Radioanalytical techniques

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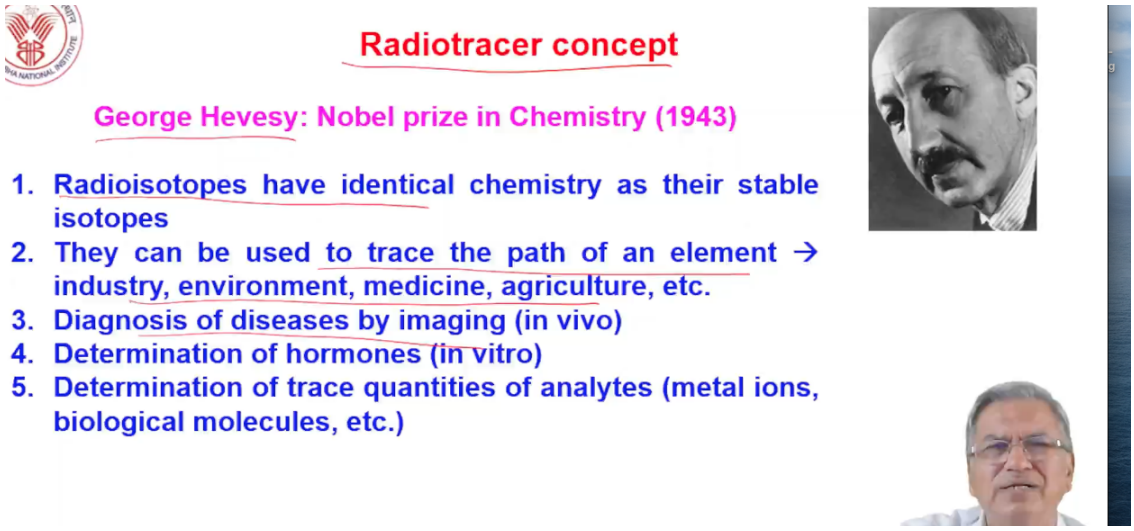
Lecture-16, Module-2

Hello everyone. In the previous lecture, I discussed about different radiochemical practices. Like you know, you have good laboratory practices GLPs, for a chemistry laboratory. We have good radiochemical practices GRPs for a radiochemical laboratory. I also discussed some of the radiochemical separation techniques. In this present lecture, I will discuss the radio analytical techniques which employ radioisotope tracers to study different chemical processes and also some of the applications in many areas.



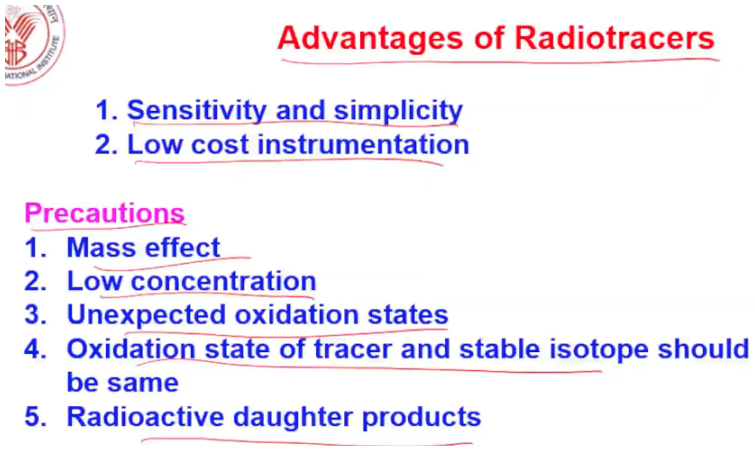
So let us first discuss the radio tracer concept. The father of radio tracer concept is George Hevesy. In 1920s, early 1920s, George Hevesy developed this tracer concept and there is a very nice story about George Hevesy, how he first time used radio tracers, I would like to share with you. George Hevesy was actually staying as a paying guest in a house and the paying guest, you know, means the landlady or landlord will give you not only the accommodation but the food also.

So one day, he realized that their landlady is serving the leftover of the previous day to them for breakfast. And so he wanted to do an experiment to scientifically prove that they are being served the leftovers. So what he did, he took a tracer of, that time, there was no artificial radioactivity, so you have lead-210, so lead compounds contain lead 210. And he just added that lead-210 in the meals. And next day when he was served the food, he took a portion to the laboratory and counted it for beta activity. And he found that lead-210 was presented in those samples. So Pb^{210} is 20 years half life and you can

separate it from the uranium series. So then he conclusively proved that the landlady is serving the stale food to them. So he actually traced the path of food using ^{210}Pb as a tracer. That is what the meaning by radio tracer.

So the concept is like, radio isotopes have the same chemistry as their stable isotopes. So you can use them to trace the path of an element. You have, for example, you have a process where sulfur is being used. You can have radioactive sulfur, sulfur-35, mix with the reactants and you can know where the radio sulfur is going. You can, from outside trace the path by radiations.

So they can be used in industry, environment, medicine, agriculture, you can use them to trace the path of an element. You can use them in diagnosis of diseases by imaging because you can make them to go to a particular organ and then they are emitting radiation, from outside the human body you can take an image like x-ray you take. Similarly, you can take the gamma image using gamma ray counters. They are used in-vitro for determination of different compounds like hormones in our body. I will discuss that. And you can also do the trace element determinations of metal ions, biological molecules using these techniques. So some of them I will discuss in the present lecture.



Radio tracers have a lot of advantages and in fact, you will notice that many of them over a period of time when modern techniques not based on radioactivity are being developed, many of them have become obsolete or so. But sometimes you will find that they are indispensable. Some of the experiments you will find you can do only with the radio tracers.

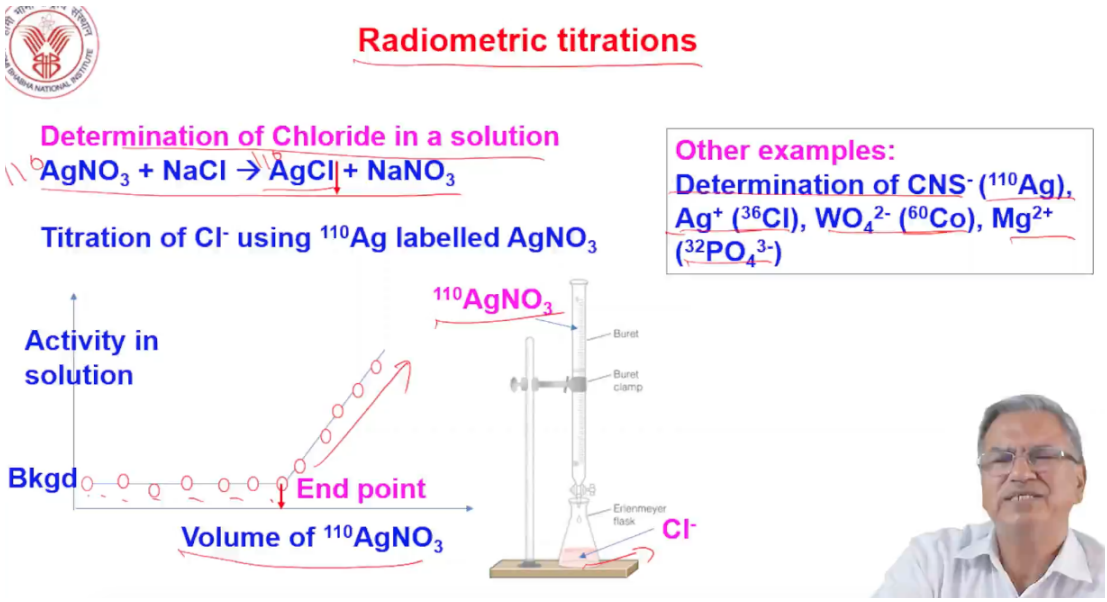
So one of the advantages of this is they are very sensitive. Suppose you are doing a counting of radioisotope emitting a particular gamma ray, then it is very sensitive because that gamma ray is unique to that particular isotope. They are very simple. Some of the detectors like GM counters, sodium iodide counters are very low cost. So you can carry out even research using this. So low cost instrumentation is one of the important

advantages of radio tracer techniques. Of course, it is said that you should use radioactivity for any experiment or research or any problem solving only when there is no alternative. So there should be an advantage in terms of manpower, time, cost and so on. So either you save time, you save money, you save manpower, then only you should be able to justify the use of radioactive isotopes in a research or in solving any problem.

So you have to take care, take precautions like mass effect means because of the very low concentrations of these isotopes, some of them are carrier free. So there is a chance that they may get adsorbed onto the wall of the container. So you have to, like I was telling you add some carriers to increase the mass so that they are not lost. So the low concentrations have their associated difficulties and challenges. When you produce a radioisotope, it may be produced in a particular oxidation state, we don't know. So you have to verify the oxidation state and then you make sure all the atoms are present in that particular oxidation state. So you need to adjust the oxidation state by using oxidizing or reducing agents. Similarly, you are using some isotopes, stable isotopes to like if you are using a tracer of an element, then you have to make sure that the tracer and this stable isotope have the same oxidation state. Otherwise, we cannot trace the path of the element in the process. So suppose tracer is in iodide form and the stable iodine is present is in iodate form. So we cannot trace the path of iodate using iodide. So you make sure both of them are in the same oxidation state.

Then many times, you know, the radioisotope may decay to another isotope, which is also radioactive as we discussed in radioactive decay chain and that may interfere in your separation. So you make sure that if you have a daughter product, then you should also be cautious about interpreting your data. You must know that the daughter product is also radioactive and you have to factor that into your final conclusions.

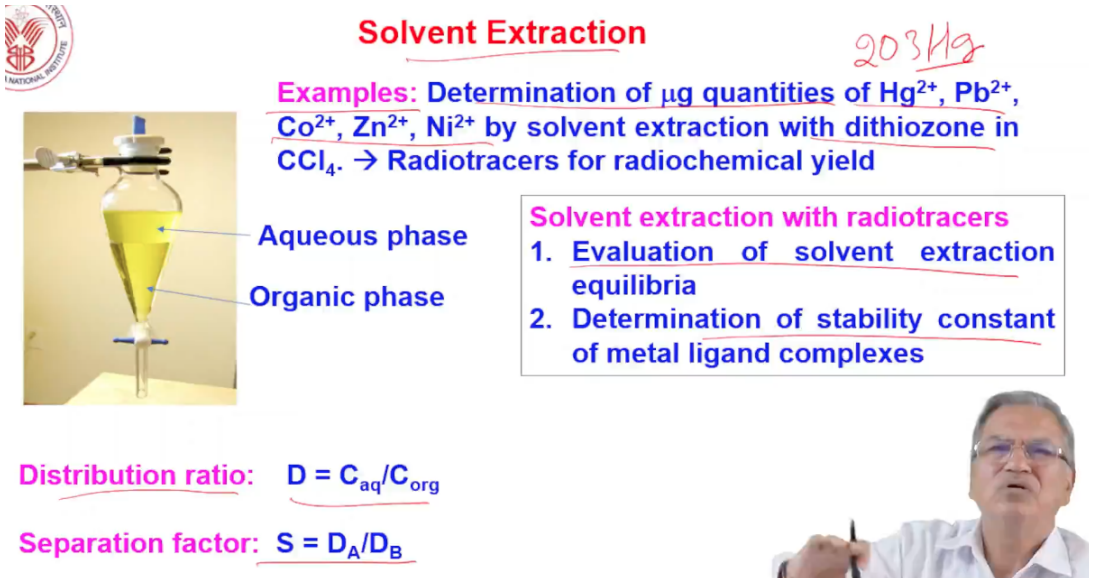
Okay, so let me discuss some of the radio analytical techniques which are commonly used and many of them you will find have become obsolete because there are now much better techniques available. So you may not, one may not need it, but it is very interesting to see and how people have developed those techniques and it is so unique about radioactivity based techniques.



One of them is radiometric titrations. So you add a radiotracer, you can do, for example, precipitation. Then you normally in gravimetric type you can do, you can precipitate, you can take the precipitate weight and find out the concentration. So you go on adding the reagent and you will find at some point of time, the equivalent point is reached. So how do you track that? So for example, you want to determine chloride concentration in a solution, like you can have sodium chloride or barium chloride and you want to do titration using a precipitation reaction. So silver chloride is insoluble. You take silver nitrate as a titrant and add, go on adding silver nitrate. This silver nitrate you tag with Ag^{110} and silver chloride will precipitate Ag^{110} and you are left with sodium nitrate in the solution. So what do you do? In the burette you take labeled silver nitrate and in the solution you have the chloride salt solution. And now you go on adding drop wise the titrant and then record the activity in the supernatant. So the precipitate will settle at the bottom, from the supernatant you take an aliquot, go on taking the aliquots and you find the activity. So what is happening? As you add the reagent, radiotracer labeled reagent silver nitrate solution, initially when silver nitrate concentration is low, all of it will be precipitated and you will see the background in the counter. When all the chloride is precipitated at AgCl, then at the end point Ag^{110} activity will begin to rise and that's because there is excess of silver activity in the solution. So you can then find out the end point, what is the concentration of silver nitrate that is sufficient to neutralize, to precipitate all the chloride. So beyond this, this silver nitrate is not separating, it is going up and up because you are just adding the one solution to another solution. So that is how you can find out equivalent point and a very simple experiment based on radiotracer counting can be done to do the determination of chloride concentration.

Same thing you can do for thiocyanate, silver ions, tungstate, magnesium and so on. So you can use the proper reagent for thiocyanate you can use silver-110, for tungstate you

can use cobalt-60, for magnesium you can use phosphate ions. So you can use the suitable radio tracer labeled titrating agent and do the radiometric titrations. But you will find that this is not very common these days because there are much better techniques you can use them for these experiments.



Solvent extractions are still very useful, very commonly used and they are used for separating the different metal ions or different organic molecules. One of the applications is determination of microgram quantities of metal ions like mercury, lead, cobalt, zinc, nickel by solvent extraction with a reagent called dithiozone. So you can do some extraction and you can use the radio tracers of these elements to see the distribution in aqueous and organic phase. So that is the beauty of radio tracers.

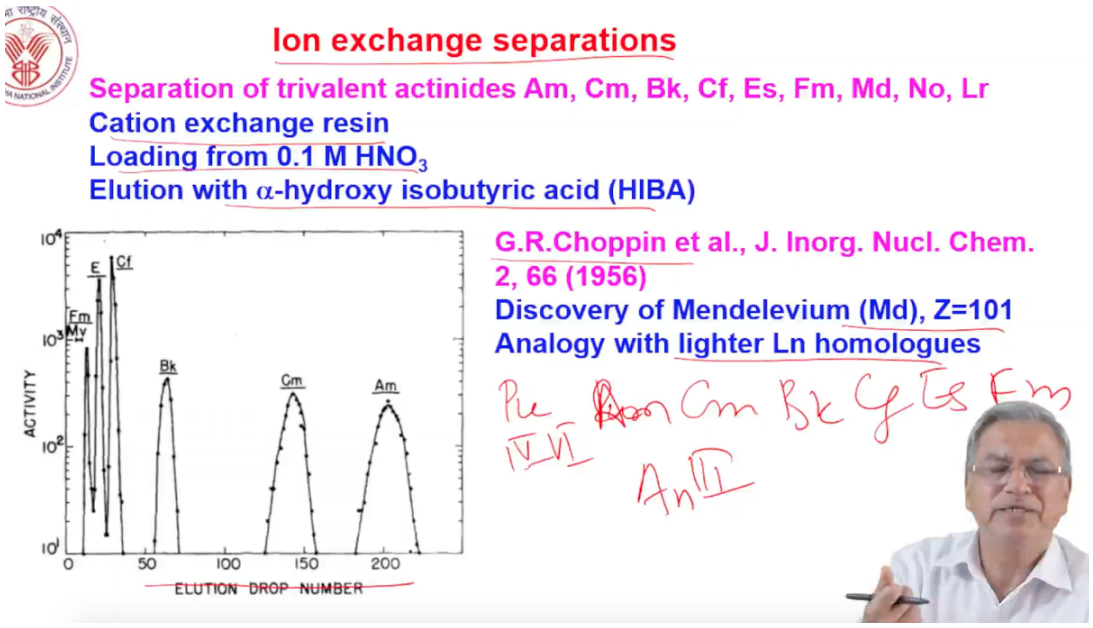
You can determine the distribution ratio. Suppose you have used mercury, so you use Hg^{203} as a radiotracer. So in the aqueous solution you have the radiotracer Hg^{203}. After the solvent extraction some mercury will go in the organic phase and you can take aliquots from aqueous and organic, 100 microliters or 50 microliters and count the activity of Hg^{203} in the two phases and you can find out the distribution coefficient. Suppose you are having multiple elements in the same solution, you can find out the D-value for different metal ions and you can find out the separation factors.

So for radio tracers, solvent extraction is very ideal system. There are now complexing reactions, you can study the equilibria. So how as a function of acid concentration, how the distribution ratio is shifting as a function of ligand concentration, how the equilibrium is shifting. All those studies can be done using radiotracers. So much so even we can determine the stability constant of metal ligand complexes.

For example, if the metal ligand is going in the organic phase, so you can do as a function of ligand concentration you do solvent extraction and then find out what is the concentration of the ligand at which you will see the metal ligand complex is getting into the extraction phase and then you can set up the equations for finding out the stability constant.

$$K = \frac{[ML]}{[M][L]}$$

This is a free metal ion, this is a complex metal ion, and this is the free ligand. So you can find out this ratio, metal ion free will be in aqueous phase and complexed metal ion will be the organic phase. Once you know this and the ligand concentration is nothing but free ligand plus metal complex concentration, total ligand is known, you can find out the free ligand and therefore you can find out the stability constant. So this is how you can use radio tracers for solvent extraction.



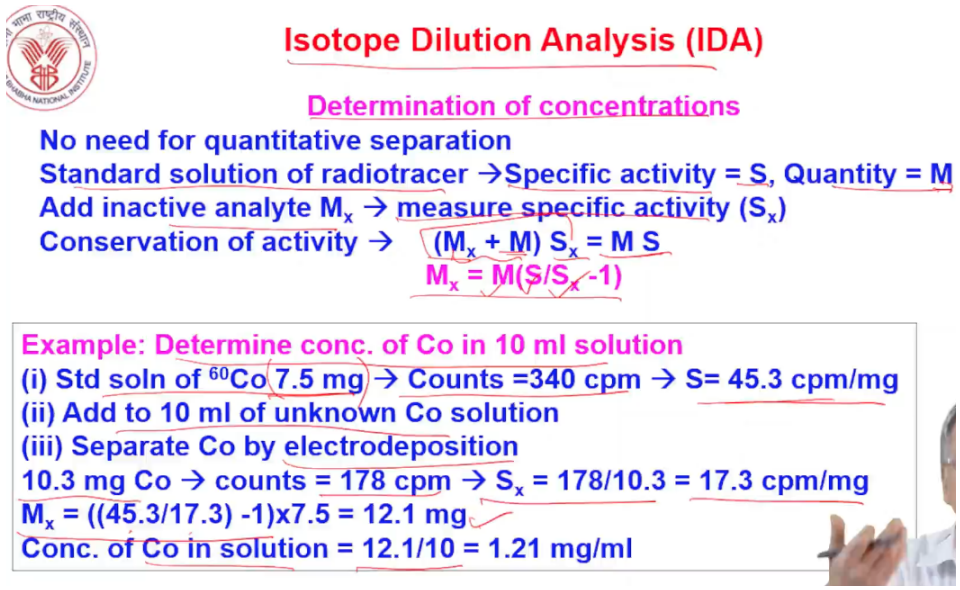
Similarly the ion exchange separations are using radio tracers very frequently. In fact, I give you some very interesting example of even the discovery of trans-americium elements if you see the actinide elements after plutonium, plutonium can exist in 4+, 6+ and so on. After plutonium, americium, curium, berkelium, californium, einsteinium, fermium and other elements. They all elements are present in the +3 state or actinides(III). Now in the late 1940s and early 1950s in the different cyclotron experiments, the trans-plutonium elements were being produced and they were being discovered.

So how to discover a new element? It was known in 1944 by Seaborg's actinide concept that beyond americium all actinides are predominantly present in the +3 states. So they

are similar to rare earths, their lighter homologs. It was already known that individual lanthanides can be separated by a cation exchange resin. You can load the solution of cations from a dilute acid into a Dowex 50 x 4 cation exchange resin and then elute with alpha hydroxy isobutyric acid which I explained some time back. One by one you will see the heavier lanthanides come first and followed by the lighter lanthanides.

Similarly these actinides also follow the same trend as the lanthanides and so the different actinide ions can be seen in the profile of the radiotracers of the different elements. Now you do not have the radiotracers of actinides, you can use radiotracers of lanthanides to see the position of individual actinides. And that is how this element Mendelevium Z=101 was discovered by Gregory Choppin, the student of Glenn T. Seaborg by analogy with their lighter lanthanide homologs and their position in the ion chromatography system was useful in discovering new elements. So this is the power of ion exchange separations you can calibrate the concentration of alpha hydroxy acid butyric acid to separate individual actinides and lanthanide elements and see their position uniquely as a function of the drop number in the elution.

That is the advantage of radio racers. In fact we have the tracers of each lanthanide element and you can mix them and do the chemistry and you can see beautiful profile of individual rare earths.



Now I will discuss some of the applications of radio analytical techniques like isotope dilution analysis. This is a beautiful radio analytical technique to determine the concentrations of some analytes and in isotope dilution you don't have to do a quantitative separation, 100% separation is not needed. Essentially isotope dilution means you dilute the specific activity of a radioisotope.

So what you essentially do is you have a standard solution of a radiotracer for which the specific activity (s) is known. Specific activity means activity per unit mass. Now suppose you have a quantity (m) so specific activity means per gram this is the activity this is the amount of the substance so total activity will be m×s. m×s is the total activity. Now you want to find out the concentration of an unknown analyte m_x.

So what you do you add this unknown analyte with this standard solution of the same element but tagged with a radiotracer and then you measure the specific activity. So you have now the standard solution and the unknown solution total you have your quantity m_x + m which will have a reduced specific activity because we have added some inactive metal ion. Now the total activity before and after the addition of unknown amount is same as the initial activity into concentration.

$m*s = (m_x+m)*s_x$

So from this equation of conservation of the total activity we can find out mx.

$mx = m[(s/sx)-1]$

m is known, the initial amount of standard solution is known and the specific activity after mixing the two is measured and you can find out the concentration in the unknown solution.

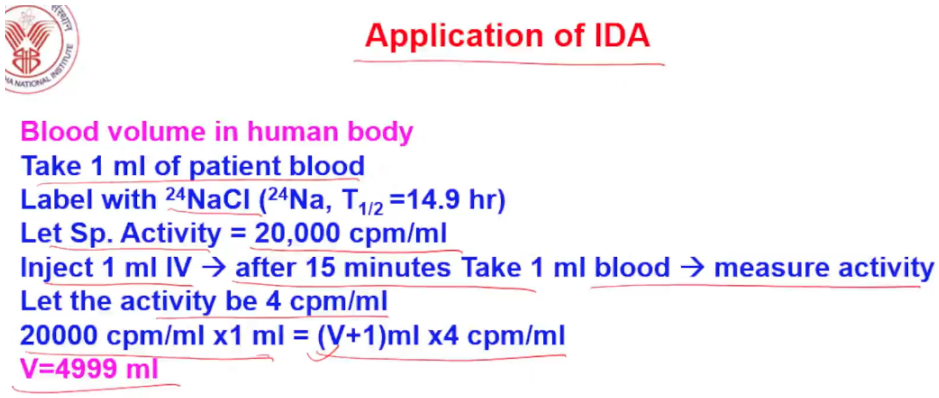
That is what is called the isotope dilution by diluting the specific activity by what factor the specific activity that got diluted tells you what is the concentration of analyte. Just to give you an example to illustrate this point more clearly suppose you have a solution of cobalt in 10 ml solution you want to know what is the amount of cobalt in this solution. So just give some numbers you have a standard solution of cobalt-60, 7.5 milligram cobalt-60 maybe very small it may be picogram quantity but amount of total cobalt in that solution is 7.5 mg you count that in that solution and you get it's a 340 counts per minute.

So the specific activity of that will be 340 by 7.5 will be 45.3 CPM per mg. Counts per minute per milligram not gram per milligram. Now you add to this solution 10 ml of the unknown solution for which you want to find out the cobalt concentration. Then you have to now do separation so you separate out cobalt by you solvent extraction or you can do electro-deposition you separate cobalt and mind you cobalt-60 and the both the solution should have the same oxidation state of cobalt. So by separation you got 10.3 milligram of cobalt so it is not necessary to have quantitative separation whatever the amount you got and you take the activity of that and you got 178 counts per minute.

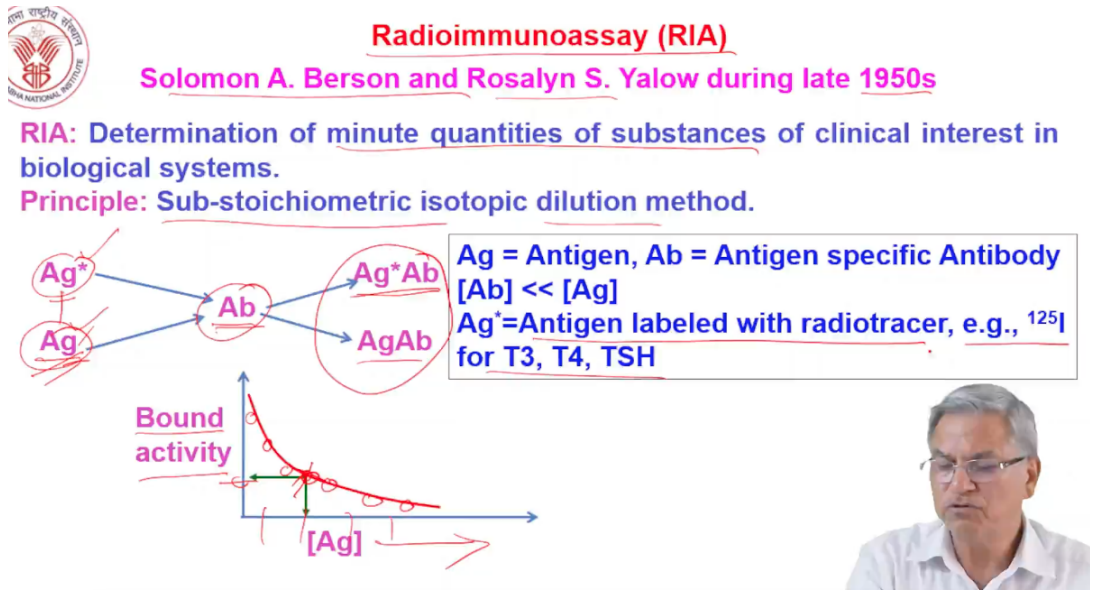
So the specific activity will be 178 upon 10.3 means 17.3 counts per minute per milligram of the separated cobalt fraction. So we can put in this equation the value so

(S/Sx-1)*7.5 will be 12.1 milligram of cobalt in the unknown solution total amount of cobalt.

So the concentration of cobalt in that will be 12.1 by 10 ml which will be 1.2 milligram per ml. Of course you can say that today you don't have to use radiotracer technique because you can put it in a ICP-AES, ICP-MS. So there are now modern techniques based on spectrophotometry or the optical emission spectrometry and so on. So such techniques you know sometimes it may not be useful but I gave an example of this one and you will really appreciate the application of isotope dilution technique.



Blood volume in human body. Suppose you want to know how much blood is there in a particular human being, a simple experiment, you can take 1 ml of your patient's blood, take out 1 ml, add sodium-24 activity, 15 hours half-life, in that 1 ml solution and find out the specific activity. You can count the gamma ray activity of sodium-24 and suppose you got 20,000 counts per minute in 1 ml of that solution. Now this 1 ml you inject intravenously into the human patient and after 15 minutes take out 1 ml. So what has happened that this 20,000 counts per minute activity is got now distributed in the entire body whatever volume we have is equilibrated with that in 15 minutes and now you take out 1 ml and measure the activity of sodium-24 and let us say you got 4 counts per minute in that 1 ml which you got later. So we can again put the initial activity 20,000 counts per minute per ml into 1 ml this is what you added and finally the volume of the blood is not known in the human body so V plus 1 ml we added into 4 cpm per ml and you can find out the V that is equal to 5000 ml. So this is the total volume. So isotope dilution experiment can tell you without much difficulty the volume of blood in a human body. So you have to find out the ingenuity in that experiment. There are some experiments we will find that radiotracers offer a unique advantage.



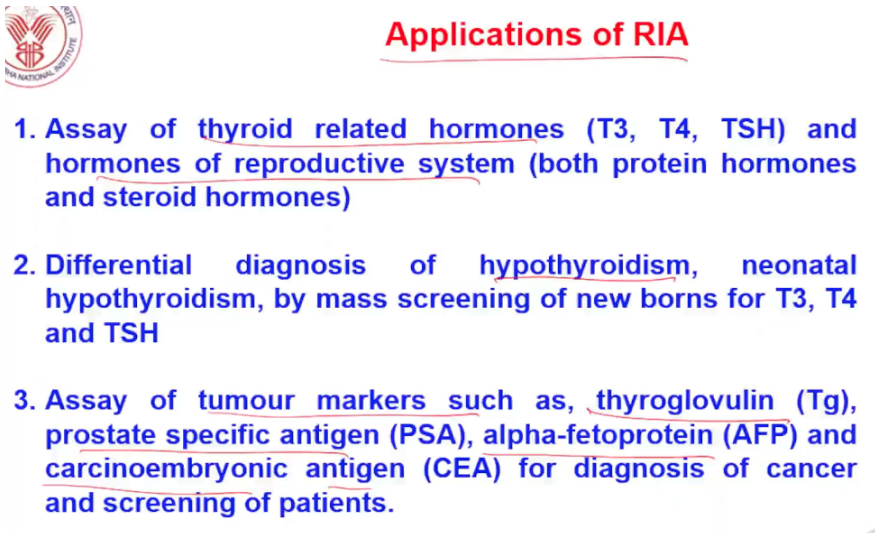
Another such technique is radioimmunoassay. Radioimmunoassay is a technique developed by Berson and Rosalyn Yalow in 1950s and Rosalyn Yalow received the Nobel Prize for this in 1977. So this radioimmunoassay is actually used to determine minute quantities of substances of clinical interest in biological systems. Our body has got certain hormones like T3, T4, these thyroid hormones or you can have many other you know in our body there are many antigens are available and you want to know their concentration as a part of the diagnosis. So this is based on the isotope dilution and is called sub-stoichiometric isotope dilution. Sub-stoichiometric means there are two reagents one is the antigen and one is the antibody and one of them is in excess other one is in very low concentration.

So they are not stoichiometrically being added. So the concept behind RIA is that suppose you want to determine the concentration of this antigen (Ag) it is not silver it is called antigen like T3, T4, TSH or prostate specific antigen in the body different glands are excreting different hormones you want to determine their concentrations. We take out a sample from a person and you add to that a labeled antigen. So suppose you have thyroid hormones they contain iodine you can take an iodine-125 tracer and make sure that they have the same oxidation state of iodine in the both. So now you have a small quantity of this antigen you add the two and now you react them with an antibody.

So the antibodies are specific to a particular antigen but the antibody is in a very small concentration with respect to antigen. So the radioactive and the inactive antigen will compete with each other to bind this antibody. And so later on you will find that you will form a complex which you can centrifuge and count. So what you are doing now you are counting the labeled antigen with antibody and find out the concentration of antigen that is labeled. So in the total what fraction of antibody is bound that is called the bound

activity and as a function of increasing concentration of antigen. So you have to do multiple experiments. So you do this is like a calibration graph you take different concentrations of the antigen and generate this graph. As you increase the concentration of antigen the activity bound will be reducing because increasing the fraction of inactive antigen will decrease the fraction of labeled antigen.

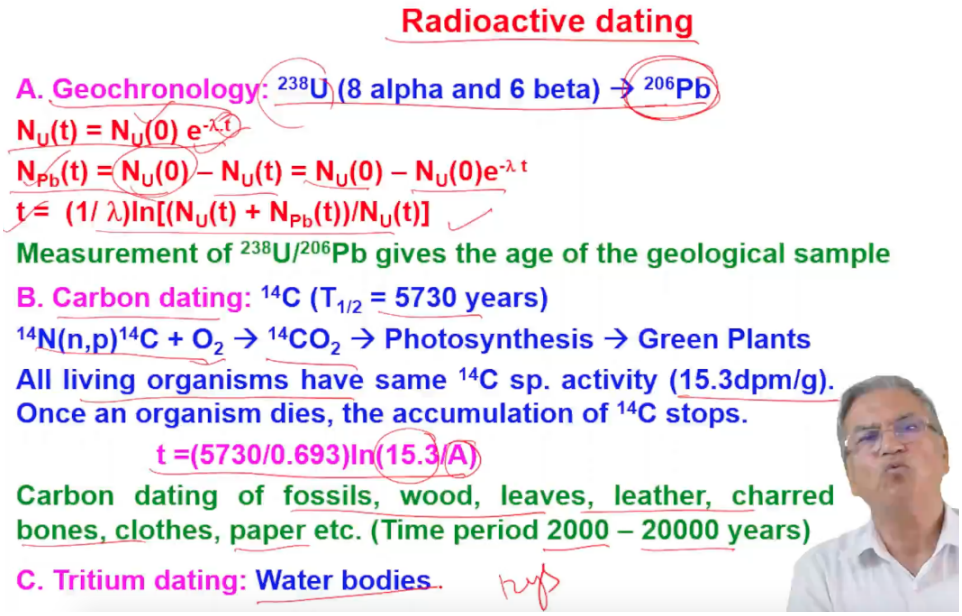
And this is now calibration graph. So for example you have an unknown sample you find out the fraction of labeled antigen and then you find out what is the concentration from this calibration graph. So it is a sub-stochiometric IDA, antibody is in a very small concentration with respect to antigen and then the decrease in the concentration of the labelled antigen tells you what is the concentration of antigen in the unknown sample. So antigen labeled with the radiotracer-125 iodine you can use and then you can find out concentration of different hormones in the blood samples.



The applications of radio immunoassay are like for example thyroid related hormones and hormones of reproductive system, protein molecule steroids, differential diagnosis of hyperthyroidism, neonatal hypothyroidism, etc.

You can screen the newborns for T3, T4, TSH, etc. Very quickly you can do experiments and there are tumor markers such as thyroglobulin, prostate specific antigen, alpha-photoproteins and carcinoembryonic antigen for diagnosis of cancer in screening of patients. In fact nowadays radioimmuno assay actually has taken a backseat because there are now chemiluminescence, fluorescence-based techniques which can also reach the same sensitivity as RIA. The RIA can tell you the concentrations in nanograms, nanomoles and so now the other techniques are advancing and they are competing vigorously with the radioactivity based techniques. So you'll find that these techniques are also slowly being phased out because if you can do the same experiment with a

non-radioactive technique with the same sensitivity and same cost of course, then there is no need for such techniques. But still many laboratories follow this technique.



And lastly I want to tell you the applications of radioactive tracers in dating. Dating means finding out the age of an object. One of the objects like for example the rocks geochronology. So you want to find out the when that particular rock was formed and this is based on the natural radioactivity series ^{238}U decaying by series of alpha and beta to ^{206}Pb. So you find out the uranium content and the lead content in the rock.

Initially let us assume that there was only or even if there was some lead then you will know because all isotopes are not formed by uranium decay. So you can find out what was the initial lead present in the rock. So the uranium present today can be given in terms of initial concentration of uranium exponential decay e raised to minus lambda t. This is the time which we want to find out.

$$N_U(t) = N_U^0 e^{-\lambda t}$$

Lead present today is uranium initial present minus uranium present today.

$$N_{Pb}(t) = N_U^0 - N_U(t)$$

You can find out lead by mass spectrometry and by solving these two equation you can find the time when this rock was formed. So if you measure the ratio of ^{238}U to ^{206}Pb you can find out the age of that particular geological sample.

And lastly the carbon dating. Carbon-14 has half life of 5730 years and it is being formed in the atmosphere by cosmic ray neutron bombarding nitrogen-14 and this carbon is

reacting with oxygen to form carbon dioxide. By photosynthesis, green plants assimilate this carbon-14 and so much so that all living organisms including our body contain a specific activity of 15.3 Disintegration per minute (DPM) per gram. One gram of any living organism will give you this much specific activity. But once an organism dies then this activity is no more being accumulated and so there will be a decay. And from that decay you can find out what is the time.

So suppose the activity is A today and initially till it was live was 15.3 by the similar equation you can find out what was the time that this particular organism died. So this is being used for carbon dating of fossils, food samples, leaves, leather, charred bones, clothes, paper so on. And since the half life of carbon-14 is 5700 years about four half life of carbon 14 is 20,000 years and about half a half life of carbon-14 is 2000 years. This is the time zone which you can scan using carbon dating. If you want to go more than 20,000 there are other techniques.

Similarly tritium half-life is 12 years. So tritium dating can be used for water bodies whether they are in contact with the rainwater or not. The isolated water bodies will have less concentration of tritium.

So these three isotopes are used for dating of different applications. So this was just a glimpse of applications of different radioanalytical techniques. I will stop here, take up the next lecture next time. Thank you.