Applied Environmental Microbiology
Dr. Gargi Singh
Department of Civil Engineering
Indian Institute of Technology, Roorkee

Lecture – 53
Biosensors I

Dear students welcome back to applied environmental microbiology. Today we are going to see a very interesting and promising method to utilize whatever we have learned so far in applied environmental microbiology curves. So, that we can fulfil our very important need to be able to detect contaminants pathogens and other risk agents in our environment faster and with higher reliability. So, what we are going to talk about today are biosensors.

Now, sensors as a word in itself tells you if something that you use to sense or pick up data either quantitative semi quantitative or even qualitative now, we must be very careful and about this particular topic because, while a lot of research is currently underway to develop better biosensors, better sensors there is a dearth of sensors that can be used at low cost for robust and reliable measurement of environmental contaminants. Now, in a country like India or in any other developing country we have a problem with we have the problem of overpopulation and pollution.

As a result, we do not even know what kind of contaminants, what kind of pollutants are present in our water in our air and our soil or definitely on our food. And not only environmental contaminants, but also pathogens are a big challenge in a developing highly populated country such as India. Because, somehow Indian subcontinent is the perfect hotbed for emergence of antimicrobial resistance as a result the pathogens that we have in India might have or in anywhere else in the world actually might have higher levels or elevated levels of antimicrobial resistance.

So, it is not only important not only sufficient for us to know that there are particular pathogen is present in the sample or not, but also to find out rapidly whether the pathogen if present is resistant or not. If you remember what we studied in our previous lectures on antimicrobial resistance, one of the key theme was that it is not just important for us to pinpoint the pathogens that have the ability to resist antimicrobials, but we

should be aware and alert about all kind of microbes that have the potential to display antimicrobial resistance genes.

(Refer Slide Time: 02:49)

So, for us we have emerging contaminants such as AMR antimicrobial resistance and antimicrobial resistance genes, then we also have highly contaminated environment. And now, this is coupled with high demand on resources and public health burden. So, think about it dear students in our country we have a highly contaminated environment. So, it is very important for us to be very cautious and proactive to ensure public safety on environmental health.

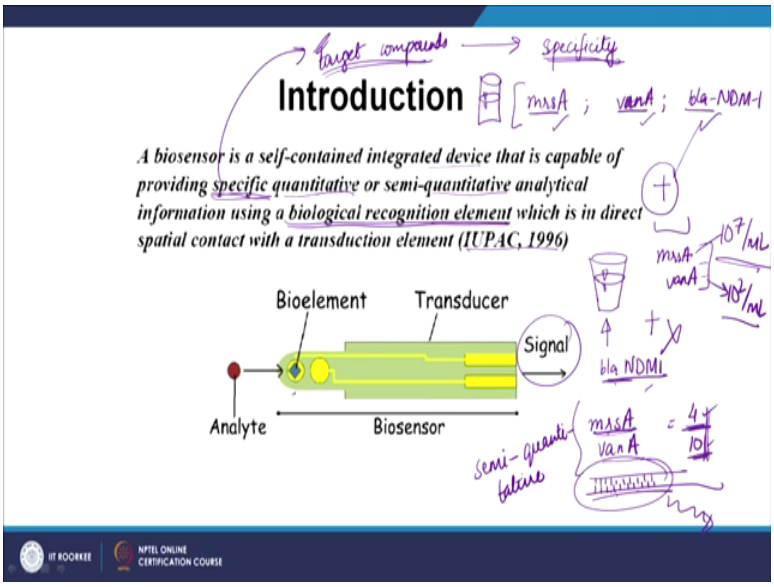
Next is we also have very high demand on our resources. So, we only have limited amount of resources and we have to make sure that we use all that we have to it is optimum. And as such we cannot afford to waste our resources. The other thing is we have a very high public health burden from infectious diseases, as a result it is important we need urgent data collection.

So, rapid data collection is required. So, we need rapid, reliable and robust data collection methods. So, basically this is the need of the hour today in our country rapid, reliable and robust detection techniques for environmental contaminants as such biosensors are a promising avenue. And this is beautiful part of biosensors that not only do they help us detect our environmental contaminants, but also, they use the biology our understanding of biology to create very specific very sensitive and reliable sensors.

For example, we know that if you go back to our lectures on central dogma where I talked about genes how they translate into proteins? And how they code in code for all cellular activities so, in this you would remember that genes they follow a very strict sequence pattern which has some leeway here and therefore, degeneracy, but mostly as a reliable strict sequence pattern.

Now, we can create such very specific sequence patterns that will only that will fold or that will behave in ways, that will only react respond or give a signal when it attaches to a target, as such the whole potential to become biosensors. Same is true with proteins and same is true with antibodies and many other biological entities that can be used that are being used to make biosensors. So, let us progress let us go ahead and study more about biosensors.

(Refer Slide Time: 05:55)



Now, what is bio sensor? Now, this is definition that has been taken from IUPAC 1996 standard journal. It says a bio sensor is a self-contained integrated device that is capable of providing specific quantitative or semi quantitative analytical information using a biological recognition element, which is in direct spatial contact with your instruction element, all right? Let us break this down break this definition down.

Basically, biosensor is self-contained integrative device. It is self-contained it is complete in itself we do not need to attach it to some other instrument. It is enough for it is complete in itself it is integrated device. So, all different parts of any sensor which is a

receptor a transducer and a signal receiver all of them are integrated in one device. It is capable of providing specific this is very important word, now the word specific here says that we are we have some target compounds that we are interested in.

For example, if I ask you the question is the water dirty? Is the water if you give me a glass of water and ask you is the water dirty? It is a very general question because, you might be it might say counter asked me a question saying well girl give what do you mean? Do you mean does it have microbes, does it have dust, does it have something that will harm you.

Do you mean biological agents chemical agents for physically? Physical properties are not good enough what are, you looking for. Do you want 100 percent pure water or what some impurities are ok? What impurities are not ok? So, there are many questions follow up questions that, we can ask to clarify very generic question or is the water clean or is the water dirty?

However, biosensors or any sensor especially biosensors are helpful when we are very targeted in our questioning not very generic. For example, if I ask you or if you ask me that hey are there anti-microbial resistance genes in this water, and then we might even go ahead and say well I am interested in metal is methicillin resistant staphylococcus aurous are they present in the water, or is our the genes that confer methylene methicillin resistant are they present in the water.

So, this is a very targeted question, and thus the compounds that I am looking for are very targeted. Which brings me to another point we will cover it later, but let us hear about it anyway where you are targeting particular compounds a particular set of compounds, we are very interested in specificity.

So, specificity basically says that your sensor will give a positive signal only when the target is present. Let us say I have mrsA gene now, mrsA is a gene that confers resistance to methicillin antibiotic. So, any microbe that has mrsA and it is actively translating it actively expressing proteins related to mrsA gene then, that particular microbe would be resistant to methicillin. Let us say, I am also interested in vancomycin resistance ok. And let us say I know that there is another form of resistance present in my water, all right? So, let us say I have drinking water a glass of drinking water and I want to find out what

the mrsA is present in it whether vanA is present in it because, these genes are marker for methicillin resistant and vancomycin resistance.

Now, let us say my bio sensor or my sensor gives me a positive signal yes, your target is present. So, it is quite possible that it has it is giving me signal for mrsA or it is giving me signals for vanA, but if it also gives me signal for bla-NDM-1 when had only designed it to give me single for mrsA or vanA then, this is not specific it is (Refer Time: 9:48) and let us say, there is another glass of water another glass of water and let us say in this we only have NDM-1 resistance gene. So, does these that give resistance to betta lactamase now, and this is new Delhi metallo beta lactamase by the way. So, only this particular genes are present in this water and if I put my sensor in and it gives me a positive signal this is not specific because, even when the target and mrsA and vanA were not present it gave me a signal.

So, it is very important at my bio sensor only gives me signal when my target compounds are present. So, that is what the specific word is here. Now, quantitative or semi quantitative analytical information ideally by sensor should not only tell me if mrsA or mrsA vanA are present or not, but it should also be able to tell me how much how many mrsA copies are present? How many vanA copies are present?

For example, if I have 10 to the power 7 per mL copies of mrsA and 10 to power 2 4 mL copies of gene copies of vanA then, my sensor should be able to give me an estimate for this sometimes it is not quantitative per se, but it is semi quantitative now what does semi quantitative it is just try to understand that.

Now, in semi quantitative my sense I may not be able to tell me exact value how much per mL of mrsA gene copies are present? And how many vanA gene copies are present? Well it might be able to give me an idea like this, an idea such that the ratio of mrsA gene copies to 16SrRNA gene copies is something.

Now, this is semi quantitative it is giving me relative abundance or maybe it is giving me an estimate of which is more the mrsA is more or 16SrRNAis more well. Let us take another example. So, let us take another example, let us say it is giving me an idea of if you let us say the semi quantitative and the sensor is giving me an idea of mrsA ratio vanA and it says 4 ratio 10. Now, because it is semi quantitative I cannot say that well I

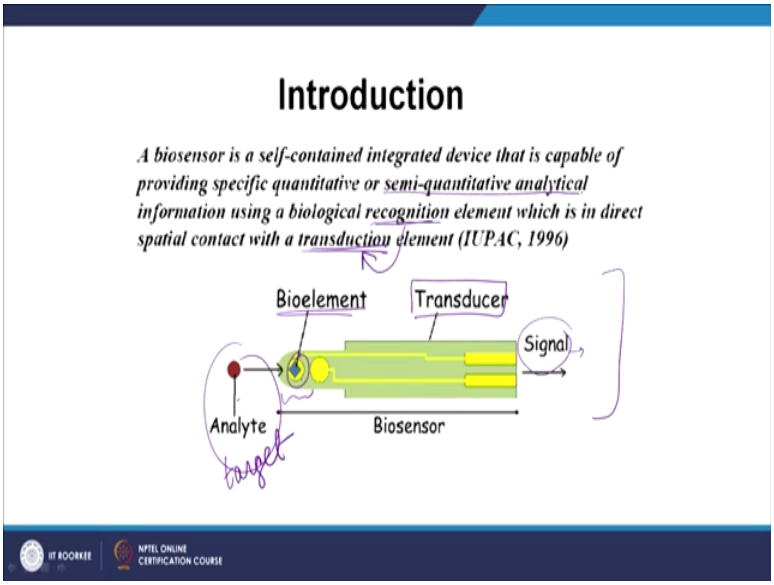
have 2.5 as many copies of vanA genes as mrsA, but what we can say that perhaps vanA is more at least twice as much as mrsA.

So, this is because there is semi quantitative we cannot rely on these numbers completely, but it does give us an idea of the relative abundance it does give us an idea of the trends. So, if over time the mrsA decreases or vanA increases I know the trend. So, that is the importance of semi quantitative. And because, it is a biosensor it uses a biological recognition element. Now how would I do that? For example, we know a particular sequence for mrsA we have already sequenced it we have also sequenced vanA.

So, one very simple technique would be we have a an optimal poly nucleotide with the sequence that is perfect complement to mrsA and somehow, we can make it specific to mrsA, and when if it attaches to mrsA if it does when we need to do a lot of troubleshooting for that. So, that is a part of research. And if the complement they complement perfectly and then after this pairing it should give me a signal and that signal should be read by my detector. If I can do this then I have my sensor ready biosensor ready, all right.

So now, let us go ahead and the biological recognition element is in direct spatial contact with the transduction element. So, the recognition element gives signal via transduction element and they are in physical contact with each other.

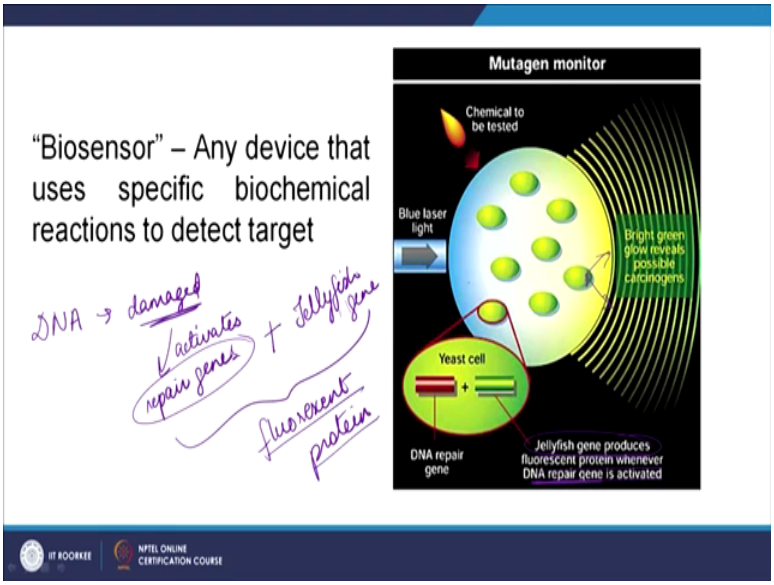
(Refer Slide Time: 13:43)



So, this is your typical bio sensor here is your analyte which is another way of saying your target, whatever contaminant or whatever you are interested in here is a receptor bio receptor. So, this is your bio element and because this is bio sense it got to be bio element it can be protein, it can be an antibody, an enzyme, it can be some genetic material like RNA based optima or DNA based optimer and then you have transducer this is very important.

So, what transducer does is? When analyte when the target interacts with the bio element either they aggregate or they behave in particular peculiar ways, then transducer creates a signal. It can be an electrical signal, it can be an optical signal and as we will go for it can be some other kinds of conductivity based signal and other kinds of signal, and then the signal is red. And when the signal is red and processed you get information. Yes, target is present no target is not present, and if target is present we want to know how much target is present or at least get some semi quantitative information all righty.

(Refer Slide Time: 14:39)



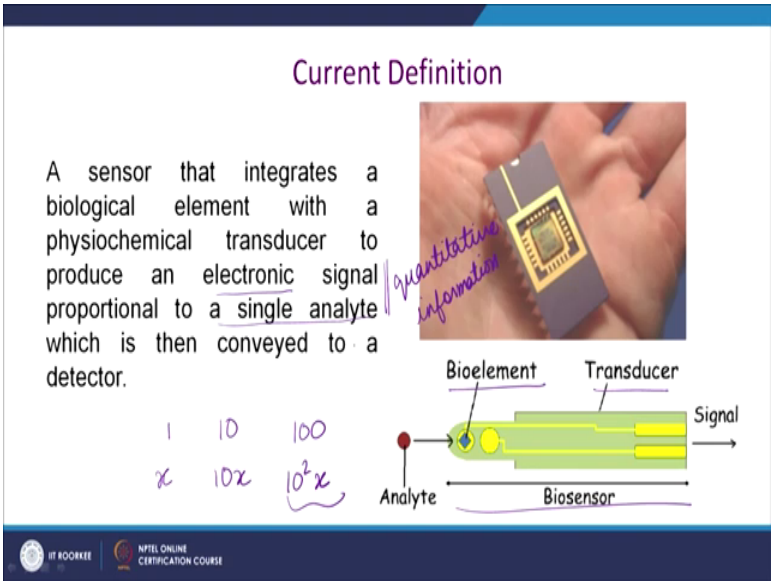
So, briefly we can say a bio sensor is any device that uses specific biochemical reactions to detect target, and this is one particular example which is a mutagen monitor. So, here you have your microbial cell here you are shining laser light. Blue light and the once if the cell has some kind of mutation in it, the kind of mutation you are looking for in Carsin carcinogenic mutation then, it will emit light of different colour, the blue will

appear to us green. So, because your sensors are actually jellyfish gene produces fluorescent protein whenever DNA gene repair is activated.

So, you are using this gene from jellyfish. And so, why would DNA repair gene be activated? Because, there is damage in the DNA. So, the way the mutagen monitor works is that there is DNA it gets damaged, and when DNA gets damaged what it does is, it activates repair genes. Because the damage, because the damage could be deadly for the cell or not helpful for the cell. So, it activates repair genes and the repair genes when they contact with when they interact with gel advertently gene from jellyfish. Let us just call a jellyfish gene, then together they are fluorescent they produce a fluorescent protein.

So, when they interact with each other the protein that will be expressed will be a fluorescent protein. Now, this fluorescent protein will change the light of the colour of the light, and will be able to tell whether the repair genes were triggered or not whether they were active or not. So, this is how we can detect mutagenesis in cells and this is a bio sensor.

(Refer Slide Time: 16:33)

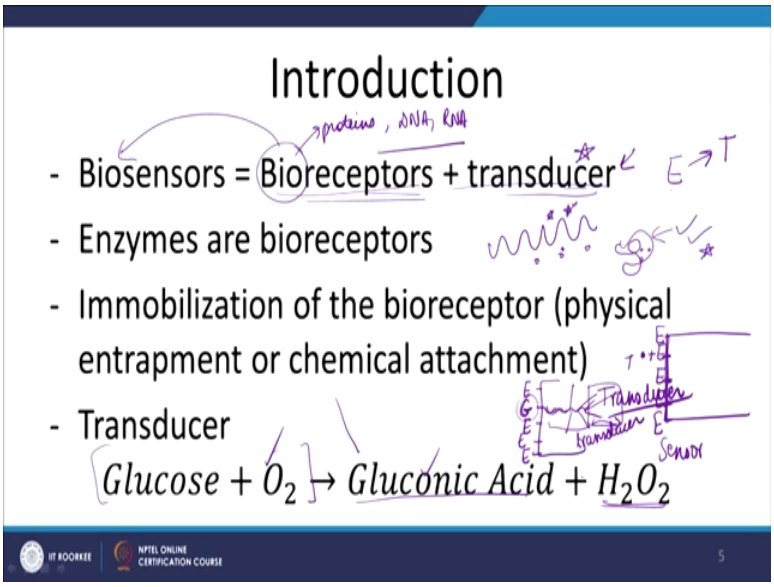


And this is a bio sensor because, we are using we are using biological agents for detecting cancer or mutation mutagenesis. Now the current definition of biosensor is a sensor that integrates a biological element with a physical chemical transducer to

produce an electronic signal proportional to a single analyte. So, this is my attempt to get quantitative information, all righty.

So, sensor integrates biological agents. So, there has to be a biological agent we can call it by a receptor, and it is in contact with a transducer which is physicochemical and it producing it produces an electric electronic signal notice not electrical signal, but electronic signals something that can be read by our computers. And the signal should be proportional to a single analyte basically, if you have one target you get let us say x amount of signal you get x amount of signal. So, if you have 10 you should get 10 x among amount of signal, if you have 100 targets you should get 100 this much amount of signal 100 times more signals. So, it will becomes quantitative and then, this is converted to the detector which feeds to a computer and we can read data all righty.

(Refer Slide Time: 18:03)



So, basically, we can say that biosensors are bio receptors plus transducers. So, there is a receptor and this is bio and this is the part of bios and biosensors that give the bio part to the biosensors. So, these are the biological agent transducers are basically physicochemical, and they can be anything you can have proteins enzymes you can have DNA genetic material, RNA, polynucleotides anything the sky is a limit here. And then there is a transducer which converts the interaction between bio receptor and the analyte into an electronic signal. So, transducer is very, very important.

Now, as an example enzymes or bio receptors. Now, if you go back to our previous lectures you would remember that we talked about what enzymes do. Basically, as a revision enzymes catalyze reactions in our body that in a microbial cell also in a cell of a big organism such as, human beings what they do is? They catalyse the reaction that would not happen otherwise at room temperature. So, they reduced the overall energy and they allow an easy jump over the reaction in energy barrier and how do they do that?

For example, very simple question when we eat food and we taste our food we start salivating. So, in this process tasting the food salivating or thinking about food and salivating the certain enzymes that are at work and these enzymes what they do is they interact with your food and they know what kind of food it is. So, they have been inform your body need to produce more insulin or less insulin produce more of this enzyme less of this enzyme.

Now also and then the insulin enzyme will interact the glucose in your blood. Now regardless of which enzyme is working where there is one thing that is common to all enzymes that enzymes are very specific. For example, of their amino acids floating in your blood after having a good hearty meal insulin would not react with them it is very specific, but if there is glucose in the blood then the insulin will interact and do it is job what it does.

Does the enzymes are very specific and how come they be specific? How does an enzyme a protein know who it is interacting with? In order to know that for protein the structure of protein behaves like a biosensor. Let us say, this is your enzyme and let us say insulin hormone and now here, you have molecules of sugar. So, it. So, happens that they to fit like lock and key and when they fit they come in a perfect shape whatever that shape maybe I do not know I am just making a schematic here, but in this perfect shape now insulin can do it is job, right?

But, if it interacts with other things like amino acids no change no change in configuration, no change in conformation thus enzymes behave like bio receptors. And no wonder one of our first biosensors were based on proteins, were based on antibodies and antigen antibody react interaction, were based on enzymes enzymatic activity. So, if I have the right enzyme I know it is specific it is the chemistry and the physical properties of this enzyme are specific to a particular target.

So, if I want to detect the target let us say, I want order to detect glucose I can take enzymes that specifically interact with glucose because, they know there they change a conformation they change their structure in the qualities once they have interacted with glucose. So, they are perfect by receptors. And to make a biosensor bio receptor let us say I have chosen a bio receptor some enzyme E, and I know this enzyme E interacts with target T. Target T is what I am interested in because, it is harmful in it is in the environment when it is in the body I know that enzyme E acts with target T.

So, what I need to do is I need to have a way of physically entrapping immobilizing the enzyme on the surface of my sensor. So, this is my sensor that I am making bio sensor. So, I need to find a way through which I can make sure that the enzyme E attaches to the surface in which I am going to put the sample. So, one of this is one of the first challenges that comes when we make bio sensor which is immobilization of bio receptor it can be done in 2 ways, one is physical entrapment we physically force the enzymes to be struck here, the second is chemical attachment. So, they can be touched through a chemical bond.

Now, next is transducer. Now basically, once the enzyme and this is analyte, the analyte that target T. So, once they interact they undergo a change. So, what was E and T separate will now look like this. So, this is still E these are still E, but this particularly E has interacted with T. So, it becomes something else and now when this becomes something else there goes a signal.

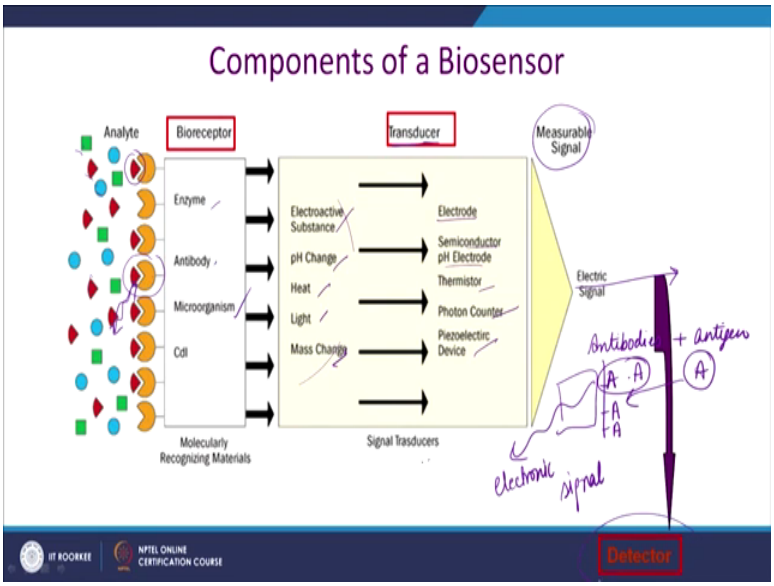
So, there is some change whether it is change in structure, change in chemistry, change in pH change in conductivity change in the optical properties. Now, this is converted into an electronic signal by a transducer all these changes in any one important property is converted translated by transducer.

So, an example would be if I am looking at oxidation of glucose, I am very interested in seeing microbial oxidation of glucose I have glucose and I know there is oxygen reaction happening. And I just make gluconic acid and hydrogen peroxide I have 3 options. So, once the once the reaction has happened glucose has reacted with oxygen, I have changed the property of this particular enzyme. It has interacted with the target with the analyte. So now, this can be read them various properties of this changed conformation

of g that can be read and can be converted into an electronic signal for example, again oxidation of glucose forming laconic acid and hydrogen peroxide I have 3 options here.

I can have a transducer here, whose job is to read the amount of oxygen available in environment. So, as oxygen drops it knows that glucose oxidation is happening. So, it can detect oxygen or it can detect gluconic acid. So, as gluconic acid is present more and more transducer creates more and more signal. So, we know now the reaction is moving towards the right side or we can a hydrogen peroxide detector. So, all these 3 things are subject for detection, and either one of them will give me information on oxidation of glucose ok.

(Refer Slide Time: 24:34)



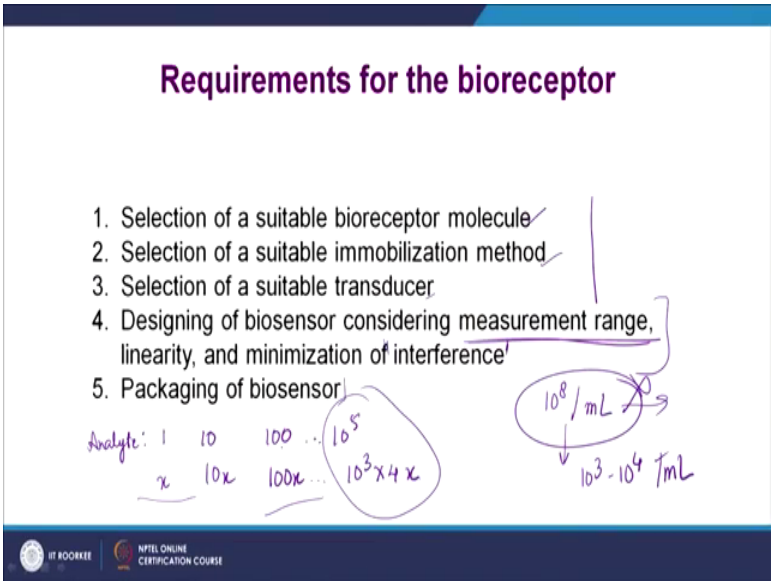
Now, let us look at components of biosensor in little bit more detail. So, this is your these are your enzymes, now coming back to the whole enzyme business enzyme antibody microorganism or capacitors. And now, these are very specific to a particular target. So, in your sample you might have different kinds of compounds present. So, we have the green circle squares red pies and blue circles. Now, all of these will interact with the enzyme, but only the red pies would fit perfectly into the enzyme, and once they fit perfectly it undergoes a conformational change. Now, this could be a change in electro active substance it could be change in pH in heat light even mass.

Because, it might release some other thing of away or gain some mass; obviously, and either of this can be detected and converted into signal using a transducer, which can

behave like an electrode like a semiconductor or pH electrode thermistor for heat and for light a photon counter for mass a piece of electric device and so on. And so, forth this is the job of transducer, and then to a transducer has converted this conformation change into measurable signal and then you can read the electronic or electrical signal. Now, let us look at some of the bio receptors we have enzyme your talked about it antibodies. Remember antibodies are part of human defence system. So, antibodies are very specifically complimentary to antigens.

So, antibody A will be specific to antigen A. So, if you want to detect antigen A immobilized antibody A on the surface bring in contact with the sample. Whenever you will interact with antigen A it will under conformational change have a nice transducer which can translate this into an electronic signal, and you have detected antigen A. All righty then we have microorganisms a certain microorganism that also behave errors by receptor. And then we have CDR as transducer I have already talked about these things let us move on this is by receptor, this is transducer and the electrical signal will go to your detector which is convert it into data all righty.

(Refer Slide Time: 26:43)



Now, what are the requirements or bio receptors we have so many options we can use ends enzymes we can use optimers poly nucleotides, we can use DNA based, RNA based by receptors we can use antibodies-based bio receptor microbial bio receptors. So, the requirement for bio receptor would be we need to detect, we need to select a suitable bio

receptor molecule, right? Which is specific sensitive and we will talk more about this. We need to also select a suitable immobilization method all righty I found the best optimal for detecting a contaminant I am very interested in, but how do I immobilize this receptor without damaging it is business I mean damaging it is function?

Next is I need to select a suitable transducer if I know that one particular contaminant is fits perfect lock and key with a particular bio receptor, I need to find out what would be the changes in their properties and what transducer would be able to convert this change in properties into an electronic electrical signal, then I need to design bio sensor considering measurement range linearity and minimization of interference these are very important. Many people have already crossed the first second third barriers, but the 4th barrier is what is my measurement range?

How what is my sensitivity? For example, if I tell you I can make us make a sensor bio sensor that will give you values of 10 to power 8 gene copies of antimicrobial resistant genes for mL and this is the lowest limited can it take and above. This might not be very important and very valid in developed countries such as Britain which are very clean and have in general lower levels of AMR and also in our country we know that the public health threats even of 10 to power 3 to 10 to power 4 gene copies per mL of AMR genes is can be tricky and dangerous.

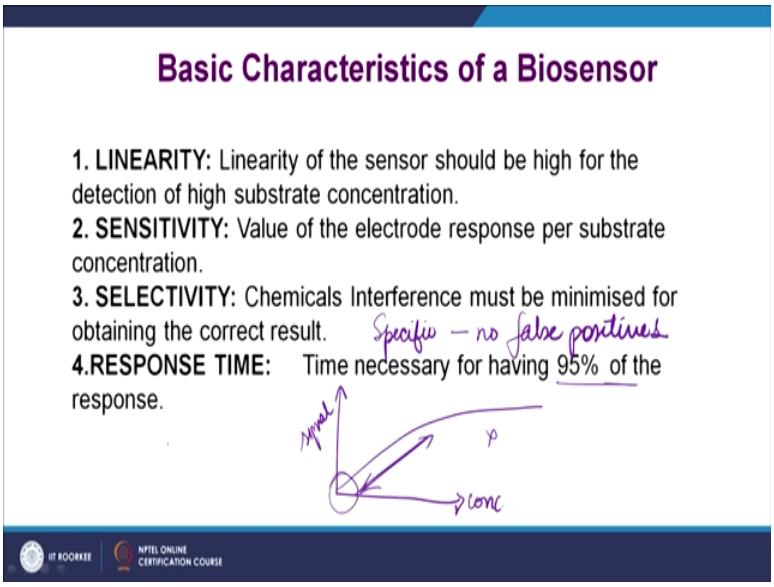
So, measurement range is very important this is not very useful if it is higher then what I am interested in. Next is linearity. So, remember in previous slide I was telling you that if I have 1analyte present this is analyte by the way then I get x signal, if I have 10 analyte I get 10 x signal if I have 100 analyte I get 100 x signal. After a time what we notice is that what we notice is that the linearity damn it goes away see for 10 to power 5 analyte I only got 4 thousand x signal not 10 to power 5 x signal.

So, this is the vanishing of linearity and this limits my upper range and also my lower range. So, this is also very important measurement range and minimization of interference. So, in lab and we make up I have sensors or in human body also when we have our enzyme doing their job it the human body is is arranged in such a way and other my life form. So, I arranged in such a way that the enzyme that is specific to a particular analyte will find each other most more often otherwise life cannot continue.

So, and they would not find other kind of crazy contaminants that might interfere with the lock and key perfect fit mechanism between enzyme and analyte, but in environment is interference business is very, very important. For example, you might have heavy metals that might completely shatter your bio receptor and then bio receptor does not work because, they because of interference or they might be other contaminants presenting present in the water or in soil in the sample in the food for example, humic acid another natural organic matter, that will interact with your bio sensor bio receptor and then you will not be able to get a good reliable data. So, in we want to minimize interference.

Next is we want to package biosensor in such a way that it is compact, robust definitely cost effective and that we can use over and over again.

(Refer Slide Time: 30:36)



So, the basic characteristics of biosensors should be linearity. So, as the concentration goes high. So, this is concentration on x axis and this is the signal on y axis. So, ideally it should be a straight line, but as a concentration goes high it plateaus. So, this should not be there should have a very nice very reliable linear range. The other is it should be very sensitive which means, I should be able to detect lower and lower values. Next it should be very selective means very specific.

So, basically, I do not get false positive, and the response time should be than necessary having 95 percent of the response, the response time is very important. Again, in the

beginning of this lecture I mentioned that how it is very, very crucial very, very important that the whatever biosensors or whatever detection techniques, we use to deter contaminants in our environment we can reduce them rapidly because, we have a crunch of resources and we have a severe public health burden.

So, we cannot afford the delays that is importance of biosensors also because, some detections for example, tuberculosis detection micro bacteria might take really long time in the lab to grow and by the time we get a positive result from the lab the patient might suffer a lot. So, quick detection techniques are very, very important and required.

So, dear students this is all for today's lecture, in the next lecture we will explore more about biosensors we will even explore different kinds of biosensors.

Thank you.