Interpretative Spectroscopy

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Lecture-01
Various Analytical Techniques and their applications

Hello everyone, I am sure you are all having an excellent time. It is a lecture series of 60 lectures. So, this is the first lecture. This as I had mentioned, is about interpretative spectroscopy. So, what I would do in next 60 lectures; I will be discussing about some of the very important spectroscopic methods such as NMR, IR, UV visible, EPR and also mass spectrometry and Mossbauer spectroscopy. So, in this one, as I already told in the introduction, without going deep into the theoretical aspects, but giving some fundamentals and basics, I would try to make you familiar with the interpretation and elucidation of the structures.

So, with this, let me start my first lecture. The spectroscopic methods are also come under analytical chemistry. So, what is the role of analytical chemistry? Analytical chemistry involves measuring the chemical composition of natural and synthetic materials. Analytical techniques are essentially used to identify the substances which may be present in a material and determine the exact amount of the identified substances.

That means, it involves both qualitative and quantitative analysis of an unknown sample or samples obtained from natural resources or it can also be mentioned in a different context. The resolution of a chemical compound and its proximate or ultimate parts that is the determination of its elements or of the minor impurities, byproducts or substances it may contain. So, that means, to know how pure the sample is, and what are the composites of that one, and also what are the constituent elements. So, all these details would come from analytical chemistry and spectroscopy is one of the analytical methods. So, how to classify these analytical methods? We can classify broadly into two methods.

One is qualitative analysis, the second one is quantitative analysis. What is qualitative method? It provides information about the identity of atomic or molecular species or the functional groups present in the sample that we are analyzing. That means, the determination of the components of an unknown sample. So, how we do it? The best way is to go for spectroscopic methods, and what is quantitative method? In contrast to qualitative method, it gives numerical information as to the relative amounts of one or more of these components. Unless we know what, it is? it is not advisable to go for quantitative method or quantitative method is of little use unless we know what it is.

So, from that point of view, qualitative method is very important and once of knowing what the substance is to quantify, we should go for an appropriate quantitative method. So, that means, the determination of the quantity of the component in a sample. So, how to do it? We can do it in several methods. I would come back to that one. The separation of analytes by using one of the methods, we use like precipitation, extraction or distillation and qualitative analysis means reaction.

How we do qualitative analysis? By reacting analytes with reagents that yielded products that could be recognized by their color, boiling or melting points or solubilities, optical activities or refractive index, if they are optical active compounds. Quantitative analysis can be performed by either gravimetric or titrimetric methods. What is gravimetric method? Determination of the mass of the analyte or some compound produced from the analyte. So, in titrimetric method what we do is, we will measure the volume or mass of a standard reagent required to react completely with the analyte. So, indirectly we will be knowing the quantity of the substance.

This is a typical analytical arrangement or analytical instrument. Whether you take analytical instrument or spectroscopic instrument, some of these things are imbibed in the instrument. So, first we have to look for a right signal source and then we have to put the sample and then the signal, the electromagnetic passes through that one and then we will

be having signal analyzer and then signal detector and transducer will be there and then it will be fed to data processor and then eventually we get the result. So, this is how the components work in a typical instrument. Then let us try to classify different types of analytical methods and what we are going to get from those methods. So, in gravimetric method, weight of pure analyte or of a stoichiometric compound containing it and volumetric volume of standard reagent solution reacting with the analyte in spectrometric intensity of electromagnetic radiation emitted or observed by the analyte the unknown sample and radiochemical intensity of nuclear radiation emitted by the analyte and in mass spectrometry abundance of molecular fragments derived from the analyte along with the molecular mass and chromatographic method, physicochemical properties of individual analytes after separation and in case of thermal method, physicochemical properties of the sample as it is heated and cooled; what would happen to a sample with temperature variation.

So, then where exactly we use it? In all disciplines of science, we use it: chemistry, physics, biochemistry, medicine, forensic materials. In case of chemistry invariably it is used in organic chemistry, organic chemistry as well as physical chemistry. Then what are the applications: the first one and utmost important one is fundamental research that means we always make new molecules and then or we look for analysis of natural products or drugs and the product development in industries and pharmaceutical companies, development of alloys polymer composites resins or new materials and also in industries; product quality control analysis of raw materials and finished products. So, this is where analytical instruments are heavily used and what are the applications. Yes, monitoring and control of pollutants very important that means heavy metals and pesticides contamination and then the metal content of ores. How much quantity of metal is there in a given ore, whether it is worth extracting or economical. In medical and clinical studies, indicators of physiological disorders and in food analysis and also labeling and origin. So, then that means: analytical technique and chemical analysis is an indispensable component of modern technology and always these two components have developed hand in hand. So, here the analytical techniques can be classified again as classical techniques, instrumental techniques, and historically derived and artificial.

Analytical problems and their solutions come from analytical methods. For all analytical problems, the solutions whether qualitative or quantitative follow the same basic pattern which can be described using seven entities. What are those seven entities? Yes first: the choice of the method.

So, after having an unknown sample in hand, to know the nature of the sample, from which source the sample obtained, we have to choose a right method. So, choice of the method is very very important. For example, if we take organic molecules, first thing we should do is, go for NMR and then to know its mass from mass spectrometry, like that. But sampling is equally important, how to prepare the sample for different instruments and the preliminary sample treatment is also important and then the separation. Components have to be separated and final measurement has to be made and method of validation has to be looked into. Then eventually elucidation, assessment of the results and elucidation of the structure. So, these are all fundamental entities one should look for, while using analytical methods to analyze unknown samples and instrumental methods for the measurements of physical properties of analytes such as conductivity, electrode potential, light absorption or emission, mass to charge ratio and fluorescence, are essential in the quantitative analysis of a variety of organic, inorganic, organometallic and biochemical analytes. The instrumentation is divided into two categories: 1) one is the detection and then quantitation, and some of these units one should be familiar with, we extensively use ppm. That is parts per million and ppb parts per billion, apart from those, we also use some of these units such as giga, mega, kilo, deci, centi, milli, micro, nano, pico, femto, and atto. So, we should know the symbols and also the corresponding units like giga is 10 raise to 9 (10^9), whereas atto is 10 raise to minus 18 (10^{-18}).

So, we should be familiar with these terms and then in different instruments the energy is presented in different forms. For example, it can be Hartree, electron volts, centimeter minus (cm^{-1}), kilo calories per mole (Kcal/mol) or kilo joules per mole (KJ/mol) or degree Celsius ($^{\circ}C$) or joules or even hertz (Hz). So, this table gives the conversion of one unit into another one very nicely and then what are the objectives of analytical chemistry?

First thing is to know what sample we have in our hand, that means identification and qualitative analysis. These two are very very important. First, we have to identify the substance and then we have to do qualitative analysis to know what it is and then to know how much it is, we have to go for quantitative analysis, and then spectral interpretation follows. This pattern here you can see, once we have pure compound in hand, we can use several instruments at our disposal to know what it is. For example, we can use CHN, MS and NMR, that would give you molecular composition and if you go through NMR, IR and UV you can identify functional groups and if you go for NMR it can tell you about substructures. For example, if you take ethanol, it can easily tell there is a methyl group, methylene group and OH group or a amine is there, if there is an NH_2 group or NH group or N group and of course, X-ray would come at the end and then molecular formula is there and then we can use hydrogen deficiency index of course, even with the mass when we see molecular ion peak we can also arrive at molecular formula using rule 13 and then once we tentatively come up with a molecular formula, we can look into unsaturation using hydrogen deficiency index, and then we can write possible structures getting information from these methods and then once we have structural formula, we can start writing all possible isomers and then we can also write all possible structures and then go back to again NMR, mass IR. We can arrive at the precise and correct structure and then with this one again if you analyze through NMR, we can give reasonable structure and of course. If it is a solid sample we can use X-ray diffraction to know the correct molecular structure of the substance. If molecular formula is known, this is how we revolve around these spectral methods to interpret and elucidate the structure of a new substance. Now, how to approach spectroscopic method for structure and who use this one: synthetic chemists, biological chemists, natural product chemists, forensic chemists, environmental chemists.

So, then how can I determine the structure of the molecule? Yes, there are several steps one should follow to identify new chemical compounds and mechanism of biochemical processes natural products from plants and animals, drugs toxins and explosives materials, soil, water or the atmosphere in all these things we use extensively to identify the sample and the right kind of chemicals. So, spectroscopic approach follows this pattern to identify molecules. One can use an array of structural information extracted by

all these spectroscopic methods and it is possible to get the information as rapidly as possible and one can deal with all phases of matter: mixtures and pure components that is the strength of these methods. Many spectroscopic methods will provide ways to analyze the sample in their own state: whether it is solid, liquid or gaseous. The process of determining the structure is very deductive and is much like solving the puzzles. One or more spectroscopic experiments have to be carried out and by analyzing the data, we can hopefully determine the correct structure, the most common methods for structure determining at present we use are mass spectrometry, nuclear magnetic resonance spectroscopy, electron paramagnetic resonance or electron spin resonance, infrared spectroscopy and electronic spectroscopy and Mossbauer spectroscopy. Each method provides its own special kind of data that we can apply to molecular structure determination and then here we should also know about approximate time scale we come across with different techniques for structure determination. For example, electron diffraction, 10 raised to minus 20 (10^{-20}), whether you consider x-ray or Mossbauer it comes around 10 raised to minus 18 (10^{-18}), that means whatever the process that happens up to that 10 raised to minus 9 (10^{-9}) can be detected using these techniques and then it drops here. UV visible 10 raise to minus 15 (10^{-15}), visible 10 raise to minus 14 (10^{-14}), IR and Raman 10 raised to minus 13 (10^{-13}), ESR is 10 raised to minus 4 to 10 raised to minus 8 (10^{-4} to 10^{-8}), and NMR 10 raised to minus 1 to 10 raised to minus 9 (10^{-1} to 10^{-9}). If any dynamic process that is happening in the molecule beyond this one, much slower than 10 raised to minus one (10^{-1}) or faster than 10 raised to minus 9 (10^{-9}) cannot be detected. In that case, we should alter by rising the temperature or by cooling the temperature of the substance. Fast kinetics 10 raised to minus 3 (10^{-3}) to 10 raised to 2 (10^{2}) and physical separation at least they should be stable for 100 seconds for doing physical separation and then the different type of electromagnetic radiation we use in the different instruments is shown here. For example, microwave we use it, and radio waves we are using in infrared, we use visible light, ultraviolet, x-rays and gamma rays we use in Mossbauer spectroscopy and then this can tell you where exactly this radiation is used. In NMR we use here and EPR we use microwave and also in rotation spectroscopy far infrared and vibration spectroscopy and near infrared to vacuum we use UV visible spectroscopy and also photo electron spectroscopy, and then this is x-ray and then gamma

($^\gamma$) rays we use in case of Mossbauer spectroscopy for looking into nuclear transitions and this is the NMR periodic table that gives a lot of information about NMR active nuclei present in various elements, their isotopes. Some of them may be 100 percentage, some of them may be less and some of them have I equal to half (1/2) or three by two (3/2) or some of them have I = 3. All this information is given here. So, another beautiful periodic table is here. This shows right here I equal to half (1/2), this yellow is three by two (3/2), and then orange is one (1). so that means most of the elements we see in the periodic table can be analyzed through NMR and multi-probe NMR are quite common nowadays we can do NMR for any of these elements shown here with non-zero nuclear spin value and this gives information about different isotopes so this also very good helps in analyzing using mass spectrometry looking into the mass fragments whether we get m^+, $[m+1]^+$, $[m+2]^+$, $[m+4]^+$ etc., all this simply by looking into what kind of elements present and how many isotopes are there and ratio all those things are quite helpful in interpreting the data. We obtain from mass spectra and then of course elemental. We can also get information from Mossbauer technique apart from iron that is extensively used, we can also use some of those things shown in red using Mossbauer technique in the contribution from different form of spectroscopy should be looked into it and here nuclear magnetic resonance spectroscopy is very popular among not only organic and inorganic chemists, organometallic chemists, and also for biochemists it is quite useful and pharmaceutical industries heavily use NMR experiments. Apply to nuclei that have the quantum mechanical properties of the spin for example if you see nuclear spin I equal to half (1/2), we have several very well-known ones ^1H, ^{13}C, ^{31}P, ^{15}N or with one is ^2H and ^{14}N and also we have three by two (3/2) for boron 11 (^{11}B) and also boron 10 (^{10}B) has 3 and NMR provides information about the types, numbers and connectivity of a particular atom for example NMR can show, if you just look into NMR spectrum of ethanol, it can tell you, it has CH_3 group it has CH_2 group and also it has one OH that means it can also tell you that there are two type of carbon in the ratio one is to one (1:1) and three types of hydrogen atoms are there in three is to two is to one ratio (2:1), and CH_3 and CH_2 groups are bonded together and CH_2 is connected to OH. All this information comes very nicely from the ^1H NMR spectrum of ethanol. Similarly for isopropanol if you look into it, shows that two CH_3 groups are there and one CH group is

there and then OH is there so it will tell you about isopropanol and then if you take normal propane, it can tell you that one CH_3 is there next to that there is one CH_2 is there next to that there is one more CH_2 is there and that is connected to OH so that means it can give you almost precise information, provided we know how to interpret and elucidate the structure. For many molecules, the entire structure can be deduced, no matter how complicated it is provided it is in its purest form and routine NMR experiments are performed in solutions of the molecules dissolved in deuterated solvents such as $CDCl_3$, C_6D_6 and D_2O using a few milligrams of samples of course how much quantity of sample we should use also depends on the abundance of the isotope for example ^1H is 100 percentage, ^{31}P is 100 percentage, ^{19}F is 100 percentage , Small quantity is sufficient, whereas in case of ^{13}C, we have only 1.1 percentage . We need substantial amount of sample. NMR can be used to study mechanisms for intermediates of chemical reactions in solution and also to monitor using NMR especially, ^{31}P NMR, if you are using, a phosphine or a phosphorus-based compounds, in a homogeneous catalysis, for a particular organic transformation. NMR is a very general method but it is most useful spectroscopic approach for determining the structures especially when you have organic samples or even inorganic material in it, and infrared spectroscopy is equally important. IR spectra result from the absorption of infrared radiation that causes vibrations of the molecules that means if you look into a diatomic molecule and it is not rigid, the bonds can be stretched okay, bond can be bent, bond can be waggled, so all kind of things happens and that can be monitored using simply passing infrared radiation over a molecule in a proper way. The spectra are typically presented as percentage transmittance or absorption. The peaks carry information such as functional group identification, structure information and even symmetry. IR is a very sensitive method and is used widespread in part due to its easy sample preparation and also equipment is not at all expensive and compact and simple to use, that single spectrum can be run in just a few minutes. The presence of functional groups give rise to distinct features that can be identified within well-defined range of the spectrum, but IR is only limited to determine the presence and identification of functional groups, but it cannot tell you how many groups are there sometime or how much it is there. All this information you cannot get it but you can identify the presence of those functional groups. In case of NMR the energy

difference is proportional to the magnetic field strength that is very simple and of course what we do is, we take NMR active nuclei keep it in a magnetic field, what happens, some of them will be aligned with the magnetic field some of them will be opposing the magnetic field and they start precessing with respect to the magnetic field. When they are precessing, the frequency with which they precess is called Larmor frequency. Since it is angular, it is not ν it is omega (ω), Omega equal to 2pi ($\omega = 2\Pi$), therefore if you take nu then it will be half 1/2 pi and this Larmor frequency is directly proportional to the applied magnetic field and then we bring a constant that is called gyromagnetic ratio and then once if you want to look into the frequency, the frequency will be gamma over 2 pi into Bnaught ($\gamma/2\pi B_o$), so that is a very simple equation one should remember and gamma is very unique for each nucleus it is called gyromagnetic ratio for example in case of hydrogen it is 26.753 radians per tesla per second ($rad \cdot s^{-1} \cdot T^{-1}$) and that means in a 14092 gauss field a 60 megahertz (MHz) proton is required to flip aproton so that means you have to apply a radio frequency of 60 megahertz (MHz) in a direction perpendicular to the applied magnetic field to flip the proton or to see the transition spin excitation that means the 60 megahertz (MHz) is equal to the Larmor frequency of that nucleus and this is comes in the low energy radio frequency and then this can show you different type of stretching modes for example symmetric stretching modes or it is anti-symmetric and then this is called scissoring and this is called rocking and this is called wagging this kind of twisting so all kind of this vibrational motions can be assessed using IR spectroscopy as I mentioned they behave like two spheres attached by a spring and then what the equation we should remember only one equation then how to correlate this vibrational frequency can be correlated with the reduced mass of two atoms between which that bond is there and also the force constant so here we can use this is the fundamental one this is simplified to 130.3 into square root of 'f over mu' ($130.3\sqrt{f/\mu}$) or it can be mu (μ) equal to 4.12 into square root of 'f over mu' ($4.12\sqrt{f/\mu}$), where f is the force constant and mu is the reduced mass reduced mass will be $m_1 m_2$ over m_1 plus m_2, where m_1 and m_2 are the two atoms that is holding in the diatomic species. That means by knowing the force constant we can predict the stretching force stretching frequency or if you know the stretching frequency the corresponding force constant can be determined for example here I have given reduced mass for this OH combination and NH. Various bonds and

force constant are also given and frequency is also given, you can use these equations to verify whether this data is correct or not. Then when it comes to UV spectroscopy what we should remember is only, we should remember only simple Beer Lambert's equation A equal to log 10 into Inaught over I ($A = \log_{10}(I_o/I) = \varepsilon Cl$). That is equal to epsilon Cl (εCl). i) A longer path length through the sample will cause more UV light to be absorbed, ii) the greater the concentration of the sample the more UV light will be absorbed and iii) UV-visible spectrum consists of absorbance, A on y-axis and wavelength on the horizontal x-axis. The number of arrangements of electrons in a given sub shell for a given electronic configuration, especially when you go to transition metals, is called microstates. According to Hund's rule, ground state has, for example three electrons in d orbital, all will be singly occupied; up to five, the sixth one will be getting paired, whereas when we look into excited states, we can have all different possibilities, that is so many different possibilities are there for arrangement of the electrons. The number of microstates that is given by n factorial (n!) over factorial (r!) into n minus r factorial n! /r! (n-r)! where n is the capacity of the sub shell. For example, d orbital, it is 10 factorial (10!), f it is 14 factorial (14!), and p is 6 factorial (6!), and if you take s orbital, it is 2 factorial (2!) and n is the total number of orbitals in a sub shell or total electron capacity. r is the number of electrons in the sub shell, d^7 means it is 10 factorial. It is 7 factorial and then the different types of electron transitions anticipated is shown here. For example, we have sigma, pi bonding, n and then pi star (π^*) and sigma star (σ^*). The transition should happen between these and the corresponding energies also you can assess from here and sigma (σ) to sigma star (σ^*) is much greater, because they are farther and then to n to sigma star (σ^*) and then pi (π) to pi star (π^*) and the n to pi star (π^*). So, these are all unoccupied levels, and these are all occupied levels. The highest occupied molecular orbitals and lowest unoccupied molecular orbitals, we call them and we see this kind of transition. Also seen here sigma to sigma star (σ-σ^*) in alkenes, sigma to pi star (σ-π^*) in carbonyls, pi to pi star (π-π^*) unsaturated compounds and n to sigma star (n-σ^*) as in halogens and then n to pi star (n-π^*) in carbonyls and d-d transition is another one that we see in case of transition metals. What is the rule of 13? For example, we get molecular mass in a spectrum that gives a number, from that number we should approximately get an idea about what are the elements present in it. Once if

you know what are the elements present in it we can come up with a tentative molecular formula. What we do is take this m plus mass and divide by 13 this gives a quotient and a reminder. The quotient will be number of carbon atoms and quotient plus reminder will be number of hydrogen atoms. For example, you take m plus $[M]^+$ is 78 and 78 divided by 13 is 6 (78/13 = 6) this is n and there is no reminder so n is 6 so C_6H_6 this is benzene. If you take 161, divide 161 by 13 (161/13) we get 5 as reminder and then if you add 5 it becomes 12 plus 5 =17. So, it becomes $C_{12}H_7$. but if you have other heteroatoms, what you can do? For example, N is there, its atomic weight is 14, so you can take CH_2 or if you take oxygen CH_4 like that you start eliminating those groups and you can add this one so that you can get the corresponding molecular formula and then of course multi-nuclear NMR is very interesting. For example, if you see here this molecule, we have one two three four five different NMR active nuclei: ^{19}F ^{31}P ^{15}N ^{1}H and also 29-silicon. For example, here one spectrum I have shown here, that of ^{31}P NMR. How it looks? First it splits into a triplet because phosphorus to fluorine coupling is very strong and then each triplet line is split into a doublet (this is called splitting tree) because I = half (I = 1/2). I will be elaborating when I start digging into NMR spectroscopy. Next comes H, it will split further these signals into a doublet here and then eventually these three protons would split this into (n +1) lines so we are getting this beautiful 48 lines and then you can see very nice spectrum. We get this one like this many interesting molecules. We also sees look into lot of problems on IR and also in UV-visible and also mass spectrometry as well as EPR. So, let us start making ourselves familiar with interpretation of data obtained from different spectroscopic methods and the elucidation of the structure.

See you all in my next lecture. I will begin my next lecture with NMR. I wish you all the very best and see you soon. Thank you.