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Review Article

Pharmaceutical analysis for Novel drug formulation

Highlights of Spectroscopic Analysis – A Review

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Abstract: Spectroscopic techniques are broadly employed to study the chemical structure of an analyte accurately. The electromagnetic radiation used in each method is allowed to interact with the molecule. The electric and magnetic property of the radiation is interacted with the similar properties of the chemical substance. Hence, the analyte is identified and characterized for the presence of atoms, bonds, functional groups, basic nucleus, molecular formula and molecular weight. The study is highly relevant to have a reference covering most of the important analytical techniques. The investigation implies content of basics for the interpretation of a spectrum, rules/laws followed, sampling techniques, sample cells, mechanism involved in analysis, units of measurements, spectral ranges, light sources, nature of samples to be analyzed, kind of solvents utilized, appearance of spectra and major uses of individual spectroscopic analysis. This also includes every significant aspect of various spectroscopic methods and represents qualitative & quantitative parameters which insists for the research and experiments carried by the specific spectral process. Thereby, review regarding combination of entire spectroscopic principle, instrumentation and applications will be giving fruitful information to all analytical persons Pharma students.

Keywords: Atoms; Molecules; Spectrum; Interpretation; Applications.

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1. INTRODUCTION

Spectroscopy is the evaluation of the interaction between matter and electromagnetic radiation. Reliably, it emerged through the study of visible light dispersed accord to its wavelength by a prism. It is associated with the absorption, emission or scattering of electromagnetic radiation by atoms or molecules. The consequence of such an interaction has researchers contemplating analytical findings[1]. UV-Vis Spectroscopy is accustomed to conclude analyte concentration by the absorption of light over the range through a liquid sample. Infrared spectroscopy is choicely used in industry besides in research. It is a conservative and reliable approach for measurement, quality control and dynamic measurements[2]. It is applied to determine functional groups in molecules. Generally, stronger bonds and light atoms will vibrate at a high stretching frequency. Nuclear magnetic resonance spectroscopy is the widest use to determine the structure of organic molecules in solution and used in advanced medical imaging techniques, such as in magnetic resonance imaging (MRI)[3]. Atomic absorption spectrometry is an analytical technique that quantifies the concentrations of components. It is thus sensitive that it can work through parts per billion of a gram in a test. The technique comprises wavelengths of light specifically absorbed by an element. Flame emission spectroscopy allows quantitative measurement of the optical emission from excited atoms to examine analyte concentration. These extreme temperature atomization sources provide sufficient energy to endorse the atoms into high energy levels. The atoms crumble back to lower levels by emitting radiation[4]. Mass spectrometry is an analytical weapon beneficial for estimating the mass-to-charge ratio (m/z) of one or more molecules present in a sample[5]. Fluorescence spectroscopy is valuable in applications such as detecting and quantifying organic compounds. Industrial applications include testing surface quality and cleanliness. Laser-induced fluorescence spectroscopy permits exciting lasers to energies fluorophores in the proposed constituents which are emitted during relaxation within a reach of nanoseconds[6]. Electron Spin Resonance spectroscopy determines the absorption of microwave radiation resemble to the energy splitting of an unpaired electron when it is placed in a strong magnetic field. X-ray powder diffraction is a constructive tool for the identification of unknown crystalline materials and determination of solids which is decisive to studies in geology, environmental science, material science, engineering and biology[7]. From the correlation of entire spectroscopic analysis, the aim and objective of this review is to accomplish content of learning about each method in a single article. Therefore, the highlights of every important feature of spectroscopy are focused in this study.

2. METHODS/STUDY

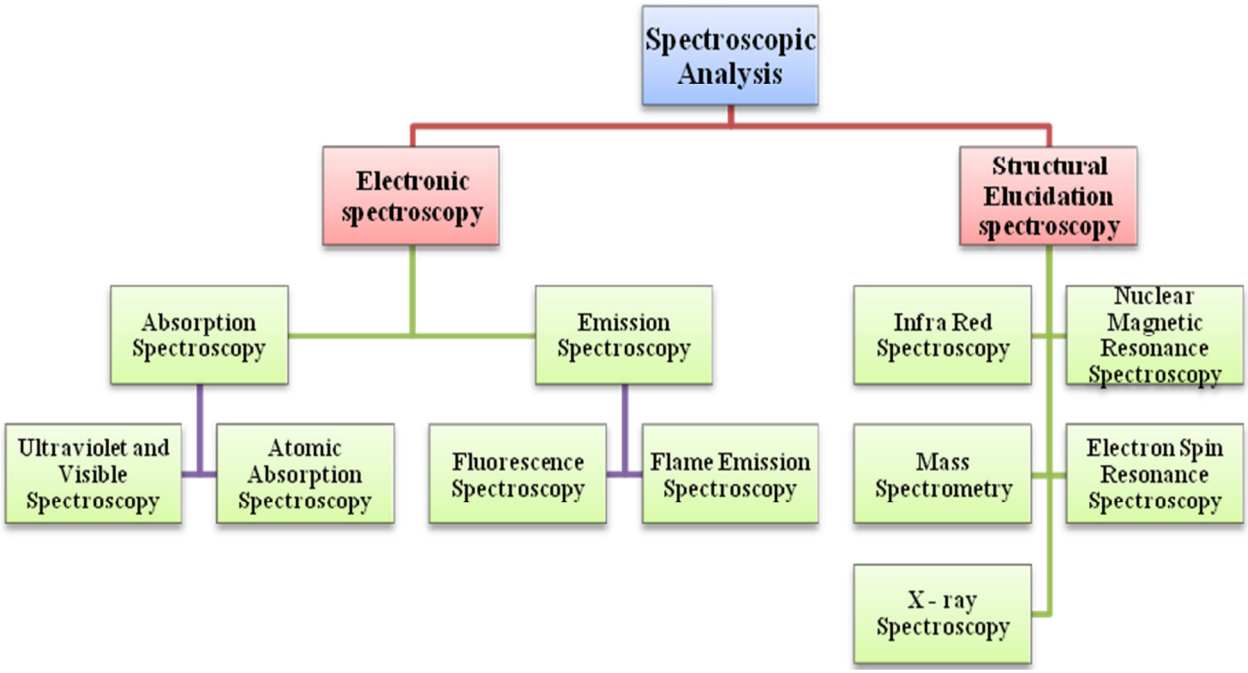

Fig 1. Classification of Spectroscopic Analysis

2.1 Comparison and Difference of Principle, Instrumentation and Applications between various spectroscopic analysis (Figure 1 and table 1)

2.1.1 Electronic Spectroscopy [8-12]

Table 1. Comparison and Difference of Principle, Instrumentation and Applications between Electronic Spectroscopy

S. No.	Parameters	Electronic Spectroscopy			
		Absorption Spectroscopy		Emission Spectroscopy	
		UV/Visible Spectroscopy	Atomic Absorption Spectroscopy	Fluorescence Spectroscopy	Flame Emission Spectroscopy
01	ElectroMagnetic Radiation used	Ultraviolet and Visible radiation	Ultraviolet and Visible radiation	Ultraviolet and Visible radiation	Ultraviolet and Visible radiation
02	Light Source	D2/ W lamp	Hollow Cathode lamp	Mercury arc/ Xenon arc lamp	No radiation source is used
03	Monochromators	Filters/ prisms/ grating monochromators	Filters/ prisms/ grating monochromators	Filters/ prisms/ grating monochromators	Filters/ prisms/ grating monochromators
04	Sample cells/ Cuvettes	Cylindrical/ rectangular Quartz/ Glass cells	Cylindrical flat bottomed Glass cells	Cylindrical/ rectangular Glass cells	Cylindrical flat bottomed Glass cells
05	Phase of Sample analyzed	Monophasic Colourless/coloured diluted solutions	Monophasic diluted solutions	Monophasic Colourless diluted solutions	Monophasic diluted solutions
06	Solvents used	Polar solvents like Distilled water and Methanol	Polar solvents like Distilled water	Polar solvents like Distilled water and Methanol	Polar solvents like Distilled water
07	Concentration range of analysis	µg – mg	µg – mg	µg – mg	µg – mg
08	Physical properties of sample analyzed	Absorption	Absorption	Emission	Emission
09	Chemical properties of sample analysed	Chromophores	Valence electrons	Fluorophores	Valence electrons
10	Principle	Change occurs at electronic level of a molecule	Change occurs at electronic level of an atom	Change occurs at electronic level of a molecule	Change occurs at electronic level of an atom
11	Mechanism	Moving of electrons from HOMO (ground state) to LUMO (excited state)	Moving of electrons from HOMO (ground state) to LUMO (excited state)	Moving of electrons from LUMO (excited state) to HOMO (ground state)	Moving of electrons from LUMO (excited state) to HOMO (ground state)
12	Detectors	Photo tubes, Photovoltaic cells and PhotoMultiplier tubes	Photo tubes, Photovoltaic cells and PhotoMultiplier tubes	Photo tubes, Photovoltaic cells and PhotoMultiplier tubes	Photo tubes, Photovoltaic cells and PhotoMultiplier tubes
13	Appearance of spectrum	Absorbance versus Wavelength (Figure 2)	Absorbance versus Wavelength (Figure 3)	% Fluorescence intensity versus Wavelength (Figure 4)	% Flame intensity versus Wavelength (Figure 5)
14	Qualitative respect	λ_{max} (λ_{ex})	λ_{max} (λ_{ex})	λ_{max} (λ_{em})	λ_{max} (λ_{em})
15	Quantitative respect	Concentration versus Absorbance	Concentration versus Absorbance	Concentration versus % Fluorescence intensity	Concentration versus % Flame intensity
16	Applications to analyze	Chromophoric substances	Metallic salts	Fluorophoric analytes	Metallic salts
17	Interpretation Laws	Beer – Lambert's law	Beer – Lambert's law	Beer – Lambert's law	Beer – Lambert's law
18	Interpretation Rules	Woodward – Fieser rule	Bohr's equation	----	Bohr's equation
19	Radiation Range of analysis	200 – 800nm	200 – 400nm	400 – 800nm	400 – 800nm
20	General applications	Detection of impurities Structure elucidation of organic compounds Qualitative analysis Chemical kinetics Detection of functional groups Examination of polynuclear hydrocarbons Molecular weight determination As HPLC detector	Environmental Clinical Pharmaceutical Agricultural Petrochemicals	Laser induced fluorescence spectroscopy of human tissues for cancer diagnosis Study of Marine Petroleum Pollutants Accurate determination of glucose	Environmental Clinical Pharmaceutical Agricultural Petrochemicals

2.1.2 *Appearance of Spectrum*

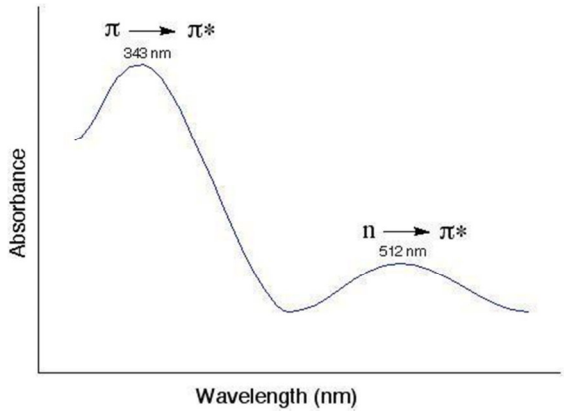

Fig 2: UV/Visible spectrum

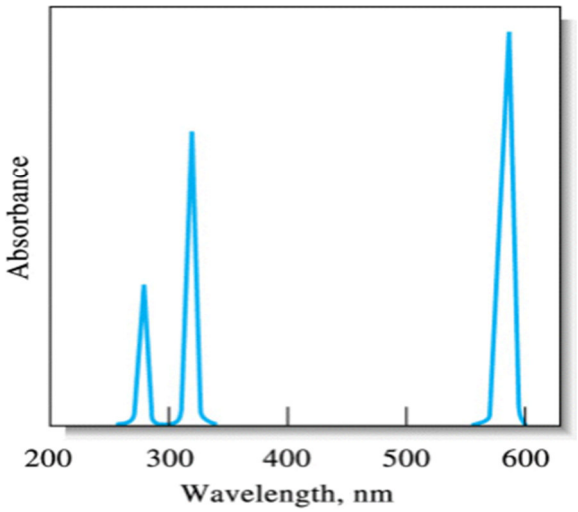

Fig 3: Atomic Absorption spectrum

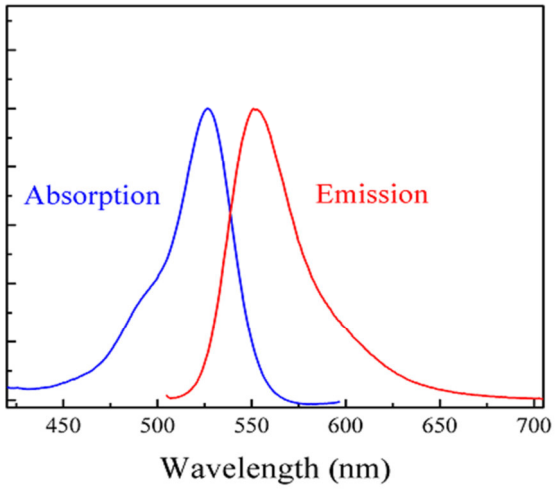

Fig 4: Fluorescence spectrum

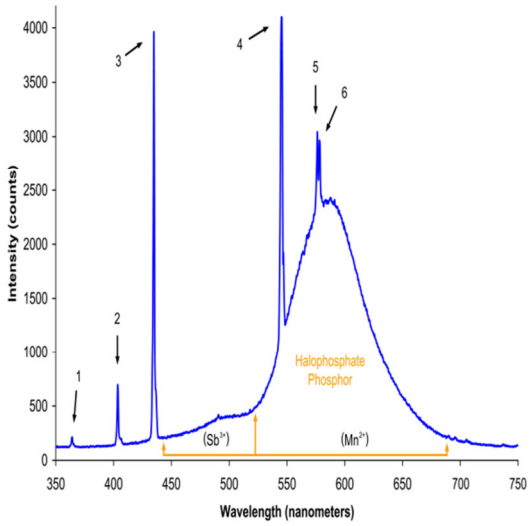

Fig 5: Flame Emission spectrum

2.2 *Structural Elucidation Spectroscopy (Part I, table 2):*

Table 2. Comparison and Difference of Principle, Instrumentation and Applications between Structural Elucidation Spectroscopy (Part I)[13,14]

S. No.	Parameters	Structural Elucidation Spectroscopy		
		IR Spectroscopy	NMR Spectroscopy	Mass Spectrometry
01	ElectroMagnetic Radiation used	Infra red	Radio Waves	No radiation is used
02	Light Source	Nernst Glower Globar Source	NMR Probes	High energy electron beams
03	Monochromators	Filters/ prisms/ grating monochromators	Filters/ prisms/ grating monochromators	Electric and Magnetic field Analyzers
04	Sample cells/ Cuvettes	Circular shaped Alkali metal halide windows	Cylindrical Glass cells	Septum type Injectors
05	Phase of Sample analyzed	Solid/ Liquid/ Gas	Monophasic diluted solutions with Tetramethylsilane as internal standard	Gas
06	Solvents used	Potassium bromide/ Nujol mull (Solids), Carbon tetrachloride/ Carbon disulphide (Solutions)	Deprotonated/Deuterated Solvents	Volatile solvents
07	Concentration range of analysis	ng – µg	µg – mg	ng – µg
08	Physical properties of sample analysed	Molecular Vibrations	Nuclear Magnetic Resonance	Ionization/ Fragmentation
09	Chemical properties of sample analysed	Force Constant/ Bond strength and Atomic mass	Protons (Odd Mass number)	Positively charged ions
10	Principle	Change occurs at vibrational energy level	Spin changes occurs at nuclear level	Acceleration of ions based on m/e ratio
11	Mechanism	Stretching and bending vibrations	Flipping of nuclear spin	Arrangement of ions as per m/e ratio by analyzers
12	Detectors	Thermopiles/ Thermoelectric/ Thermocouple detectors (MCT detector)	PhotoMultiplier tubes	Ion detectors Electron Multipliers Faraday cups Ion – to – Photon detectors Micro channel plates
13	Appearance of spectrum	% Transmittance versus Wavenumber	Intensity of signal versus Chemical shift	% Relative intensity versus Mass to Charge ratio
14	Qualitative respect	Wave numbers in Fingerprint region and	Chemical shift	Mass to Charge ratio

		Functional group region		
15	Quantitative respect	Concentration versus % Transmittance (Figure 6)	Concentration versus Intensity of signal (Figure 7)	Concentration versus % Relative intensity (Figure 8)
16	Applications to analyze	Molecules with asymmetric and Dipole moment	Structural elucidation of Organic molecules	Molecular formula and Molecular mass
17	Interpretation Laws	Hooke's law	-----	-----
18	Interpretation Rules	3n – 5 (Linear molecule) 3n – 6 (Non – Linear molecule)	n + 1 rule Pascal's triangle Coupling Constant	Nitrogen rule Ring rule Rule of 13 Stevenson's rule Even Electron rule Isotopic rule
19	Radiation Range of analysis	4000 – 667cm^{-1}	60 – 200MH_z	Depends on Molecular weight
20	General applications	Identification of substances Studying progress of reactions Determination of molecular structure Detection of impurities Monitoring the structural plasticity of plant cell walls Protein quantitation Non-invasive blood glucose monitoring	Protein hydration Ionization state Protein folding Molecular dynamics Solution structure	• Pharmaceutical analysis • Bioavailability studies • Drug metabolism studies • pharmacokinetics • Characterization of potential drugs • Drug degradation product analysis • Screening of drug candidates • Identifying drug targets • Biomolecule characterization • Proteins and peptides • Oligonucleotides • Environmental analysis • Pesticides on foods, Soil and groundwater contamination • Forensic analysis/clinical

2.2.1 Appearance of Spectrum

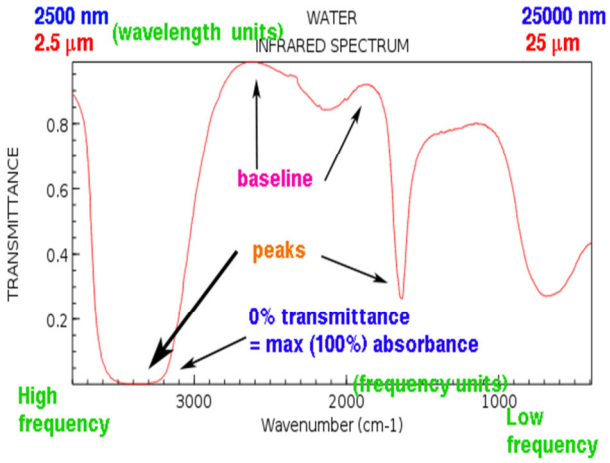

Fig 6: FTIR spectrum

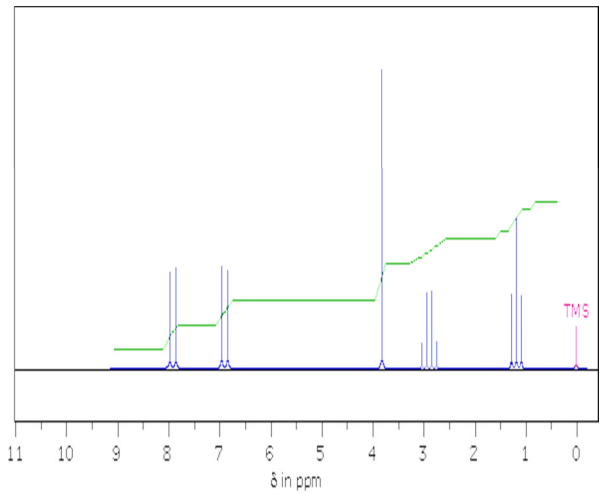

Fig 7: NMR spectrum

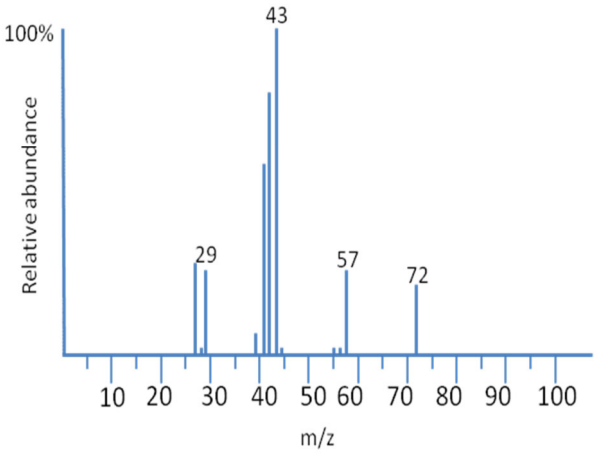

Fig 8: Mass spectrum

2.3 Structural Elucidation Spectroscopy (Part II, table 3)

Table 3. Comparison and Difference of Principle, Instrumentation and Applications between Structural Elucidation Spectroscopy (Part II)[15-17]

S. No.	Parameters	Structural Elucidation Spectroscopy	
		ESR Spectroscopy	X – ray Diffraction Spectroscopy
01	Electro Magnetic Radiation used	Microwaves	X – rays
02	Light Source	Klystron source	Metal atom with high energy electron beams
03	Monochromators	Isolator and Attenuator	Crystal type monochromator
04	Sample cells/ Cuvettes	Cylindrical Glass cells	Metallic pan
05	Phase of Sample analyzed	Monophasic diluted solutions with Diphenyl Picryl Hydrazyl radical as internal standard	Solids
06	Solvents used	Polar Solvents	-----
07	Concentration range of analysis	µg – mg	µg – mg
08	Physical properties of sample analysed	Electron spin Resonance	Diffraction
09	Chemical properties of sample analysed	Free radicals/ Lone-pair electrons	Electrons in crystal planes
10	Principle	Spin changes occurs at electron level	Diffraction of X – rays by electrons in a crystal plane
11	Mechanism	Flipping of electron spin	Analysing diffraction pattern
12	Detectors	Square law detector at low energy levels	Photographic detectors

		Linear detector at high power levels	Coulter – Counter methods Geiger – Muller counter Scintillation counter (Semi – conductive detectors)
13	Appearance of spectrum	Intensity of signal versus External/Applied magnetic field	Intensity of signal versus Angle of Diffraction
14	Qualitative respect	Applied magnetic field	2θ (Angle)
15	Quantitative respect	Concentration versus Intensity of signal (Figure 9)	Concentration versus Intensity of signal (Figure 10)
16	Applications to analyze	Structural elucidation of Organic molecules	Particle size and crystal structure determination Polymer characterization
17	Interpretation Laws	-----	Bragg's law
18	Interpretation Rules	2nI + 1 rule (No. of Hyperfine lines) Pascal's triangle Coupling Constant	
19	Radiation Range of analysis	9,000 – 10,000MH_z	0 – 100Å
20	General applications	Biology (as spin – labels or spin – probes to study dynamic organization of lipids in biological membrane) Chemistry (in chemical reactions) Physics (Electrochemical systems and in materials exposed to UV light)	Crystal perfections, Crystallite size, % Crystallinity Quantitative analysis, Phase identification, Change in lattice, Atomic substitutions

2.3.1 Appearance of Spectrum

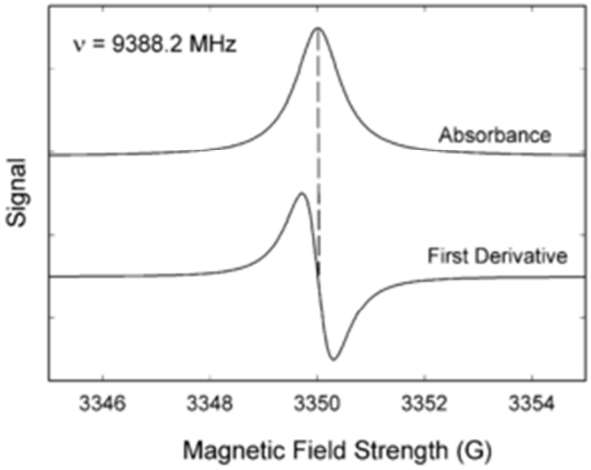

Fig 9: ESR spectrum

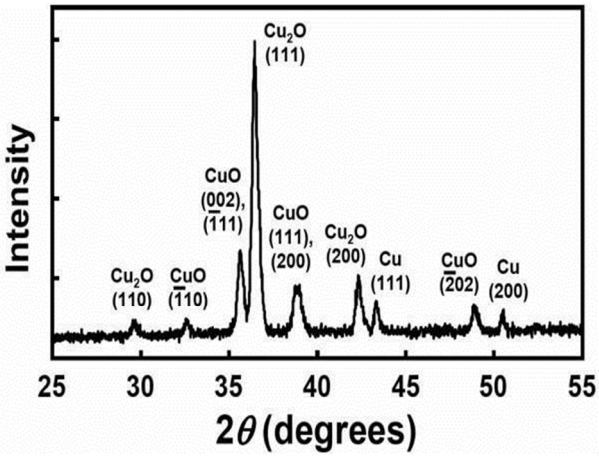

Fig 10: XRD spectrum

3. CONCLUSION

The study includes information on different electromagnetic radiations, their range in EMR spectrum, the part of instrument to convert it into monochromatic light, individual sample cells, actual principle and mechanism of sample and radiations, various detectors, laws governing each analysis, rules used for interpretation and general applications of all basic spectroscopic techniques. Absolutely, this will guide to get idea regarding the fundamentals of each method in concise format. Benefiters are researchers, scientists, chemists, pharmacy students, pharmacists, clinical chemists and most importantly competitive exams appearing candidates.

4. ACKNOWLEDGEMENT

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5. AUTHORS CONTRIBUTION STATEMENT

Sasikala M, Mohan S and Swarnakumari S contributed maximum to complete this study and bring it to publication.

6. CONFLICTS OF INTEREST

Conflict of interest declared none.

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