Optical Sensors

Prof. Sachin Kumar Srivastava
Department of Physics
Indian Institute of Technology, Roorkee

Lecture - 02
Sensor Fabrication and Characterization
Functionalization Methods and Performance Parameters

Welcome to the second lecture of Optical Sensors course. In the first one, we saw what sensors and bio sensors are; what are the components involved in a sensor; what are the roles of each of the components in the sensor and then we discussed various transduction methods. And, from there we come to the point that you name as sensor based on the transduction method.

If it is electrical transduction method, you can call it an electrical sensor. If it is an optical transduction method, what it means is that the interaction between the analyte and the bio recognition element leads to change in the optical read out: that is an optical sensor. And, then we discussed what are the parameters which need to be probed to make an optical sensor.

For example, there was electric field of an EM wave. I wrote this equation and you saw that, it can either be amplitude or intensity and then the phase part. So, that is all. Now, we are going to discuss the sensor fabrication and characterization, what are the functionalization methods and other performance parameters which need to be seen.

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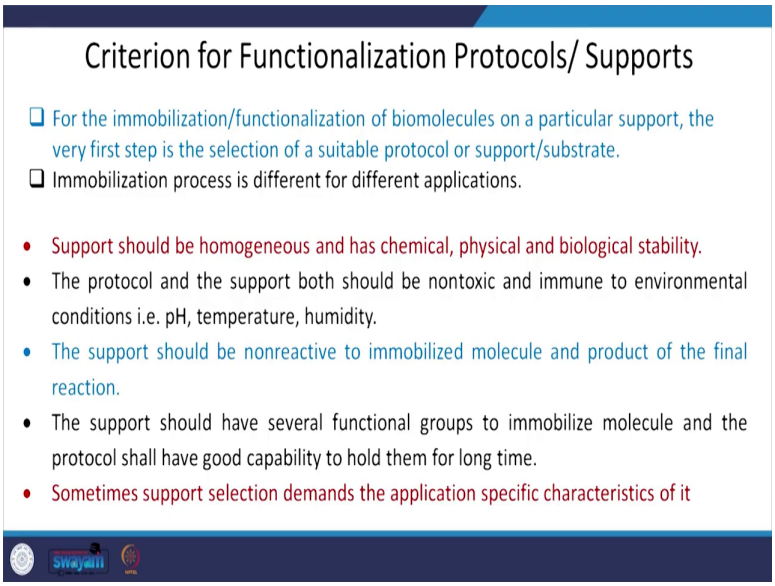

So, criteria for functionalization protocol is that for the immobilization or functionalization of biomolecules on a particular support, the very important thing is the selection of a suitable protocol or the substrate. And, then we use immobilization or functionalization process which is different for different kind of applications.

So, what is the meaning of this functionalization or immobilization? The meaning itself is hidden in the word itself. It means that you block or you restrict the mobility of a molecule on a surface: that is - immobilization, that is in terms of the molecule. When, you say in terms of the substrate or surface on which you put the molecules basically you do it by certain functionalization of this surface.

So, this is the meaning of immobilization - you restricted the mobility of the molecule by attaching it to on the surface. There are certain criteria for this surface - that this surface should be homogenous, and it has to be stable- be it chemical stability, be it physical stability or biological stability. And, the protocol and the support both should be non-toxic and immune to environmental conditions, say for example, pH or temperature or humidity. So, it should not be like that as this protocol changes with temperature or substrate or surface changes its properties with, say, humidity. Then, this will not be a good sensor. Why? Suppose, you have a sensor and it is working fine in normal weather conditions. Now, it started raining, the weather becomes humid the property of the sensor will change. Then, you will need to characterize it again and again.

So, the best way to avoid this is to choose a support and a protocol for binding of this molecule, which is not affected by ambient conditions - OK. And, the support should be non-reactive to immobilized molecule or the product of the final reaction.

What it means? It means that, you mobilized a molecule on the substrate and then it starts reacting with itself, then your sensor is not good, because this will lead to faulty signals. Also, suppose you attached this molecule - this is a BRE. You functionalized this, you attached it, immobilized it on the surface ok. It does not react with the surface, but then comes now analyte. It interacts with the BRE - it leads to another product, which starts reacting with the surface. This is also not good. So, the choice of the protocol for sensing and the substrate is very important. The substrate should have several functional groups so that you can immobilize the molecule and the protocols have good capability to hold them for long time. It is not that ok, if it came, got attached and after certain time it goes back.

So, sometimes the supports selection demands the application specific characteristics of it. So, one has to be very specific. According to the application, one has to choose the support and, also the transduction mechanism. For example, you cannot use a wooden surface for optics, ok.

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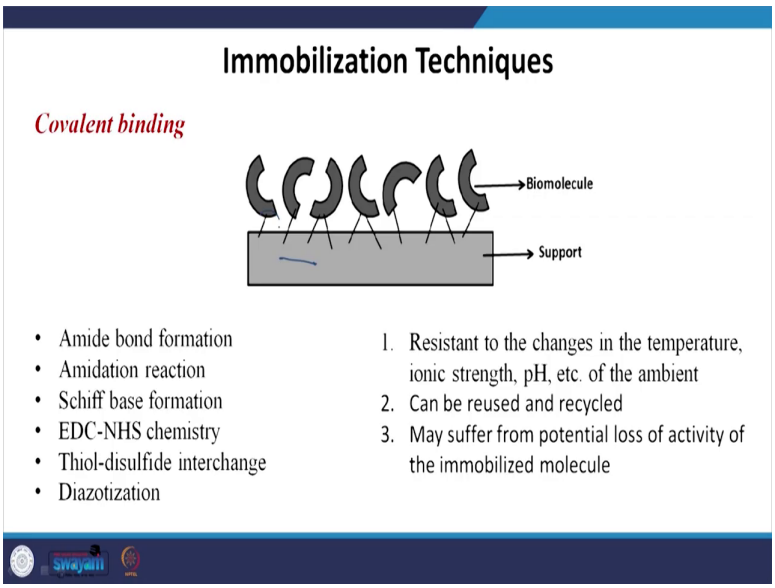

So, let us go to the immobilization techniques. Covalent bonding - One of the immobilization techniques very favorable is covalent bonding. Why? because covalent

bonds are very good. So, if you have a support like this, you attach the molecule using covalent bonds. It can be any of these bonds- it can be an amide bond, or amidation reaction, or Schiff base formation, EDC-NHS chemistry. This a very good - I told you the antibody and anti-antigen reactions, this is very good for attaching them.

Thiol disulfide interchange, thiols and disulfides bonds are good for bonding on metal. and diazotization - why it is good? because it is resistant to the changes in temperature, ionic strength, pH, etc. of the ambient. It can be reused and recycled, but the problem is that it may suffer from potential loss of activity of the molecule.

Why? because it is attached firmly. After certain time, there is a possibility, that as the sensor surface becomes older and older, it will start losing it is activity. That is the reason that there are certain limitations. There are various kinds of covalent bonding say thiol bonding. Thiol is basically organo sulfur compound, which contains carbon bonded sulphydryl SH group.

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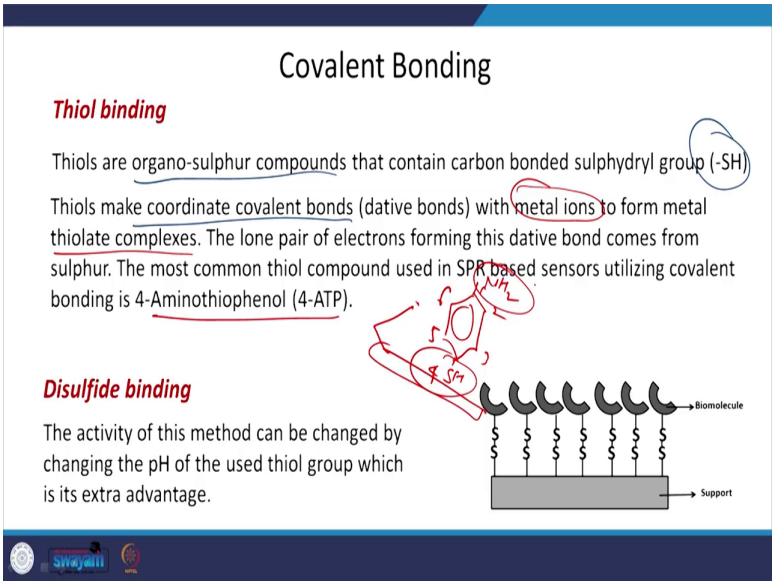

And, this makes very good bond, that is basically coordinate covalent bond and it makes a very good coordinate covalent bond - very strong bonding with metal ions to form a metal thiolate complex.

So, the lone pair of electrons which this sulfur has - it is donated to the metal to form a bond. So, people generally use 4-Aminothiophenol. It has basically this kind of structure one side you have SH, another side you have NH 2.

So, if you count from 1 2 3 4 5 6. So, this is called 4-Aminothiophenol. So, on the 4th one you have a metal surface here and this NH 2 is free for binding of other molecules ok. Then you have disulfide bonds. Disulfides are other molecules which have 2 thiols; one binds to the substrate and the other binds to the molecule. So, very strong bond ok.

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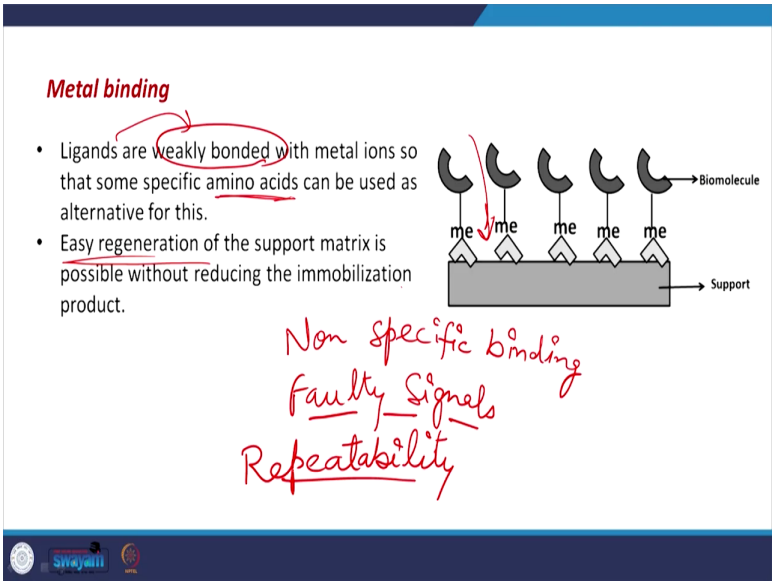

Then you have metal bonding, it is really weak bond. So, ligands can bond sometime directly. This is very good for, you know, specific amino acids and it possesses easy regeneration of support matrix. Because it is loosely attached, if you want to regenerate the surface for sensing again and again you can easily do it.

Sometimes analyte can go directly bind on here very easily, but the problem is that this will lead to something called nonspecific binding. So, when you have nonspecific binding then it will lead to faulty signals. So, one has to be very careful. I mean this metal binding is not very appreciable for making real sensors, because it does not have very good response. Why: because it is loosely bound.

So, the regeneration - I mean repeatability is a problem. Repeatability-I will come to what is repeatability and all these things. So, repeatability is a problem, it will not have a

repeatable signals. Sometimes there are 5 and sometimes there are 4 molecules one washed away, because it is very loose bond-weak bond. So, it is not very good.

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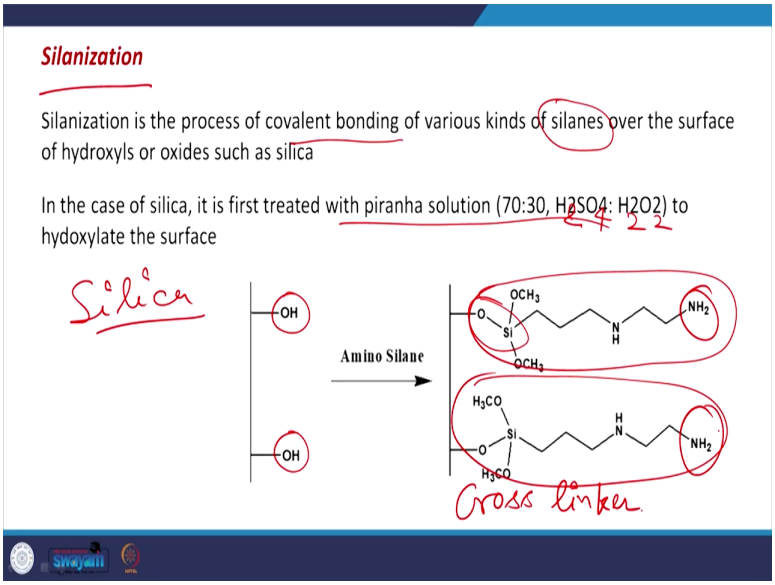

Then there is Silanization. This is the process of covalent bonding of various kinds of silanes. These are kind of molecules, which are attached as they have oxyl groups here, which leads to attachment on some surface like silica. Silica, you know, silica like glass. It is something very difficult to attach anything on, but what happens actually that if you treat the glass with piranha solution, actually it should be like H 2 S O 4, H 2 O 2 what happens actually that it gets hydro-oxalated- OH groups.

And, on this OH groups these silanes go and bind on. Then you have this works as a cross linker. In the first lecture, I told you that sometimes there you need a molecule as an adhesive, which attaches basically the bio recognition element to the surface. So, for example, this kind of silanization works for this purpose. So, you have free NH2 molecules, where you can bind your BRE - bio receptor, ok.

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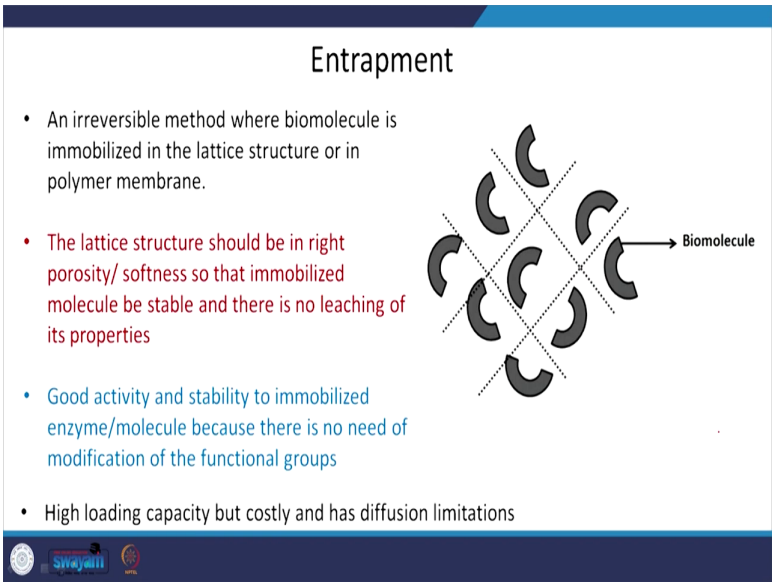

Then you have entrapment. Entrapment is also an irreversible process, where biomolecule is basically immobilized in the lattice structure of a polymer membrane. What it means? It means, like you have a polymer, where these molecules are everywhere embedded and this polymer has to be porous.

So, if it is porous then what will happen that the analyte will tunnel through reach to this molecule and there will be a change in the signal. So, that is how a sensor based on entrapment works. So, it is very good activity why because the molecule is not immobilized - basically it is free almost. So, it is good, it does not lose it is character, when it is immobilized like this. Because, it is not firmly attached - this is somewhere moving in a polymer matrix.

So, you can put more and more molecules. So, it has high loading capacity, but the problem is it has diffuser limitations. So, suppose the pore size is small and the analyte sometimes is bigger. So, I mean every time you have to choose certain optimum conditions. For examples, you have a polymer and there are certain pH conditions say acidic conditions the pore size will be different, the aqueous conditions it will be different.

So, the diffusion rates are different in different conditions, that can lead to faulty signal. So, this is a very good sensor, but again there are certain limitation to it. So, it can work for certain places, but at certain places it does not work. Then you have encapsulation.

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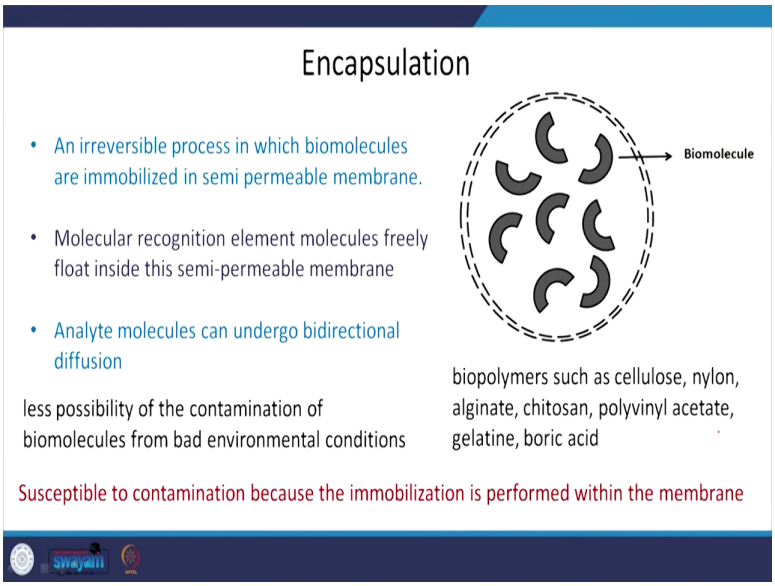

In encapsulation you have a semi permeable membrane and all the biomolecules are in it. So, again it is a loosely bound thing. Since, it is the membrane which is semi permeable, the biomolecules cannot go out, but the analyte can come. And since this is freely floating inside - the reactivity is not changed. That is good and analyte molecules can come and go. So, it is like bidirectional diffusion.

So, the probability of contamination of the biomolecules is less, because biomolecules are not going away, they are encapsulated, they are in a fixed place. At the same time, there is a contamination due to perforated membrane, why? because now the analyte is coming and getting immobilized inside the membrane with the molecules. The bacteria can also come, other things also can come and get bind there.

So, since there is a free flow you are never in control of passage of fouling agents. Say, for example, if you have a molecule- if this is big it can go through then, smaller fouling particle can also go through. So, there is certain limitation to this kind of sensor, but it is still OK. Then there is class linking. This is also an irreversible process. All of them are irreversible processes. I will tell you what is a reversible and irreversible process.

The good thing is that you do not need any support mechanism like you needed a polymer matrix for embedding in entrapment. In encapsulation, we needed a semi permeable membrane, but here we do not need any support mechanism. It is free.

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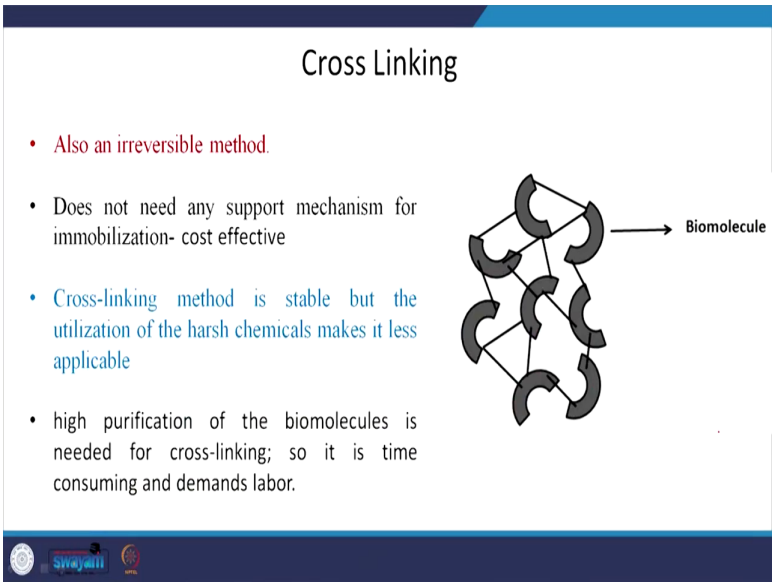

All the biomolecules bind to themselves - it is called cross linking. So, this is very stable method, but if you use harsh chemicals then it is less applicable. It is not so easy, ok. And, the molecules which do a cross linking have to be very pure or highly pure. To make a highly pure biomolecule or improve the purity, it takes lots of money, lots of time and a lot of labor.

Then there is adsorption. Adsorption is a reversible process. Molecule can come - attached on it and can go again like this. It is not like this pen. It got fixed here - it is not moving easily right, it is not moving.

But, adsorption is not something like that. Molecule can come and get adsorbed again. There is a high chance that it will again leave and again come. So, it is a reversible process, because this is based on very weak interactions - it can be van der Waal's force, it can be an ionic interaction or affinity bonding.

So, because of this it has it is a very simple process, it is low cost, retentive and fast technique. Again I told you in the begin, when I was talking about metal bonding, due to weak interactions the rate of leakage of biomolecules is high. So, the loading capacity like attachment, is random and binding is unstable.

So, the repeatability of the sensor is very poor. Sometimes you will have 100 molecules, sometimes you will have only 50 may be. Your sensor response is not so, repeatable.

Also, this is very prone to non-specific bindings, because it is adsorption: anything can come and bind. So, it leads to fouling. So, there is another problem with it.

Then comes molecular imprinting.

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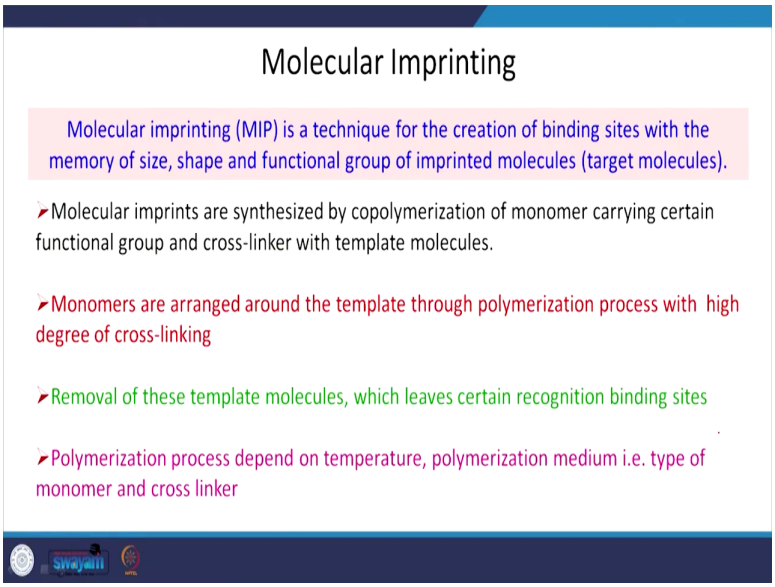

Molecular imprinting is a technique for the creation of binding sites with the memory of size and shape and functional group of the imprinted molecule. I am talking about something different. For the all the methods which we have talked, we had certain X, which went and bound to certain Y, which was on the surface. This is not the case for molecular imprinting, all the bindings which I have talked about yet, they were all based on functional group they have no memory of size or shape.

So, any molecule having similar kind of functional group could come and bind on here ok, but this technique creates binding sites, which do not only have the memory of size or memory of the functional group, but also the shape and size of the molecule. Tt is like this.

Suppose, someone is asked to [talk/teach] teach general relativity or special relativity let us say. Any teacher of physics can go and teach does not matter, what is the height? what is the shape? He can be a lean guy, or thick guy, or may be taller one, or smaller one, shorter one does not matter, that person can go and teach special theory of relativity.

But, now so, from there you can say that ok, this guy must have some Physics degree that is why he is able to teach it. Now, we come to a point ok, I do not want just this physics guy, but also want the shape and size. Let us name it. Say, I want only Albert Einstein to come and teach special relativity.

So, what you do is that he knows physics, but you again do something which takes care of the shape and size of this person, then Feynman cannot go and fit in there, ok. So, that is why what something molecular imprinting is doing? Any molecule, which has the same functional group can go and attach in other kind of bindings, other kind of functionalization method, but here they cannot because the shape and size is also defined.

So, how they are made? They are done by synthesizing copolymerization of monomer carrying certain functional group and a cross linker with template molecule. What is the meaning of this? Suppose, you want to detect some molecule, then you choose a polymer and a cross linker, which attaches to this molecule at certain sites and they are all polymerized together.

Once they are all polymerized, the template molecules, the molecules which we want to sense, are removed. So, this leaves to a recognition site or binding site, where this molecule can go and fit in. Again, because now there are 2,3 functional groups and the memory of shape and size, so it can go and fit in only in that orientation and functional group. The polymerization process depends on temperature, polymerization medium monomer or cross linker all these things.

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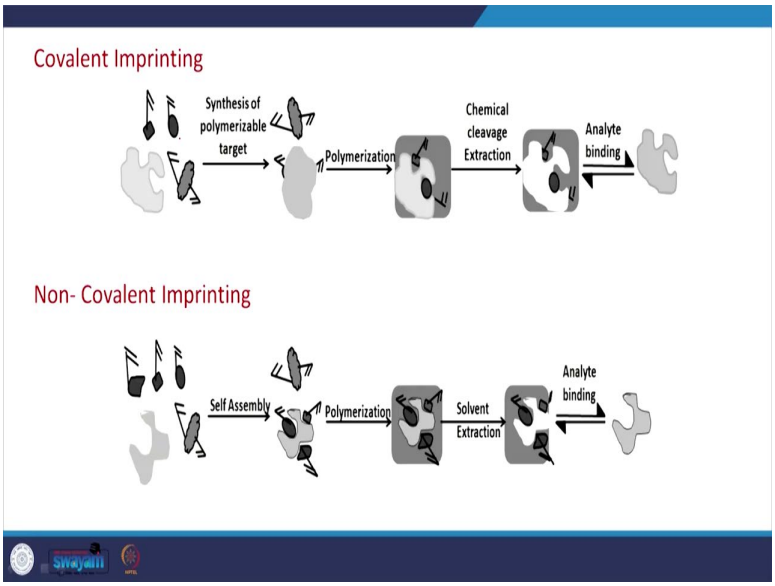

There are two kinds of imprinting: one is covalent imprinting; another one is non-covalent imprinting. In covalent imprinting what you do is that, you take the template molecule and cross linkers together. And, then first they are allowed to react and then they are copolymerized with the polymer.

So, you have these guys, who are working together and then they get polymerized. And, then you need a chemical reaction - cleavage reaction to take this out. While in non-covalent imprinting, everything is polymerized together - all of it. All of it is polymerized together and then you have a solvent, which can take it off, because it is non-covalent bonding.

So, that is good thing. What is bad about non-covalent imprinting or covalent imprinting or molecular imprinting is that, it, again, uses polymers and a polymer can swell or shrink in different ambient conditions: You change the pH, it can swell or shrink, thereby changing the shape and size of the binding site- may be not, but may be yes. And, that will possibly lead to faulty signal. Also, the shrinkages or swelling of this polymer will lead to change in the refractive index. So, if you are making an optical sensor, which is measuring the change in reflective index of the polymer due to the ambient conditions.

So, it is not like that. I mean, one has to take the calibration of this thing, if you are making this kind of sensor. Now, let us see what are the performance pa.rameter of a sensor. I mean, when you made a sensor, how do you know that if it is good or not? So,

there should be certain things - certain parameters which will tell, what are the qualities of this sensor if it is good or not?

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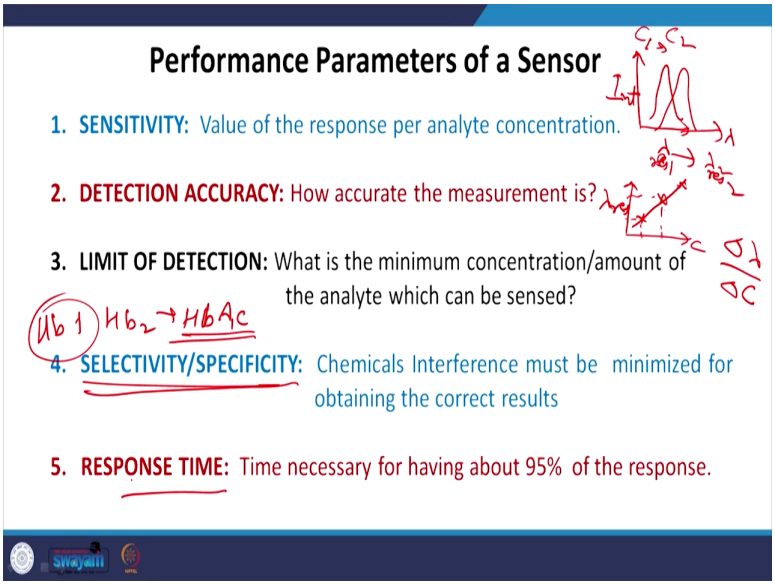

Sensitivity: the first thing that comes to your mind, if you talk about a sensor is a sensitivity. So, sometimes in many talks people ask me what is the sensitivity of this sensor? Suppose, I am making a sensor and then I am describing this thing and someone ask me what is a sensitivity of the sensor. And, then I tell them can you please repeat your question. What he says - how sensitive is your sensor is?

Now, I want to tell you that there is a very minor difference between these two terminologies - a very fine line between the question what is the sensitivity of the sensor and how sensitive your sensor is? Two different things - I will come to that.

To discuss all these things, let us consider a performance parameter: let us say, that I have a sensor, which has intensity and here is wavelength. So, I have sensor say for concentration C 1 I get this lambda 1, concentration C 2, this shifts to lambda 2, if I change from C 1 to C 2 it goes from lambda 1 to lambda 2, let us say this is my sensors.

So, if I plot I with concentration, you have concentration here and say lambda 1 and lambda 2. So, I will say lambda may be I call it lambda resonance, resonance at 1 lambda resonance at 2. So, this is lambda resonance and I see that it is changing like this.

Its C 1 C 2 C 3 you have lambda 1 lambda 2 lambda 3, ok. Sensitivity is the value of the response per analyte concentration; that means, that you have delta lambda by delta C, that is the sensitivity - the slope. Slope of this curve at particular concentration gives the sensitivity at that particular concentration.

Detection accuracy- how accurate is the measurement? Limit of detection- what is the minimum concentration or amount of the analyte which can be sensed. What is the smallest C - possible smallest C value for that you have a confident value of the parameter say lambda here that is the limit of detection. So, if someone asks you what is the sensitivity; that means, this is the slope?

But if someone asks you how sensitive your sensor is; that means, what is the minimum value that it can sense that is the limit of detection. So, these are two different questions. Selectivity or specificity: This is not a quantitative measurement. Tt is a qualitative measurement. So, this sensor, your sensor, if you do a sensing experiment, it should be very very selective to the molecule you want to sense. It should not even sense its neighbor. Suppose, you have, say, hemoglobin 1 and hemoglobin 2, that is, say, H b A 1 C. If you want to detect H b 2, it does not show the presence of H b 1- does not show any response then it is called the selectivity or specificity of the sensor. So, it is not a quantitative value, it is a qualitative response.

Response time: time necessary for having about 95 percent sensor response. You want to have a sensor response - what is the time that it will have a confident signal from it - that is the response time.

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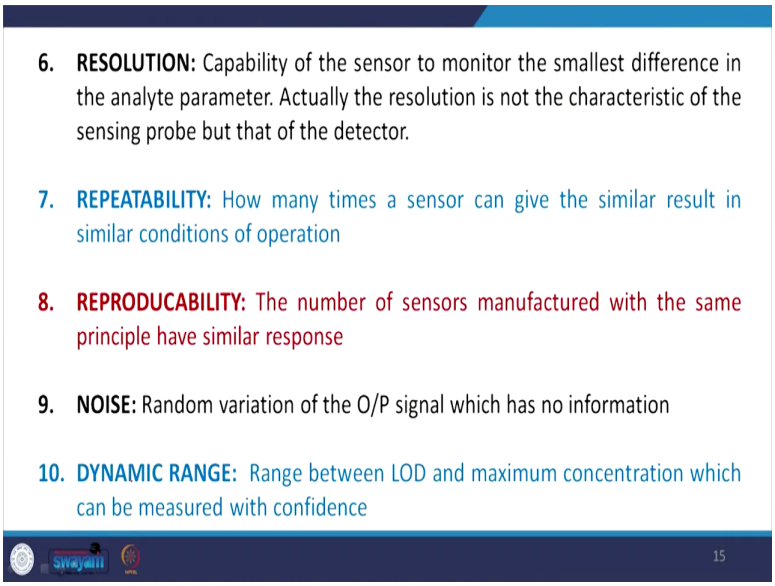

Resolution: capability of the sensor to monitor the smallest difference in the analyte parameter. So, suppose you want to measure two concentrations here, what is the difference between these - how small is the difference between C 1 and C 2; that means, how small you can measure the difference between lambda 1 and lambda 2. And, this is not actually the property of the sensor system. Actually, it is the characteristic of the detector. If, your detector has high resolution, it can resolve lambda 1 and lambda 2 very precisely and you can have good resolution.

Repeatability: how many times the sensor can give similar result in similar conditions of operation - that is repeatability. Reproducibility: if you manufacture this kind of sensor you can make this kind of sensors in the similar conditions, the response should be similar - that is reproducibility.

Noise; a sensor can have noise, which is basically the random variation of the output signal. Sometimes, you may not have an input, but you will have some output signal, that is noise.

Dynamic range - What is the range that this sensor can work in? So, you have the minimum concentration that is the limit of detection to the maximum concentration, which can be measured confidently. This range is called the dynamic range of the sensor. The sensor works in this range only.

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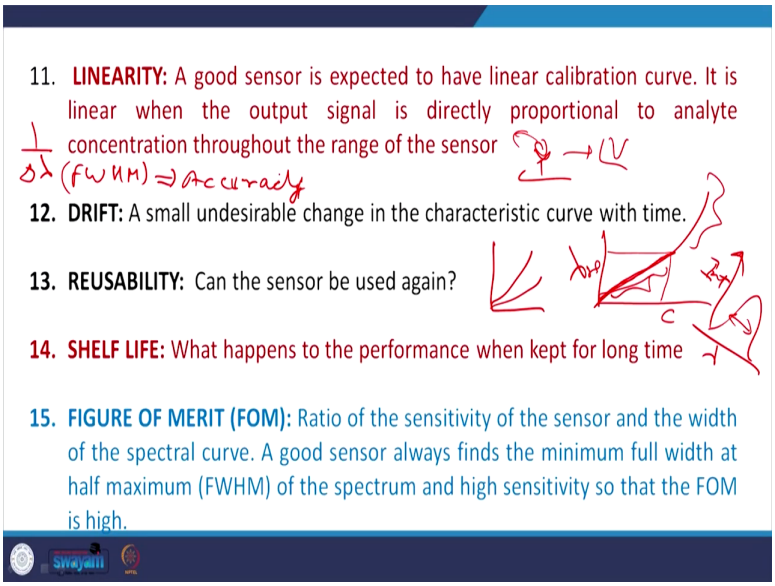

Linearity; a good sensor is expected to have linear calibration. What it means? like I told you that lambda resonance versus C curve and I showed you this kind of thing. Suppose, you have like this it is not linear here.

So, basically you want to have the sensitivity, if you report the sensitivity of a sensor, I told you that this is the slope. You want to have a constant sensitivity that is the linear part of this response. That is what most of the people are concerned about, because you want to have something which has constant sensitivity and there is a good calibration curve. So, better the linearity, better the sensor.

Drift: you operate a sensor for long time and after certain time, may be, the characteristic curve will change. So, it was showing like this. Now, it has started showing this. So, this is undesirable, actually, but it happens. As the sensor gets older and older there is a drift, but you must know what is the drift? Because, if you want to measure using it, say after 6 months, you must know that how much my sensor response should have changed. So, you correct your value accordingly, ok.

Reusability: can a sensor be used again? it is like this - you have a sensor, it got attached something here, you got a signal and then what happens if you wash it? can I use it again and again?

So, that is also good concern, but for financial, I mean for commercialization aspects most of the companies - they do not want actually the sensor to be reusable. So, that you use it, you throw it and then they will make more money. Also, it is something important that then, if you have a sensor which is not used again and again, then you will not have the problem of this drift and all those things. So, it will have a good, I mean confident, response.

Then the shelf life. What happens to the performance, when kept for long time? So, you can keep the sensor for long time and then what will happen to it? I mean if it is kept on the shelf; you are not using it even then is it possible? Because the biomolecules and the sensor surface may get degraded, you can have a poor response. So, that is the shelf life that up to that life, the performance of the sensor is not changing.

Figure of merit: this is the ratio of the sensitivity of the sensor to the width of the spectral curve. One of the width of the spectral curve say if you have a spectral curve like this, I told you that this is this is the intensity and this is lambda 1, over the width delta lambda F W H M, this gives the accuracy.

What it means? It means that, the sensor will be accurate when you have very fine peaks. The figure of merit - it means that a sensor is good, when the full width half maximum is small and the sensitivity is large. That is how we define it. So, let us summarize the talk today we discussed what are the various functionalization methods for sensor fabrication. And, what are the performance parameters for the sensor. If you want to make a sensor, you must know what are the things which need to be kept high and low.

Thank you.