Spectroscopic parallelism in structural skeletonization and standardization of pharmaceuticals

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ABSTRACT

Spectroscopy based pharmaceuticals chemo fingerprinting and standardization is an essential intent to portrait molecular structures as well, a cemented platform to harvest diversified physiochemical characteristics of therapeutic chemo entity. Compared to classical wet techniques, the spectroscopic-framed-chemical analysis meritoriously distinguished from former in terms of sensitivity, accuracy, precession, rapidness, detection limit, spectrum, versatility, result reliability, intuiting data, and automated operation. Quest for "ideal medicine" is still a misnomer, however, may comply if being assisted with well planned and excellently executed spectroscopy methodology. The present paper is design to explore various prospective of different spectroscopic technique and their role in chemical evaluation and standardization of pharmaceuticals.

Keywords: Spectroscopy, ideal medicine, standardization, chemical identification, chemical shift, Quenching

INTRODUCTION[1, 2, 3]

Undoubtedly, quantum change in technological aspects, in-depth picturization of human pathophysiology, better understanding of proteomics & genomics paved the way round the globe for pharmaceutical industries in designing and development of *ideal medicine*. During the recent years, an integrated conjoint multidisciplinary effort of science and technology including internationalized research surplus the therapeutic armamentarium of globe from plethora of uncountable bunch of chemicals, pharmacologically active in one or other aspects. Instead of this fruitful universal momentum in design and development of novel medicines; risk benefit ratio, standardization, impurity profiling, chemical purity, dose formulation, generalize chemical instability, toxicological & metabolic profiling has to be pegged to a bare minimum level to explicit & equalize the chemical entity in terms of medicine and platform the same to meet global regulatory affair. Chemically, drugs constitute diversified class of essential organic, to a lesser extent inorganic compounds differing among in presence or absence of specific elements, their position, number, nature of functional groups & their orientation. Furthermore, nature of bonding, chemical structure, and molecular configuration or conformation practically imposes challenge for their unambiguous identification. As a social instrument, the reliability index of pharmaceutical is expressed in terms of standardization; defining safety, efficacy, quality, and quantity of drug-in-formulation as a prerequisite parameter for their global validation hence commercialization. To an extent, classical methods of chemical analysis provides answers to some of the suspected questions, but pictorially fails to engrave exact chemical portrait of a molecule. In addition, estimation of certain physiochemical aspects such as boiling & melting point, chemical test induced functional group determination certainly aids up in identification and purity justification, but again their genuineness is questionable for unknown/newer chemical entity. Beside these, large sample size, lack of sensitivity, detection limit, result reliability, long analytical time, and non-automation are some of the additional limitations associated with classical method of analysis. In recent years, spectroscopic methods are dynamically accepted as a newer analytical curricula globally, when conjunct with chemical method focally act as an indispensible tool to achieve the desired chemometric evaluation of pharmaceuticals including their stereochemical, and topochemical profiling. The superiority of spectroscopic technique is not only limited to drug analysis and their standardization, but also explores its utilization in the fields of novel drug design and

development. Quick & multidimensional evaluation of pharmaceutical samples irrespective of their nature, high sensitivity, ease of sample handling, small sample size, greater detection power, wide spectrum, reliability, and overall automation are some of the handsel of spectroscopic techniques. The present paper is design to summate various prospective of different spectroscopic technique and their role in evaluation and standardization of pharmaceuticals.

PRINCIPLE OF SPECTROSCOPY [4, 5]

The technique of spectroscopy is based on the simple phenomenon of interaction of electromagnetic waves with matter and to study various molecular and electronic changes associated with same on such interaction. On the basis of nature of interaction, the technique can be well categories broadly into absorption and emission spectroscopy. Since each pharmaceutical active ingredient including auxiliary components has unique molecular/ionic structural pattern hence their electromagnetic wave absorbing/emission properties are also unique thus aiding-up in their characterization. Currently, the technique of spectroscopy is preferred analytical as well as quality control tool universally over classical methods of pharmaceutical analysis.

COLORIMETRY [6, 7]

Colorimetry also known as visible spectroscopy is a type of absorption spectroscopic technique relies on the principle of light absorbing capacity of chemical or a biological system as a function of its concentration. Since the technique correlates color as a component of concentration thus conveniently termed as colorimetry. The technique shade-in 4000-7500 A° of electromagnetic spectrum virtually accessible by human eyes; a reason for its verbal pronouncing-visible spectroscopy. Ease of sample preparation, rapid analysis, universal acceptance, less sample quantity, and hassle free lower analytical cost are some of the key features of this technique.

The pharmacopoeial acceptance of this technique is still questionable however in assistance with suitable chromogenic reagent (color forming reagent) such as ferric chloride the technique is used in analysis of Doxycycline & Oxytetracycline at 490nm, likewise alcoholic extract of digitoxin can be efficiently determined colorimetrically at 495nm using alkaline picric acid as chromogen. Apart from this, several pharmacologically active therapeutic moieties such as Rifampicin, Dithranol, Clonidine, Folic acid, Ergotamine, Riboflavin, and Clofazimine can be analyze by this technique by incorporating appropriate chromogen during estimation. Clinically, the colorimetric philosophy is used in biochemical profiling of body fluid to establish associated pathological abnormalities. Chemical identification, purity justification, structure elucidation, molecular weight determination, estimation of pka value of indicator are some other, but lesser known applications of visible spectroscopy.

U.V. SPECTROSCOPY [8]

UV spectroscopy is yet an another spectroscopic technique based on the phenomenon of absorption of waves especially of ultraviolet region of electromagnetic spectrum thereby inducing electronic transition of valence electrons from lower energy level to higher energy level within a molecule, generating characteristic absorption peaks unique for their quantitative and qualitative estimation. The *σ to σ*, n to σ*,* π to π*, n to π*, and d to d* are some of the common electronic transitions associated with UV spectroscopy. On the basis of absorption of specific wavelength in a particular electromagnetic spectrum of ultraviolet region by a compound, the technique is further be subdivided into two main categories, far or vacuum UV spectroscopy (<200nm) and near UV spectroscopy (200-400nm). In compared to colorimetry, the UV spectroscopy is universally accepted and officially valid tool for identification, analysis, and quality control of numerous pharmaceuticals. Normally, UV spectroscopy is free from utilizing chromogen or color forming derivatives for analytical purposes and can be used for hassle free estimation of colorless organic compounds. For the purpose of analytical, quality control, and/or standardization workout sample is initially dissolved in suitable solvent and further investigation of same is done with an aid of instrument known as UV-spectroscope. Transparency, solubility, no self absorbance, polarity, purity, environmentally friendly, non toxicity, easy disposability, affordability, and availability are some of the common parameters which must be considered prior to selecting appropriate solvent.

INFRA RED SPECTROSCOPY [9, 10]

IR (infra red) spectroscopy umbrella 0.8µm to 1000µm region of electromagnetic spectrum, acting versatile tool in identification and critical examination of majority of organic and inorganic pharmaceuticals except homonuclear medicines lacking permanent dipole moment. Official compendia for this purpose provides comprehensive authentic IR spectra of pure pharmaceuticals that are used for comparative workout in analytical as well as synthetic laboratories to establish their purity justification for the purpose of commercialization. The USP-XIX and NF-XIV elaborates assays of numerous pharmaceutical dosage forms in their respective solvents such as Triprolidine HCl (cyclohexane), Methocarbamol injection (chloroform), Methocarbamol tablets (chloroform), Cyclizine lactate injection (cyclohexane), Iodochlorhydroxyquin ointment (potassium bromide pellet with Fe $(SCN)_3$), Quinethazone tablets (cyclohexane), and Simethicone tablet (carbon tetrachloride). IR spectra, from applicability point of view are conveniently categories into near IR (NIR), mid IR (MIR), and far IR (FIR) regions. The near IR/NIR region (0.8-2.5µm) also known as overtone region, is considered as high energy region associated with overtones or harmonic vibrations within a molecule. Since the region present in proximity with visible region of electromagnetic spectrum and responsible for numerous lower intensity absorption bands thus provides only limited information regarding chemical nature of pharmaceuticals. However, moisture content determination and estimation of iodine number are some of the prominent pharmaceutical applications of this region. From analytical prospective, the mid IR/MIR region (2.5-50µm) plays crucial role not only for qualitative but also for quantitative philosophies associated with pharmaceuticals, since most of the organic as well as inorganic therapeutic molecules are capable of absorbing IR frequency restricted solely to this region thereby experiencing vibrational and rotational changes (thus this region is also known as *vibrational-rotational region*) leading to generation of set of identifiable dips (IR peaks) that are specific & unique for each molecule. On the basis of nature of analytical and/or characterization workout done in assistance with differential IR frequency, the MIR-region is further be categories into group frequency (2.5-7.5µm) and finger print region (7.5-15µm) each having their own specific characteristics.

MASS SPECTROSCOPY [11]

Mass spectroscopy is highly advanced hyphenated instrumental technique devises primarily to determine molecular mass of chemical entities on the basis of their mass/charge ratio via *mass spectroscope*. Sophistication & technical advancement in instrumentation, extensive computerization of data handling technique, and refinement in methodological aspect elaborated its scope to gather information regarding chemical kinetics, structural elucidation, reaction mechanism, and quantitative multi-component analysis of pharmaceutical formulations. The technique is based on the principle of sample ionization with the means of ionizing sources (electron impact, chemical ionization, field ionization, surface ionization, electric ionization, fast atom bombardment (FAB), and matrix assisted laser desorption/ionization technique-MALDI); ions (molecular ion, fragmented ion, rearrangement ion, metastable ion, multicharged ion) so formed are separated according to their mass/charge ratio under the effect of electric and/or magnetic field via mass analyzer (single focusing, double focusing, quadrapole mass analyzer, time of flight (TOF) analyzer, ion trap analyzer cum detector) and finally detected by suitable detector system employed such as photographic plate, faraday cup, channel electron multiplier (CEM), scintillation detector, and electron multiplier. The mass spectra so obtained provide characteristic information regarding molecular weight of sample under investigation. Some of the common advantages of mass spectroscopy includes instrumental automation, less response time, accurate as well as precise molecular weight determination, handling of wide varieties of components, simultaneous analytical profiling of single and multi-component mixture, least sample size, and ease of sample preparation. Mass spectroscopy plays pivot role in various interdisciplinary scientific fields however in terms of pharmaceuticals sciences the key applicability of same includes molecular weight determination of known and unknown synthetic, semisynthetic as well as naturally occurring pure therapeutic moieties with utmost accuracy and precision however the

technique fails to discriminate geometrical and optical isomers. Impurity profiling is yet an important pharmaceutical prospective of mass spectroscopy by comparing spectral data of pure therapeutic moiety with test component. Simultaneously conjunction of mass spectroscope with other instrumental techniques such as gas chromatography and HPLC enhanced their technical applicability and analytical scope towards elaborative clinical studies including pharmacokinetic & metabolite profiling of numerous pharmaceuticals, peptidal sequencing, and forensic study.

NUCLEAR MAGNETIC RESONANCE [12, 13]

Isidor Rabi (1938) describes highly sophisticated form of absorption spectroscopy-Nuclear magnetic resonance (NMR), was awarded Noble prize (1944) for his accreditation. In contrast to other spectrometric techniques, which concentrate upon electronic transitions, the NMR mainly deals with atomic exploration of components within an electromagnetic region (4MHz-750MHz) to characterize its chemical portrait. The spectra obtained by absorption of radiofrequency wave by nuclei provide essential information such as types of proton, their number, and respective electronic environment. Chemical shift symbolically denoted as δ and expressed in terms of ppm is an essential criterion to define the position of proton with in a molecule with respect to internal standard tetramethylsilane (TMS). The value of chemical shift not only describes the position, but also enumerates the local chemical environment of proton where it resides. Shielding & deshielding of protons, electronegativity, space effect, solvent, hydrogen bonding, and magnetic anisotropy are some of the major factors affecting chemical shift. Like other spectrometric techniques, nuclear magnetic resonance has been extensively used as analytical as well as research tool however the greater cost of instrumentation somehow resist its institutional affordability. The technique is employed for assay mixed pharmaceutical formulations such as Quinidine & hydroquinidine, Meprobamate & Mebutamate, Methsuximide & Phensuximide capsules, Trimethoprim & Sulphamethoxazole, Meclizine & Methaqualone. In addition to this, NMR is used in estimation of iodine value of natural oils and results are equally comparable with Wijs method. Clinically, Magnetic resonance imagining (MRI); a highly resolved form of NMR is used as a non invasive tool to study body's internal structures. Beside these, the NMR contributes towards structure identification of synthetic as well as natural products. Study of hydrogen bonding, determining activation energy, isotopic elucidations, exploring protein-ligand complex, analysis of tautomer and conformation analysis are some other well defined applications of nuclear magnetic resonance.

FLUORIMETRY [14]

Florescence and phosphorescence are two important equivalent manifestations of photoluminescence involving bounce back mechanistic pathway for movement of electrons from higher to lower energy state thus providing rigid platform for fluorimetry; a type of emission spectroscopy. Technically, fluorescence involves emission of longer wavelength radiation for a time interval of 10^{-12}-10^{-9} seconds while phosphorescence involves emission of radiation at a time delay greater than 10^{-8}-10 seconds after absorbing electromagnetic radiation. In comparison with absorption spectroscopy, the technique of fluorimetry is advantageous in terms of selectivity as well as sensitivity, and capable of handling sample at a concentration of micro to nanograms. The main limitation of this technique is confinement to systems that are either self fluorescence or derivatize to fluorescence. Since fluorimetry measures intensity of emitted radiation as a function of concentration, thus any factor involving reduction in fluorescence intensity affects the final results making it false positive. This phenomenon of reduction in fluorescence intensity is termed as *quenching* which may be caused due to internal as well as external factors such as complexation, radiationless energy transfer, extensive collision between molecules, pH, self absorption of emitted radiation, and high concentration. In addition, temperature, viscosity of medium, scattering and absorption of radiation, nature of molecule, chemical functionalities of system, chemical structure & its rigidity, nature of bonding, and polarity of solvents are some of the other key factors modifying fluorescence intensity. Pharmaceutically, fluorimetry is used in assay of Aminacrine, Proflavine, Ergometrine, Desipramine, Quinine, Indomethacin, and Morphine. With certain

chemical modification, the same technique is used in estimation of Chloroquine, Imipramine, Isoniazid, Thiamine, Diphenyl hydantoin, and Nicotinamide.

CONCLUSION

Indubitably, the technique of spectroscopy is a powerful instrumental tool to access multidimensional chemical portrait of known as well as unknown chemical species including pharmacologically active moieties. Generally, most of the spectroscopic techniques irrespective to their applicatory measures are based on either absorption or emission phenomenon, when correlates expresses their chemical behavior. Rapid & in-depth hassle free electronic and atomic exploration of therapeutic moiety popularize the technique globally as an validated tool to access multidimensional evaluation of pharmaceutical samples irrespective of their physical nature.

↓REFERENCES

1. Taylor, J.A.; "Quality Assurance of Chemical Measurement"; Lewis publisher incl.; Chelsea, MI, 1987.
2. Christian, G.D.; Analytical Chemistry, 6th edition; John Wiley & Sons, Hoboken, NJ, 2004.
3. Sigga, S.; Survey of Analytical Chemistry, McGraw-Hill, NY, 1968.
4. West, W.; "Technique of Organic chemistry" Vol IX, In Chemical application of spectroscopy, 2nd ed., Interscience, NY, 1968.
5. Silverstein, R.M. et al; Spectroscopic identification of organic compounds, 5th edition; Wiley Interscience; NY; 1980.
6. Babko, A., Pilipenko, A.; Photometric analysis; Mir Publishers, Moscow; 1971.
7. IUPAC Commission on Spectrochemical and other optical procedure for analysis; Spectrophotometric data for colorimetric analysis; Butterworth Publisher ltd; London 1963.
8. Rao, C.N.R.; Ultravoilet and visible spectroscopy; Butterworths, London 1975.
9. Dyen, J.R.; Applications of absorption spectroscopy of organic compounds; Prentice Hall; NY; 1965
10. Nakanishi, K.; Infrared absorption spectroscopy; 2nd edition; Holden-Day; San Fransisco; 1997.
11. Pramanik, B.N.; Applied electrospray mass spectrometry; Marcel Dekket; NY; 2002.
12. Harris, R.K.; Nuclear magnetic resonance spectroscopy; Longman Scintific and Lecturers; ND; 1993
13. Bhacca, N.S., Williams, D.H.; Applications of NMR spectroscopy in organic chemisty-illustrated from steroid field; Prentice Hall; San Francisco; 1964.
14. Lindon, J.C.; Encyclopedia of spectroscopy and spectrometry; Tranter GE and Holmes J.L.; Academic, San Diego, 1999.