounds can be tentatively identified based on accurate mass, isotopic pattern and MS/MS information. In addition, significant changes in the serum level of leucine, dihydrosphingosine and phytosphingosine are noted, indicating the perturbation of amino acid metabolism and phospholipid metabolism in diabetic diseases, with implications in clinical diagnosis and treatment.

It has been proposed that serum sulphatides is a novel biomarker for cardiovascular disease in patients with end-stage renal failure (ESRF), based on the possible antithrombotic properties of this molecule. Using high-throughput analysis combined with MALDI-TOF mass spectrometry, we can measure the levels of sulphatides in the sera, liver, small intestines and kidneys in a diseased individual. As the disease progresses, the levels of sulphatides in sera, livers and small intestines decreased to approximately 60% of the original levels, while those in kidneys increased by approximately 1.4-fold. These results indicate that kidney dysfunction affects the levels of sulphatides in lipoprotein producing organs, such as livers and small intestines, and lowers the levels of sulphatides in the sera²⁰.

A high-performance liquid chromatography-electrospray tandem mass spectrometric (HPLC-ESI-MS/MS) method is also known for simultaneous quantitation of Cysteine (Cys), total homocysteine (tHcy), Sadenosylmethionine (SAM), S-adenosylhomocysteine (SAH), cystathionine (Cysta), methionine (Met), glutathione (GSH) and cysteinylglycine (Cys-gly) in plasma with N-(2-mercaptopropionyl)-glycine (MPG) as internal standard. The method had simple pretreatment (without derivatization); the chromatograms showed better separation of the eight aminothiols, the analytic time was 20 min. The method

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also successfully applied in the case-control study of patients with diabetes mellitus (DM) and diabetic nephropathy (DN). The method allows quantitating the concentrations of amino thiols in the human plasma. SAH and SAM were suggested as better potential biomarkers of DM and DN²¹.

e) Detection and quantification of uremic toxins

Mass spectrometry (MS) has been successfully applied, for the identification and quantification of uremic toxins and uremia-associated modified proteins. Uremic toxins include low-molecular-weight compounds (e.g., indoxyl sulfate, p-cresol sulphate, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid, and asymmetric dimethylarginine), middle-molecular weight peptides, and proteins modified with advanced glycation and oxidation. These uremic toxins are considered to be involved in a variety of symptoms which may appear in patients with stage 5 chronic kidney disease. Based on MS analysis of these uremic toxins, the pathogenesis of the uremic symptoms can be elucidated to prevent and manage the symptoms²².

Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) may also be applied to the direct analysis of melamine cyanurate (MC). The MALDI matrix is able to desorb/ionize melamine from MC upon laser irradiation. The method is able to detect melamine from a small amount of MC (down to 12.5 microg) spiked into urine and is successfully applied for the rapid and sensitive detection of melamine in urine stones/residues of the samples collected from patients clinically confirmed of having kidney stones associated with the consumption of melamine contained food products. The analytical time of the method for high-throughput analysis from the time of sample treatment to analysis is less than 7 minutes per sample²³.

f) In diagnosis of Maple Syrup Urine Disease

Maple syrup urine disease (MSUD) is an autosomal recessive disorder of metabolism of branched chain amino acids (BCAA) like L-leucine, L-isoleucine and L-valine and their corresponding branched chain keto acids (BCKAs). This disorder is caused by a severe deficiency in the activity of branched chain alpha-keto acid dehydrogenase complex. MSUD is diagnosed by determination of L-leucine, L-isoleucine and L-valine in neonatal blood. Tandem mass spectrometry is applied for the diagnosis of MSUD. Tandem Mass spectrometry is a most powerful tool for the diagnosis of MSUD because of its high throughput and accuracy. Trifluoroacetic anhydride, n-Butanol are used for the derivatisation of the sample into a volatile sample. L-valine, L-leucine, L-isoleucine and L-phenylalanine were reacted with n-butanol and the butyl esters of amino acid are derivatised by trifluoroacetic anhydride. The free hydroxy and amino groups are modified by n-butanol and trifluoroacetic anhydride respectively. The retention times of the L-valine, L-leucine, L-isoleucine

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and phenylalanine derivatives are 8.241, 8.983, 9.086 and 11.579 min respectively (Fig. 2 and 3)²⁴.

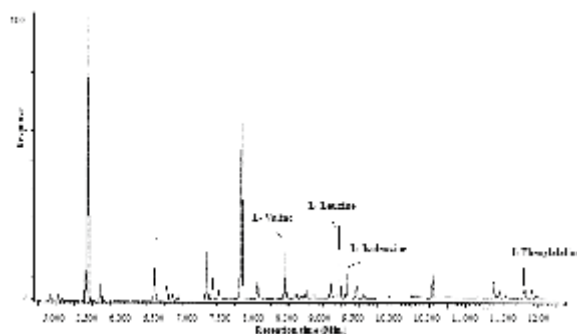


Fig. 2: Normal Chromatogram (Retention time (min) v/s Response)

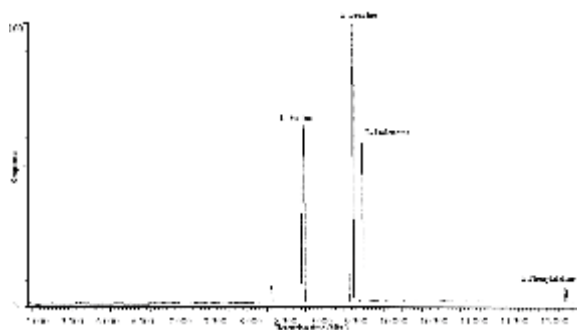


Fig. 3: Abnormal Chromatogram (Retention time (min) v/s Response)

g) Future Prospective

i. Clinical Protein Analysis

The success of mass spectrometry in the measurement of small molecules has pushed clinical mass spectrometry toward the analysis of peptides and proteins for diagnostic testing. A more targeted approach to quantitative protein analysis has emerged that differs significantly from the attempts to identify all proteins in a complex sample (termed shotgun proteomics). Quantitative clinical proteomic methods typically use enzymatic digestion of a complex sample, isotope dilution for normalization, and m/z transitions with tandem mass spectrometry. Quantitative analysis of proteins by mass spectrometry is an exciting area of laboratory medicine that faces several challenges before implementation²⁵.

NMR Spectroscopy applications

Magnetic resonance diagnostics (MRD) use an automated, high-throughput nuclear magnetic resonance (NMR) spectroscopy for the rapid identification and quantification of small-molecule metabolites in biofluid mixtures (blood, urine, saliva, cerebrospinal fluid, and others). Specifically, MRD involves using a high-field (400 MHz) NMR instrument equipped with a small-volume flow probe and robotic sample handler to rapidly load biofluid samples and to collect their ¹H

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NMR spectra. Spectral deconvolution software automatically assigns individual peaks to particular compounds and calculates concentrations from peak areas. MRD uses the principle of chemical shift separation to physically separate and identify individual compounds directly from ¹H NMR spectra, thus avoiding chromatographic separation steps (e.g., HPLC, gas chromatography, and capillary electro-phoresis). MRD is useful for rapid (<2 min per sample) qualitative and quantitative assessment of small-molecule metabolites.

NMR spectroscopy is not new to the field of clinical chemistry. Indeed several important applications have already been demonstrated in the area of diagnosis and therapeutic monitoring of metabolic disorders, in toxicologic and renal testing, and in the profiling of blood lipoproteins and cholesterol. An emerging approach to enable high-throughput in vivo toxicology is called metabonomics, which uses high-resolution NMR to rapidly evaluate the metabolic status of an animal.

A key limitation to all of these NMR approaches is that they depend on manual sample handling and/or manual (i.e., expert) spectral analysis. This has made most NMR approaches to clinical analyses far too slow or too costly for routine chemical profiling or high-throughput screening. Because MRD is fully automated (sample handling, spectral collection, and spectral analysis are all handled by robots or computers), this technique offers the potential for high-throughput, comprehensive, and inexpensive chemical analysis of a wide range of biofluid samples²⁶⁻²⁸.

a) Brain Imaging

NMR spectroscopy primarily used for the examination of the brain, where high-level contrast between grey and white matter provides anatomical detail which is not available with other imaging techniques. Similarly images are also available in the sagittal and coronal planes. A large variety of cerebral pathology has so far been investigated ranging from vascular diseases to inflammatory disorders and tumours. NMR images are also sensitive to flow and this can be manifested either by an increased signal from blood flowing into the slice or absence of a signal from blood flowing out of the slice. As a result it is possible to demonstrate occlusion to blood flow as well as abnormal blood flow in arterial malformation.

Demyelinating diseases such as multiple sclerosis are of particular interest and so examinations of infants and children where a high level of contrast between grey and white matter enables one to assess the progress of myelination. There is a rapid phase of myelination during the first two years of life followed by a slower phase continuing into the second decade. It is also possible to demonstrate the neural canal and the cord within it, as well as the intervertebral discs and the nucleus pulposus.

b) Soft tissue Imaging

Clinical evaluation to date has also extended to soft tissue structures of the thorax, such as the heart and mediastinum, and the large vessels. Organs in the abdomen (the liver and pancreas) and in the retro-peritoneal space (the kidneys and adrenal glands) are also being studied.

Soft-tissue structures in the limbs, particularly muscle, are well demonstrated by NMR spectroscopy which appears to be superior to computerised tomography or even ordinary soft-tissue radiography. Not only normal muscle and surrounding soft tissues but also tumours and soft tissues in the region of the joints are well defined. Bone has very few unstable protons and therefore does not produce a NMR signal. Nevertheless, soft tissues associated with bone, such as marrow, blood vessels and the surrounding structures, muscle tendons and joint capsules, which do produce signals are very likely to make NMR imaging important and of interest to orthopaedic surgeons. Presently, NMR study in diagnosis is very limited; with time, sufficient experience will be gained and the application of NMR in the diagnosis will be evaluated and established²⁹⁻³¹.

Fluoroscopy applications

Fluoroscopy is an imaging technique that uses X-rays to prevail real-time moving images of the internal structures of a patient through the use of a fluoroscope. The fluoroscope consists of an X-ray source and fluorescent screen between which a patient is placed. However, modern fluoroscopes couple the screen to an X-ray image intensifier and CCD video camera reserving the images to be recorded and played on a monitor. Fluoroscopy have several applications in the field of medicines to minimize radiation dosage, save working time, and prevent procedural accidents, but it is also useful to physiotherapist, veterinarians, criminologists, the army and airport security³².

Applications of PET/MRI and MRS

Nowadays, the magnetic resonance imaging (MRI) are usually used into the clinical practice, the magnitude of the operating magnetic field has depicted a slow but steady increase. The inherent increase in signal and spectral information has historically been paralleled by a general development of the scanner hardware and functioning logic, thus, greatly amplifying the information available. Hence, the standard fields for clinical and clinical research applications are 1.5 and 3 Tesla (T), respectively; meanwhile an increasing number of higher field (7-T and above) human scale scanners are devoted to the basic research (mainly related to neurosciences), and are now starting to be exploited in clinical research also. On the other hand, positron emission tomography (PET) scanners have acquired comparatively little, with research focusing, rather, on

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the development of new tracers. Both techniques deal a substantial flexibility, deriving from the physical origin of the nuclear magnetic resonance (NMR) signal in one case and from the properties of the tracer in the other. However, MRI enjoys the status of being both a structural and a functional technique, while PET scanners, even the most advanced ones, provide limited resolution. MRI is capable of imaging anatomy with very high spatial resolution, at organ and tissue level, and with high diagnostic sensitivity. By counterpoint, PET, using positron emitting radiopharmaceuticals, provides images of functional processes, at cellular and sub-cellular level, with very high diagnostic specificity and high tracer-detection sensitivity. The provable restrictions of PET studies (i.e., low spatial resolution) were initially improved, albeit partially, by combination with CT (Computer Tomography), because the building of hybrid PET/CT scanners presented fewer technical problems. PET/CT scanners, however, suffer from several limitations, including high patient dose and limited soft-tissue contrast, while the combination of PET with MR, by uniting the exquisite sensitivity of PET with the versatility of MR, allows the complete development of a much broader range of structural and functional data. Among the functional studies, complementary metabolic information can be gathered by PET and magnetic resonance spectroscopy (MRS).

Multiple research efforts are today devoted to identifying the conditions in which the advantages offered by PET/MR integration can go far beyond the simple acquisition of functional PET information under structural MRI guidance. In this context, it has been proposed that the combination of anatomical MRI, DWI and PET imaging, to get concerted data about the cellularity and biological activity of a tumor (i.e., by the use 18F-fluoro-deoxythymidine, or radiolabeled choline), may indeed result in enhanced accuracy in staging, as well as in assessing tumor spread to lymph nodes, especially not only in the pelvis but also in the mediastinum and head and neck region. MRS and PET can both be used to monitor metabolic processes and products *in vivo*. However, one has to bear in mind that these methods may differ not only in sensitivity and spatial resolution.

Moreover, PET and MRS are furnishing unique information on drug biodistribution, targeting, metabolism and pharmacokinetics/pharmacodynamics (PK/PD). PET using drugs radiolabeled with ^{11}C and ^{18}F is the technique of choice for PK/PD studies, whereas the nuclei most suitable for PK/PD studies using MRS are ^1H , ^{13}C , ^{19}F and ^{31}P . The development of a single combined hybrid PET/MRI instrument able to perform PET and MRI/MRS measurements simultaneously can allow a better study of drug kinetics³³.

PET/MRI could offer good potential in the management of diseases in which the MRS has already been shown to play an important role. PET/MRI could also be a

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powerful tool in identifying liver metastases and in characterizing pancreatic tumors, as demonstrated by previous studies on PET/MRI fusion images which showed high accuracy of this technique, exceeding the performance of PET/CT [31, 32]. Apart from these indications, the system could also be used for lymphoma, melanoma, bone and soft-tissue tumors, gynaecological, hepatic and colorectal malignancies, with the main imaging procedure being whole-body PET/MRI followed by regional MR acquisitions^{34, 35}.

In summation, nowadays MRI, functional MRI (fMRI), diffusion tensor imaging (DTI), diffusion-weighted imaging (DWI), MRS and perfusion imaging, offers more than just anatomical information. The fields of application are numerous: brain tumors, early diagnosis of degenerative diseases and other neurological and psychiatric disorders (i.e., Alzheimer, schizophrenia), oncology (prostate, breast, gynecological cancers), cardiovascular and inflammatory diseases. Further studies and with larger patient populations are required to confirm the clinical relevance of these united methods³⁶.

CONCLUSION

The implementation of spectrometry in the clinical laboratory has resulted in significant advancements in clinical pathology while adding a new layer of complexity to an already overwhelming aspect of medicine. Now, as always, extensive communication between laboratory directors and clinicians is a vital component of effective laboratory medicine and patient care. IR and Raman Spectroscopy have an application to soft tissue as well as bones and teeth, UV – Visible spectroscopy deals with enzymatic reactions, haemoglobin analysis, while Mass spectrometry is increasingly prominent in the clinical laboratory, NMR spectroscopy deals with Brain and soft tissue imaging. A basic understanding of the spectral techniques offers an aid to the diagnosis and monitoring of disease. Spectroscopy is an extremely powerful analytical tool that provides biologists/analyst/chemist with a fingerprint of the agent they are studying and whose immense future applications are only now being fully understood.

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