Bio - Microeletromechanical Systems

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Module No. # 01

Lecture No. # 24

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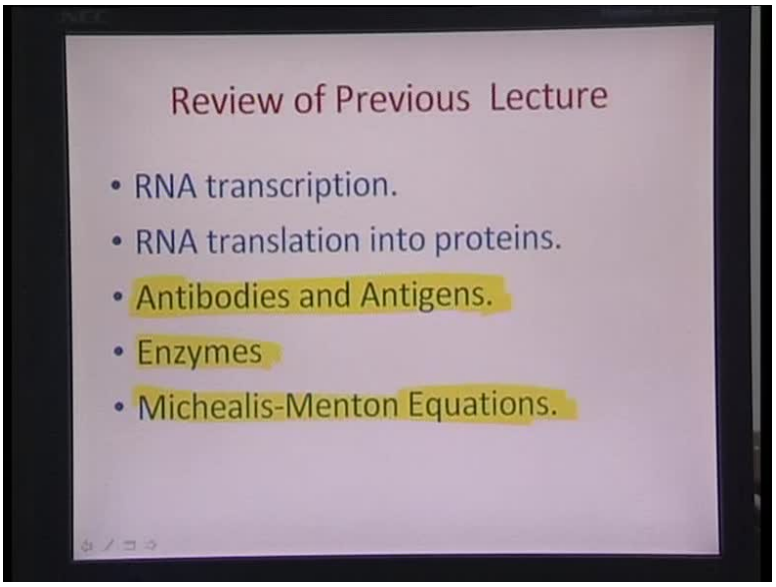

Hello and welcome back to this 24th lecture of Bio-MEMS, bio-microelectromechanical systems. I would like to have brief review of the previous lecture. We talked about RNA transcription and translation processes. Transcription is basically conversion of double stranded DNA in the chromatin region of the nucleus into a messenger RNA and the translation is the change of language from the RNA into proteins or a sequence of amino acids. We saw how beautifully this nano machinery inside the living cell works; especially the ribosome which assembles all the different messenger RNAs and coordinates with the transfer RNA which is around the cytoplasm. These transfer RNAs have aminocyl groups which conjugate with the messenger RNA on a base triplet by base triplet basis and thus there is a sequence of amino acid which is generated.

This is a very interesting process, because this coding is also responsible for the physiological state of health of a living cell. We also described about antibodies and

antigens and would be doing this little bit later in more detail. We briefly talked about enzymes where we are just about to begin Michealis- Menton equations of the formulation of enzyme substrate complex.

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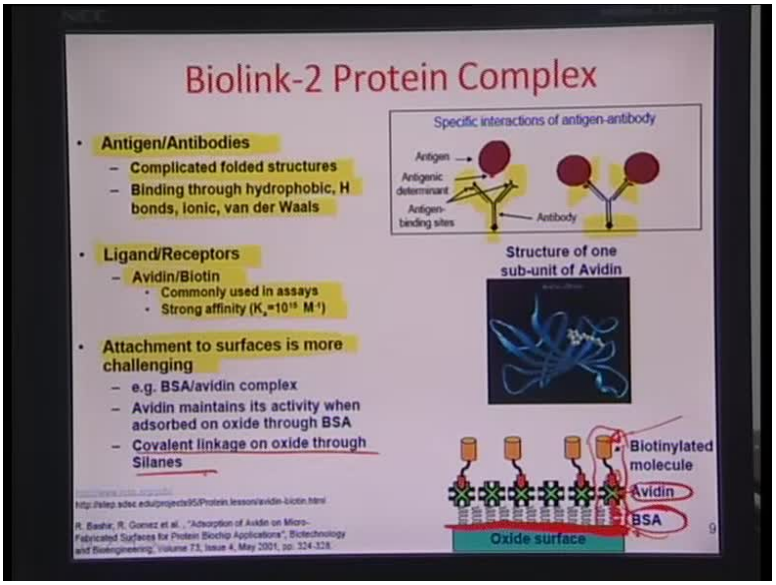

Today we will focus a little more on these three areas and then try to go ahead and derive these set of equations and see what the enzyme catalysis rate would be in a typical chemical reaction. To begin with, let us look at why all this is important. We are talking about enzymes; we are talking about proteins, we are talking about antibodies; from a Bio-MEMS perspective, it is almost always essential that we have immobilization mechanisms wherein all these different moieties are used for capture of targets in the analytes. From sensor design point of view, it is a very important aspect that these antigens, antibodies etcetera which are specific to capture of a certain reagent or certain analyte is immobilized onto the surface of biochips or bio systems.

Some of these immobilization mechanisms are for example, antigens and antibodies. As you can see here, they are complicated folded structures and their binding mechanisms are through hydrophobic interactions, hydrogen bonds, ionic, or van der Waals interactions. Typically almost all antibodies are like these y-shaped molecules and they are proteins with two heavy chains and one light chain. The light chain is at the base here and these two are the heavy chains of the antibodies. What is also important to know is

that there are these groups on the top here at both ends which are also known as epitope sites. We will be doing this in just about next slide in little bit detail.

The idea is that these sites correspond to certain molecular constitution, which can bind it to flowing specimens like cells, bacterial cells, million cells, different capture agents and so on so forth. This is definitely one of the mechanisms of immobilization or capture of a certain specimen over a surface. Assuming that you can somehow localize this antibody onto the surface of your choice along this lighter chain here at the bottom and use these two as capture points or capture hooks which can collect flowing things in a medium. Then we also have these ligands and receptors, the very famous biological lock as we popularly know this as avidin biotin lock.

These are two moieties: avidin, a protein and biotin, a vitamin. There is a strong bondage between the two. They have a very high affinity constant of the order of about 10 to the power of 15 mole inverse and whenever these two species are together, they almost always bind with each other.

Assuming that you have a biotin end group or a biotin moiety put into one of these logical entities like an antibody or let us say a protein of certain interest, then you can easily bind two such proteins by putting this avidin lock in between. You flow the biotinylated antibody in question and then put another avidin molecule in between and then you have another biotinylated antibody, it can form into a sandwich mechanism of two antibodies together. So, these molecular locks of this concept of ligands and receptors can be very effectively used for immobilization of some of these biological moieties onto biochip surface. They are very commonly used in assays and these have strong - especially the avidin biotin lock, it has strong affinity constant of the order of 10 to the power 15 mole inverse.

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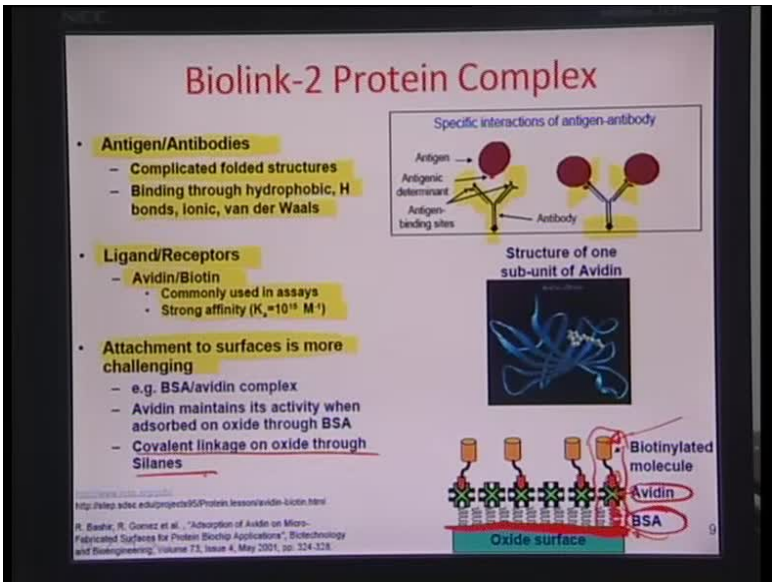

Another very interesting mechanism is how you can attach these antibodies to surfaces using the BSA avidin complex. This is very important to describe as you can see here in this particular figure. There is a silicon dioxide surface and this right here is the surface as you can see - is the oxide surface and what you do is you take this BSA molecule with a biotinilation done on to this molecule. So, BSA or bovine serum albumin is essentially a protein and there are certain protocols in which you could actually by using the differential binding of moieties to each other under certain pH, bind a biotin moiety onto the surface of a BSA molecule. You can actually pass it through a protocol, where you keep on changing pH, absorb that physically onto a silica membrane and then put this biotin n group under a certain pH so that it can protonate very near to this BSA. They have a kind of ionic attraction and although there is not a covalent bond but then there is a tendency of this biotin to bind to the BSA molecule. You have a biotin here as you can see in this end particularly - bound to this spring like BSA molecules. These spring like molecules on the surface are what is describing BSA or bovine serum albumin. You can use a similar mechanism to bind an antibody with a biotin n group. You take an antibody and you pass it through the same set of filters and use the variation in pH so that there is an ionic attraction developed between the modified biotin moiety and the antibody. There is some kind of affinity between the two and they bind ionically and so you have a biotinylated antibody.

So, you have biotinylated BSA molecule on one end; you have a biotinylated antibody in other end and all you need to do is simply put an avidin moiety inside here as you can look at in this particular figure - this right here is really the avidin- the green area that you are seeing here is the avidin and you have a biotinylated antibody on one end and another biotinylated BSA.

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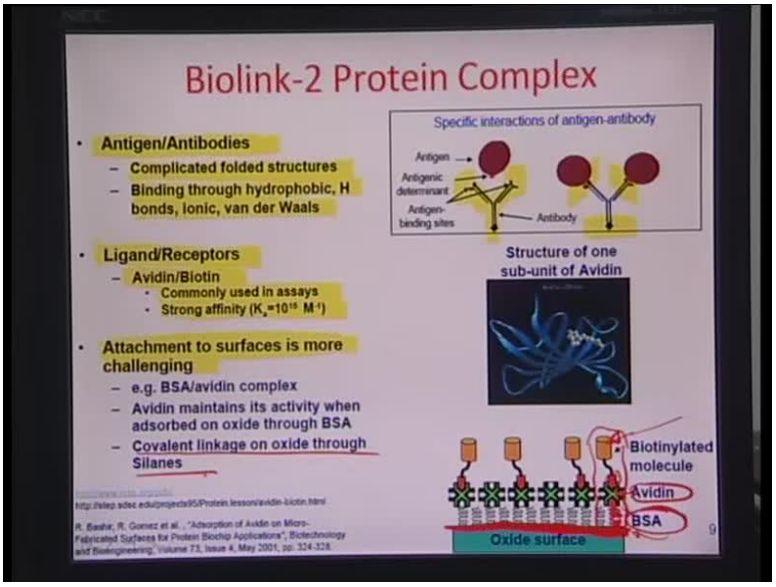

Another very interesting factor to mention here is that if you can really change the pH where the BSA binds to the surface of the silica or SiO2, you could actually develop ionic attraction between the positively charged BSA - that means you have a hydrogenated amine group, NH3 plus and the negatively charged oxidized surface, SiO minus. Therefore, there is always an ionic attraction between the BSAs other end here and the surface. So, one end has been bound separately to a biotin using a pH based mechanism and then the whole moiety is brought close to the surface and again a pH base mechanism is done in order for the BSA which is biotinylated to bind on to the surface using a similar chemistry or similar mechanism and you are putting an avidin in between and then a biotinylated antibody.

This whole structure is nothing but a molecular hook, what you get is the biotinylated molecule. It could be an antibody, it could be a DNA, it could be a protein, and therefore, this is a very good mechanism of binding. It is a kind of a covalent linkage which is

developed through this BSA avidin, biotin etcetera where you can trap or hook molecules to oxide surfaces.

Having said this, let us look into some of the binding characteristics and especially this again holds true for enzymes. By definition, enzymes are larger complex macromolecules consisting largely of proteins and one prosthetic group which may be a protein or may be some kind of a non amino acid organic or inorganic group. The enzymes play a very vital role by behaving like catalysts to move forward reaction. We will try to investigate the reaction kinetics in such a case and we can extend that to even the antibody, antigen binding in the solution or over a surface pretty much in a similar manner.

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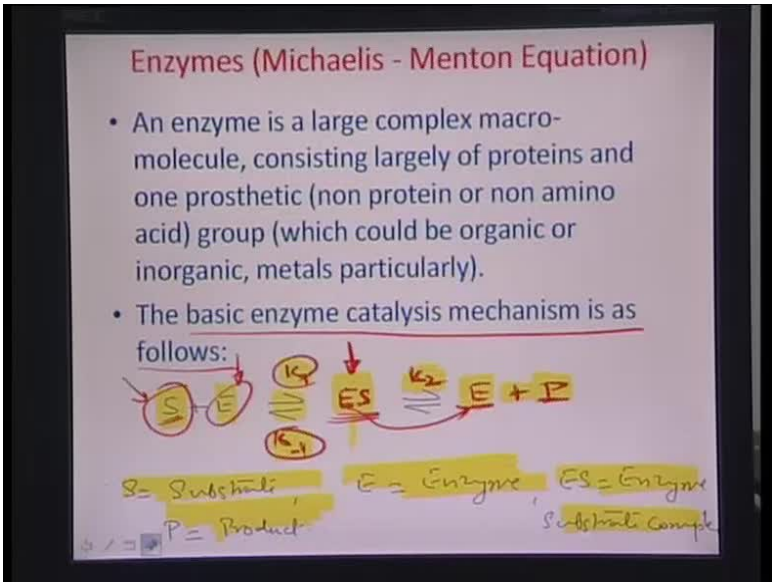

Let us look at some of these basic kinetic characteristics and equations. The basic enzyme catalysis mechanism is as follows - you have a substrate here; mind you again - substrate is the molecule or the moiety in which we are trying to bind something or convert into some other moiety. So, here substrate does not mean the physical meaning of it like a wafer. Substrate means something to which the particular biological moiety, be it an antibody or an enzyme would bind. You have an enzyme E which is binding to a substrate S here and there is equilibrium established between these two. There is an intermediate complex which is formulated as you can see here - E S, enzyme substrate

complex which is the ephemeral nature. It lasts very short and breaks down into an enzyme itself and the products, so, as an enzyme really comes out as it is.

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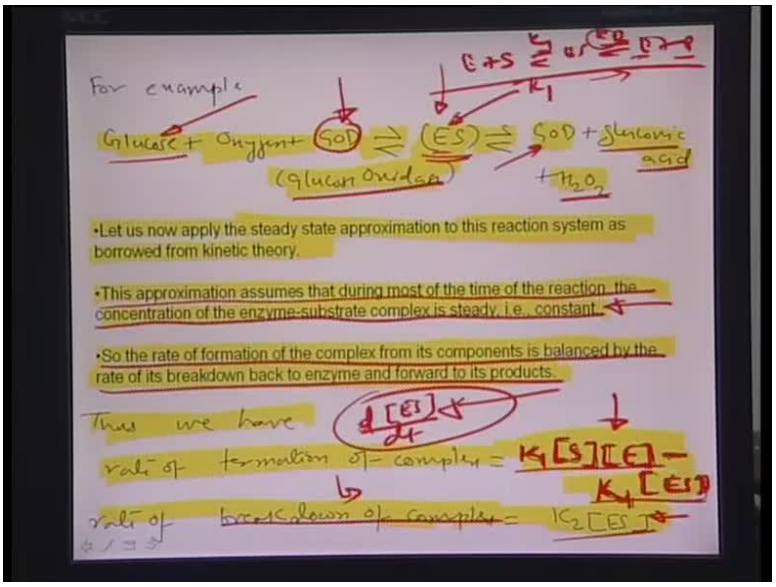

Therefore, it is just a catalyst. Although it participates physically into the reaction it is not a part of the product and it can retrieve itself back normally. You have substrate and enzyme on one side; you are actually doing the reaction here at a certain equilibrium state where maybe the forward reaction has a rate constant K 1, the reverse reaction of the rate constant K minus 1, let us say. It converts into an enzyme substrate complex and again breaks down into the enzyme and the product at a forward reaction K 2. In this kind of a situation, let us see an example of what kind of the enzyme can do what kind of behavior to certain molecule. We have many times before discussed about this famous glucose detection reaction. Glucose here is oxidized in the presence of this enzyme, GoD. Glucose oxidase in order to convert again into an enzyme substrate complex and the GoD separates out and you have gluconic acid and H 2 O which gets generated.

This is an enzymatic reaction. Similar kind of reaction chemistry can as well be used for antibody antigen kinetics. It is very important to keep rate kinetic based method to ascertain what kind of times do we need to hold these solutions on a certain substrate or may be on a certain wafer for the moieties to bind to that wafer or indirectly if you want to perform sandwiched between two molecules in a solution, what kind of time delay should you do between different steps of the chemical processes for the binding to occur?

You have to have a good mathematical idea about the rate constants etcetera and so the model that we have taken here for describing here is the enzyme substrate reaction.

If you apply steady state approximation to this particular reaction system - let us say this enzyme plus the substrate getting into enzyme substrate and then again getting into enzyme in the product and this is borrowed from kinetic theory. So, under this approximation, it is assumed that during most of the time of the reaction, the concentration of the enzyme substrate complex is steady that means you have a constant concentration of the enzyme substrate complex as you can see here. The rate of formation of the complex from its components is balanced by the rate of its breakdown back to enzyme and forward to its products. There is some kind of equilibrium between the enzyme substrate enzyme substrate complex and enzyme product. Let us say we have the rate of formulation of the substrate d ES by dt given by this particular equation.

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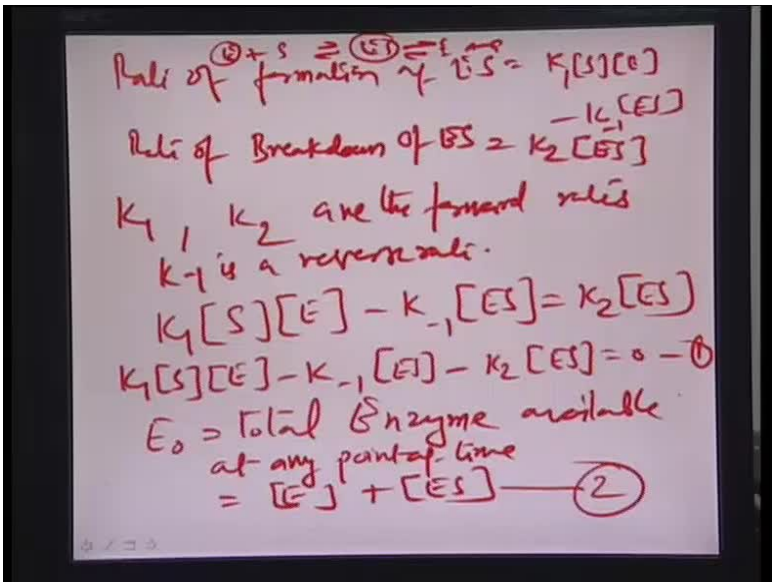

You have an enzyme E reacting with the substrate, the forward rate is K 1, reverse rate is K minus 1 and is formulating ES. Again you have another combination where the forward rate is K 2, let us say. You are converting into enzyme and product. If you look at the way it is formulated or the amount of the substrate consumed is K 1 times of concentration of S times of concentration of E from first of the kinetics and the amount of the reverse reaction that is the breakdown of enzyme substrate complex into the enzyme and substrate is at the rate of K minus 1 times of concentration of enzyme

substrate. The rate of breakdown of this complex in the forward direction is really K 2 times of the concentration of the enzyme substrate complex. Therefore, because K 2 is the forward rate reaction in which the enzyme substrate complex is broken down into enzyme and product as it can be seen here. That is the overall formation break down rate of the enzyme substrate complex. Therefore, the rate of formation of S, enzyme substrate complex is given by K 1 concentration S concentration E minus K minus 1 concentration S. The rate of breakdown of ES is K 2 concentration S. This K 1 and K 2 are the forward rates and K minus 1 is the reverse rate.

Assuming this to happen, we can say that if there is a steady state behavior of this reaction and the way that the enzyme substrate is formulated or enzyme substrate complex is formulated is same as the rate at which enzyme products are formulated and is same as the rate at which enzyme and substrate independently are consumed. Basically we can equate these two rates and then say that the rate of formation of the complex is same as the rate of degradation of the complex or deformation of the complex. If we take that into picture then we can say K 1 times of concentration S concentration E minus K minus 1 times of concentration S is equal to K 2 times of concentration S. Therefore, basically the different rates all put together, K minus 1 ES minus of K 2 ES is zero, let us call this equation 1 at this time.

So, we described the enzyme concentration in terms of the total concentration, E 0 of the enzyme as the concentration available free with the substrate and the conservation available in the complex state with the substrate. If you do that then we can write it very safely that E 0, the total enzyme available at any given point of time can be represented as the concentration of E and concentration of S. You see here in the reaction, let us say you have the reaction written here as enzyme plus substrate giving enzyme substrate complex further giving enzyme plus product - you can see here that the enzyme at certain point is the sum of enzyme substrate and enzyme.

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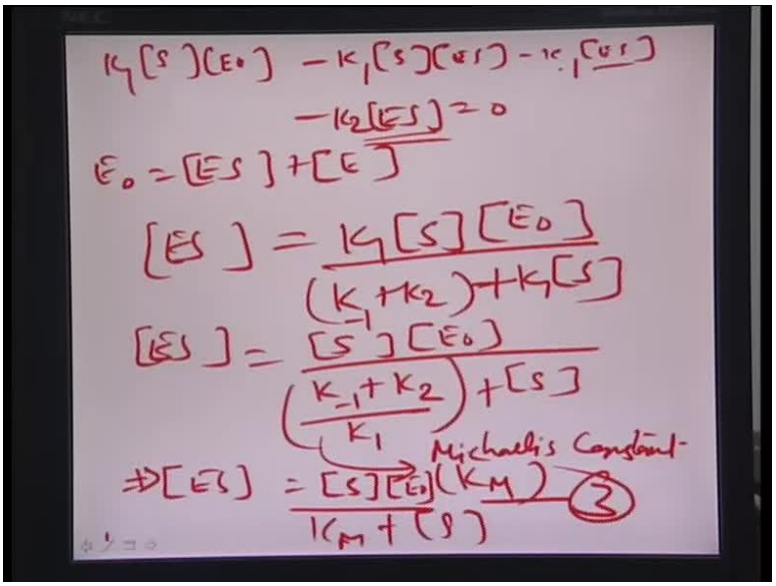

Assuming that to happen here in the whole reaction system and if we substitute this, we call this equation 2. Substitute this 2 back in 1, we are left with the equation as K 1 S times of E 0, where E 0 is total enzyme concentration at any given point of time minus K 1 times of S times of ES minus K minus 1 S minus K 2 S is zero. This is by the substitution of the E 0 value which is equal to the concentration of ES plus conservation of E at some point of time reaction. This is equation 2. Assuming this to be true, we are able to solve from this the concentration of the enzyme substrate complex at any point of time which is equal to K 1 times of concentration of S times of concentration of E 0, the total enzymes available times of K minus 1 plus K 2 in brackets- this K 1 concentration of S. If you divide by K 1 on the numerator and denominator; you are left with enzyme substrate concentration as S times of E 0, where S is the concentration of substrate, E 0 is the total enzyme concentration and this term here, K minus 1 plus K 2 by K 1 plus the concentration of S, this is known as Michealis constant - upon the name of its inventor. The set of equations are called Michealis menton equations.

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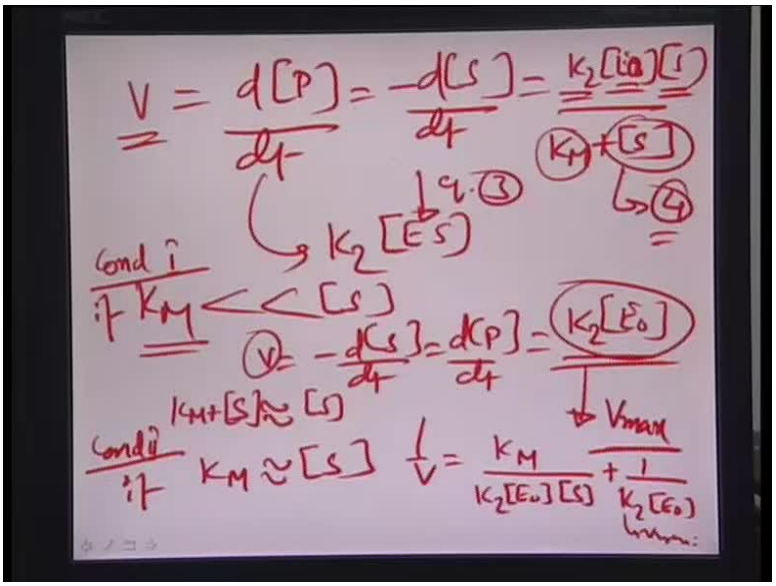

Let us call K M as Michealis constant in this particular case. Then we can represent the ES value as concentration S concentration E 0 divided by Michealis constant K M plus concentration of S. Let us call this equation 3. The overall reaction rate or rate velocity or rate of formation of products is given by equation, velocity V is equal to d times of rate of product formulation, d p by d t rate of formation of product or the rate of formulation or the rate of degradation of the substrate minus d s by dt given by the Michealis menton equation as K 2 times E 0 times of S divided by Michealis constant K M plus S. This is nothing but K 2 times of the concentration of ES, which was derived from equation three before.

The reaction velocity V can be represented by K 2 times of E 0, the total enzyme concentration times of concentration of substrate S divided by Michealis constant plus the concentration of the substrate. There are several conditions that this equation can impose. Let us call it 4. In condition one, let us suppose that this Michealis constant K M is very small in comparison to S. You have first condition called condition one, where assume Michealis constant K M to be less than S. Equation 4 really changes into V equals minus d s minus dt equals formulation of product, d p by dt as K 2 E 0 because the K M plus S can be approximated as itself.

Therefore, it is a case where the velocity of the reaction V is proportional to the concentration of the total enzyme is zero that had been given at the very beginning of the

start of the reaction when the complex was just about to get formulated. Therefore, this is also known the maximum concentry or maxim velocity V max. We call this the maximum rate of the reaction or V max. One of the reasons why that is so is- that if suppose there is some addition to K M, the value V is always going to go down. Therefore, mathematically also this can be represented as the V max or the maximum rate velocity or rate of formation of the product.

Let us assume another case little bit different, where the concentration of S is same as the Michealis constant and if I will show you later in a plot, how these different points are very significant for understanding the enzyme substrate formulation reaction. Let us say in condition two here; we assume that this Michealis constant K M is approximately equal to the substrate concentration S. In this particular case, what would happen is that you have one by V from this equation 4 can also be written down as K M divided by K 2 concentration E 0 concentration S plus 1 by K 2 concentration E 0 which is nothing but V max as we just saw little bit before.

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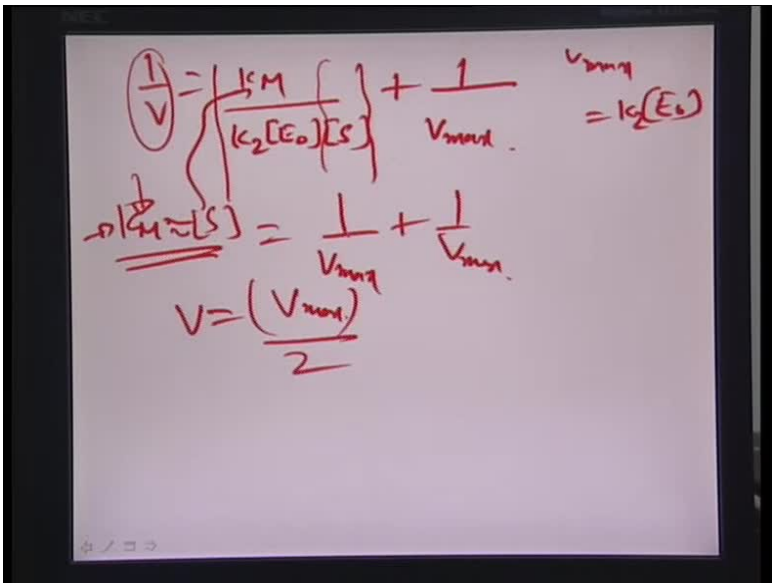

We are left with an equation of the form - concentration of 1 by V equal to K M divided by K 2 enzyme the concentration of the total enzyme time concentration of substrate plus 1 by V max, where V max is already K 2 times of concentration of total enzyme E 0. If you assume K M and S to be equal, it can be represented as 1 by V max plus 1 by V max.

If K M is same as S here and if you substitute this, the conservation of S really goes off and you have 1 by K 2 E 0 which is nothing but 1 by V max.

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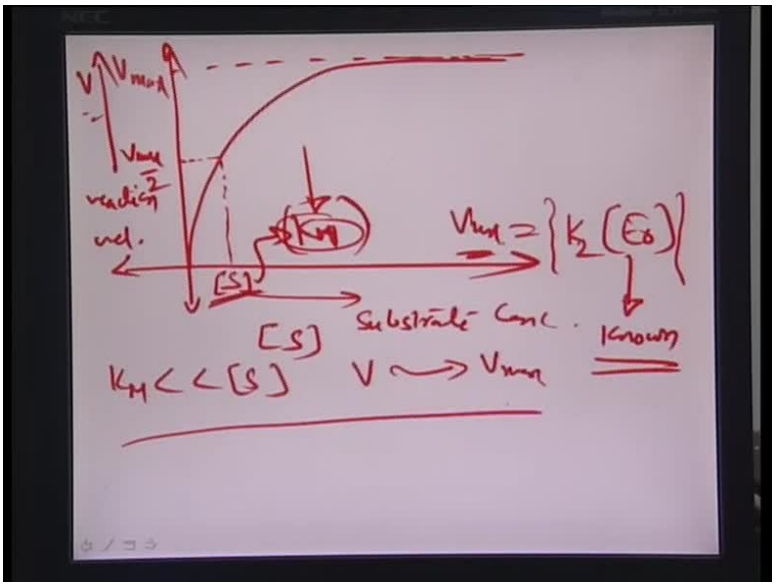

So, in this case, when the concentration of S is equal to Michealis constant K M V becomes V max by 2 -that means it is the half rate velocity of a particular enzyme substrate formulation complex formulation reaction. Let us plot now. If you look at the curve here - if you plot between 1 by V and 1 by S here, this is going to be a linear plot with the slope and intersect. The slope is V max. On the other hand, if you plot between V max in the substrate concentration S, the way this plot would go is a something like you have let us say V max or V on the y axis, here reaction velocity and the substrate concentration S in the x axis. In this particular case as we can see K M is very smaller than S and the V approaches V max.

Let us say we have a straight line here describing what we know as V max, then the reaction rate should be asymptotically approaching this V max line and that this is what the relationship between the V and S would look like. So, the plot of V max by 2 on this particular equation would correspond to a point on the substrate concentration axis which would be equal to the Michealis constant K M. It is an experimental method of determining from reaction chemistry what the Michealis constant K M would really mean at a certain substrate concentration. If you know what V max is based on your initial enzyme concentration which is known, mind you, in a particular reaction this is

known and K 2 is of course the forward rate at which the enzyme substrate is broken down into the enzyme and product, so, it is a product formulation rate, K 2 is again the product formulation rate. If you know these two which will give you an indication of V max then we could find out what the Michealis constant K M is by looking at the substrate concentration at a point of intercept on the curve corresponding to V max by 2.

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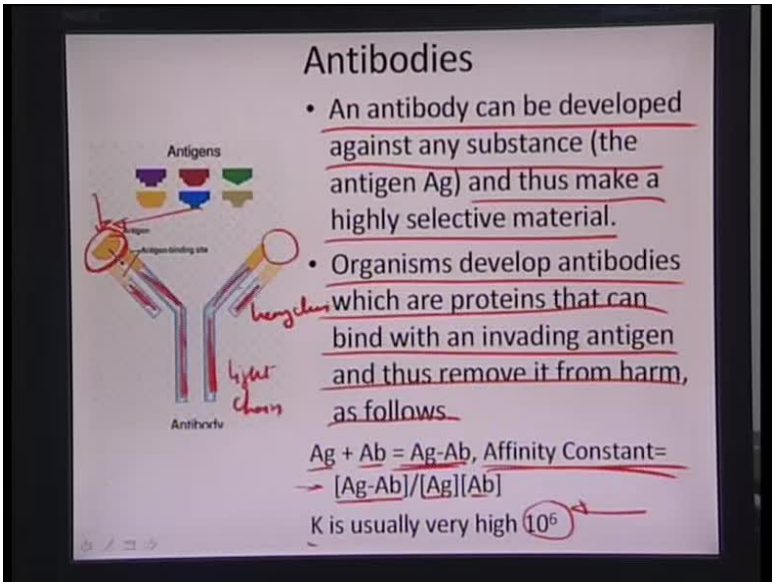

So this is how the rate kinetics of the enzyme substrate reactions happen and is a matter of fact, any antibody, antigen reaction also are governed by the same set of equations. The only thing is that in this case probably the rate the reaction kinetics is not bound by the formulation of an intermediate state, it is just a product formulating from a substrate and the participation of antibody is there on both ends of the reaction. Let us look at little more details of what really the antibody is; what their structures are in order to understand more. A very important fact is that an antibody can be developed against any substance popularly known as antigen and it can be raised. The idea is that the antibody so developed would be highly selective to the substrate. Antibody can be developed against any substrate and can be made very highly a selective material to a particular substrate. You can raise antibodies within an organism. The idea is that antibodies as we know are proteins formulated by a so called b cells within a certain living organism and the organisms can be used to develop antibodies which can be later used for the purpose of capture or specific recognition in vitro.

Therefore, organisms develop antibodies which are proteins and they can bind with an invading antigen and move it from harm as follows - the antigen and antibody binds together to form this particular complex and the affinity constant in this particular case is actually the formulation or the concentration of complex divided by concentration of independent antigen antibody. Usually it is very high not as high as the avidin part in link but again it is usually about 10 to the power of 6 mole inverse in case of antigen antibody binding which is a pretty high affinity constant.

If you look at antibodies - their y shaped mechanisms like you can see here, you have these sections here - at the stem of the y which are really light chains and the two upper sections here which are the heavy chains. The overall structure of the antibody by and large remains constant. There is not much variation except the fact that there are certain subtypes based on what these chains are in million cells or million organisms. There are about five different kinds of such subunits which can be categorized as this heavy chain light chain structures or antibodies. What is interesting though is that you know the portion here on an antibody which is actually known as an epitope site is the only variation in the whole antibody. By and large the other subtypes of antibodies which are available are only five in number but if you look at the amount of variation that these particular epitope sites would have had, there can be million different antibodies corresponding to binding or which can be capable of binding to million different biological entities. This epitope site keeps on changing whereas, the other part of the y that means these light chains, these heavy chains by and large remain similar except categorization in to a few subtypes.

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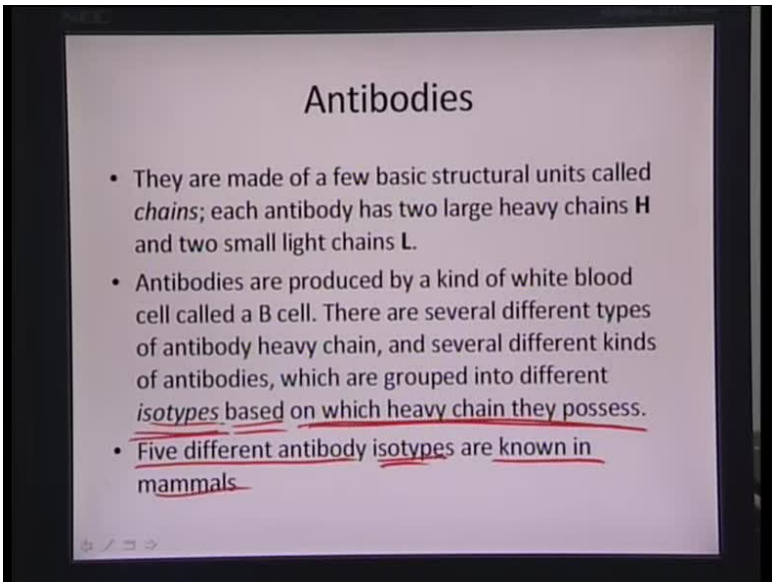

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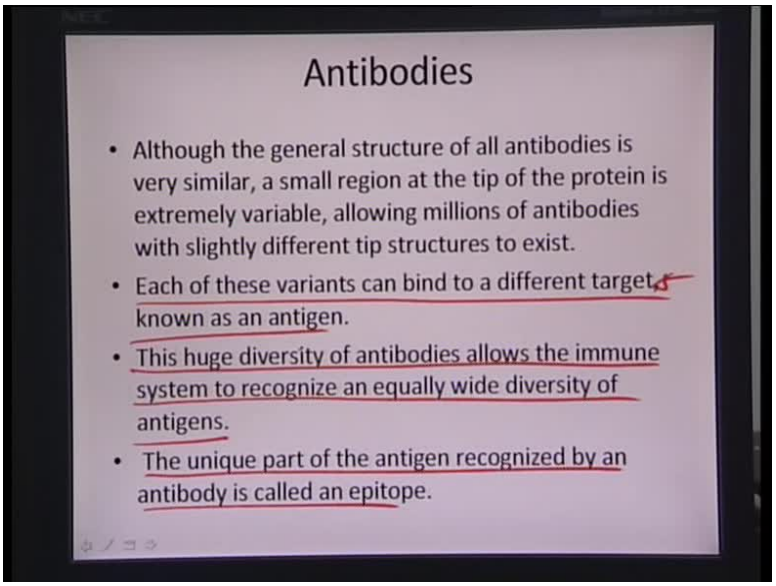

The different subtypes are also known as isotypes. Isotypes are based on which heavy chain, the antibody would process. As I indicated before, there are about five different antibody isotypes known in mammals. That is all the range that antibody could have except the fact that the epitopes are varied and numerous in region. An antibody made up of this epitope with the heavy and light chain isotype can bind to different targets and the targets that are typically bind to are known as antigens. There is a huge diversity of the antibodies which allows the immune system to recognize an equally wide diversity of the

antigenic systems. So, the unique part of antigen is recognized by an antibody called an epitope as I have indicated earlier and shown in the particular figure.

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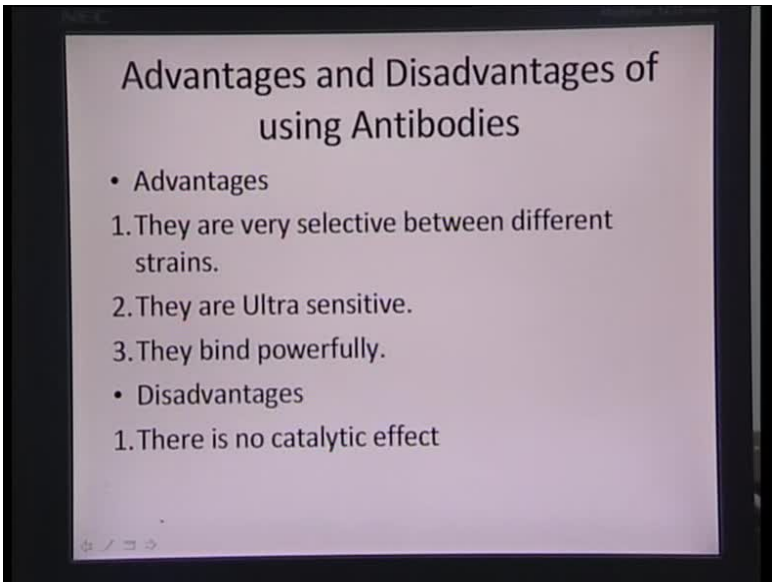

There are several advantages and disadvantages of using antibodies. One of the most prominent advantages are the selectiveness or the selectivity that these antibodies would normally possess between the different strains. Another very important advantage is that these antibodies are ultrasensitive to any kind of a small variation in the analyte of interest. I mean the binding or unbinding is typically dependent on what is the characteristic of the particular analyte. If there is a slight variation in the ambient, it can change the way that the antibody would form a complex with the antigen or bind with the antigen. Although when they bind, they do bind very powerfully and that bond cannot be easily broken up. The only disadvantage is that there is no catalytic effect in this particular binding to antigen antibody as happens in the case of enzyme.

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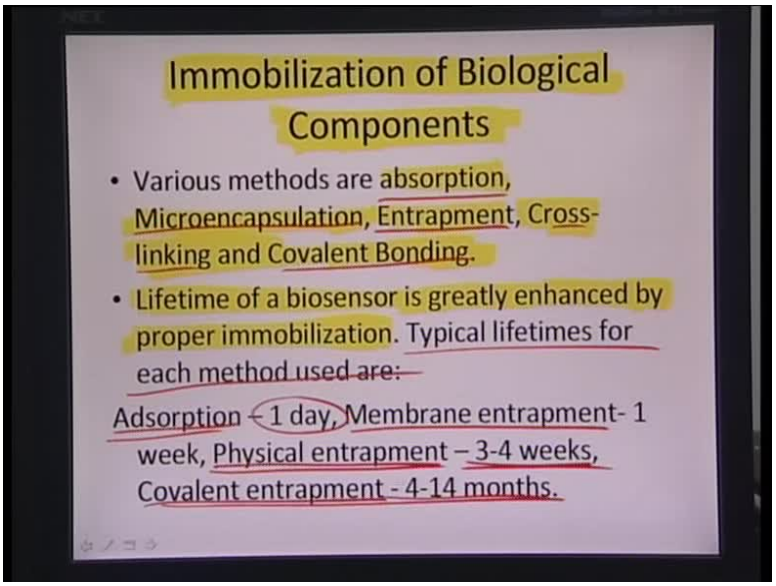

So, here whatever is bound is bound - I mean it cannot be reconverted back into pure antibody again or a pure antigenic and it is an irreversible reaction of formation of the antibody antigen complex. By and large these are some of the advantages and disadvantages of antibody antigen systems. Let us now turn into a little bit different component - how we can immobilize the different biological components. We have done a little bit of this while looking at sensors but essentially as is obvious the next question which comes into being is after we have understood the reaction kinetics we try to find out more details of the structure of these different biological moieties. How do we really use them in sensors and therefore, immobilization protocols are very important for that.

There are various methods for immobilizing, it can be a absorption, it can be microencapsulated, it can be entrapped on a particular electrode or a substrate you can cross link or covalently bond a certain biological moiety or entity. The lifetime of a biosensor is greatly enhanced by proper immobilization technique. If you can choose the right immobilization technique, you can use the biosensors many times useably without making changes onto the sensor surface, Based on the particular immobilization method from this different range of methods that I have stated before – absorption, microencapsulation, and entrapment, crosslinking or covalent bonding. The typical life time of a biosensors also vary a lot.

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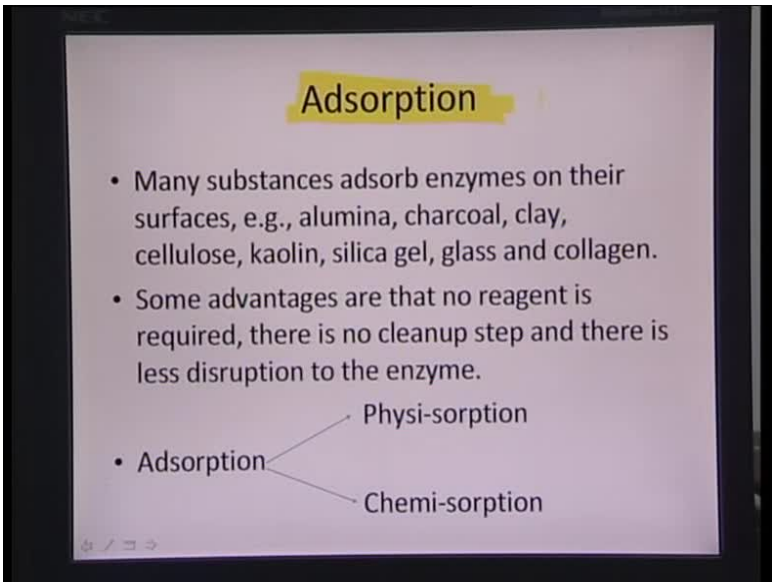

For example, in case of adsorption, it is only about a day or so. Adsorbed substances do not stay very long onto a surface they are a prone to de-absorbing based on change in the partial pressure of the atmosphere in which such a surface would be kept. The membrane entrapment that we have talked about here is typically about a one week lifetime. It is pretty stable because membranes are essentially thin perforated structures which would be able to hold these biological moieties very close to a particular substrate. For physically entrapment cases, it is about 3 to 4 weeks. The device lifetime is greatly enhanced because of the schematic wherein this entrapment is a gel kind of a network and for covalent entrapment again, it is very high because there is a covalent bond now between the particular moiety, it is about 4 to 14 months. Details of the different immobilization aspects and schemes: the first aspect which comes into picture is adsorption. What really adsorption is? It is some kind of either physical or chemical attachment over a surface by physical attachment over the surface, what we mean is, if there is a size based selection of a certain moiety on a surface that could be said as physically adsorbed on to the surface. Chemical absorption on the other hand could be because of very many reasons; one could be just ionic attraction between moiety and the surface. They are ionically opposite in nature and there is a ionic bond which is formulated between the two or due to Van der Waals forces there may be the tendency of a particular chemical to absorb onto the surface; it is a weak interaction between moiety and the surface.

So, many substances adsorb enzymes particularly on the surfaces. Some examples could be alumina, charcoal, clay, cellulose, kaolin, silica gel, glass, collagen, and etcetera. If you look at the microstructural aspect of the surfaces, they are all very highly porous in nature and more surface area is involved. Therefore, if the area is more, there is a tendency of the adsorption of a particular moiety to be more. So, adsorption is dependent phenomena on typically the number of binding sites that surface has to offer. If you have more surface area, more binding sites, and you have more adsorption.

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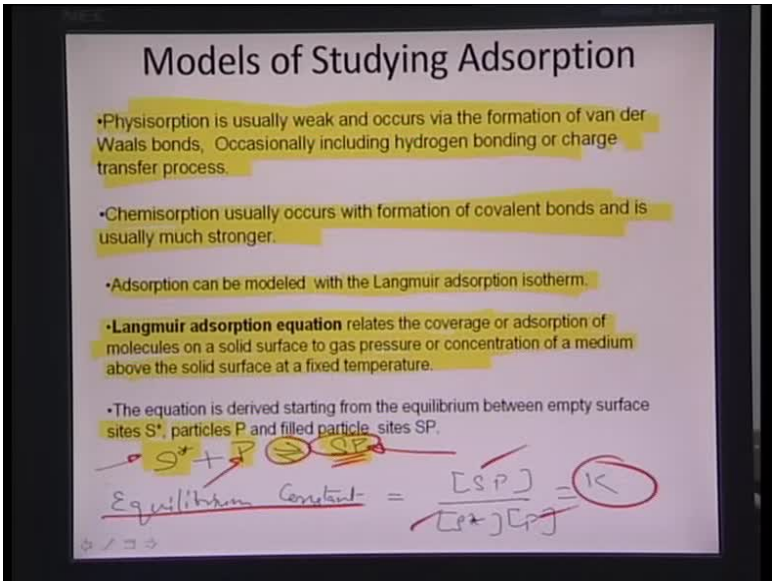

The advantage of this process is that typically no reagent is required. That is the biggest advantage that adsorption has to provide. There is no cleanup step and there is less disruption to something like a biological moiety, an enzyme or antibody, because the involvement of chemical steps is less.

Adsorption can be classified into physics option and chemist option. This is physical adsorption; this is chemical adsorption. People have been really studying this adsorption on a very modular manner as already indicated. Physics option typically called by van der Waals bonds which are usually weak and occasionally hydrophobic - hydrophobic interactions or a charge transfer process or hydrogen bonding that is how physics option would typically occur. Chemist option on the other hand would be usually by the formation of covalent bonds and much stronger process of adsorption onto the surface.

So, the adsorption phenomena can be modeled as you can see here by the Langmuir adsorption isotherm. What this equation is really relates is the coverage of adsorption to the molecules on a solid surface to gas pressure or concentration of a medium above the solid surface at a fixed temperature.

It is also known as the Langmuir adsorption equation. What is important for an adsorption process? One is the amount of active bonding sites or binding sites which is also a function of the active available surface area of a particular moiety. It is very critical. Another is especially in gas phase absorption, the vapor pressure which is over the surface is very critical to determine what is the adsorption. If the pressure is a little higher, then the adsorption rate automatically increases because you can think about it as some kind of a forcing mechanism for the molecule to seep through different vias and trenches on a particular surface.

Therefore, if the partial pressure is high in the atmosphere, P a, is high, then the adsorption rate is automatically increased. So, adsorption is typically given or described by this equation here. Let us day, S dash is the number of sites which are available on a particular surface; you are absorbing particle P onto these S dash or S star sites onto that surface and the filled particle sites are represented as SP. So, typically there is equilibrium between these two. You have S star which is the number of active available sites and P number of particles and then this is the filled site or a number of sites which are fully filled SP. There is equilibrium between these as you can see.

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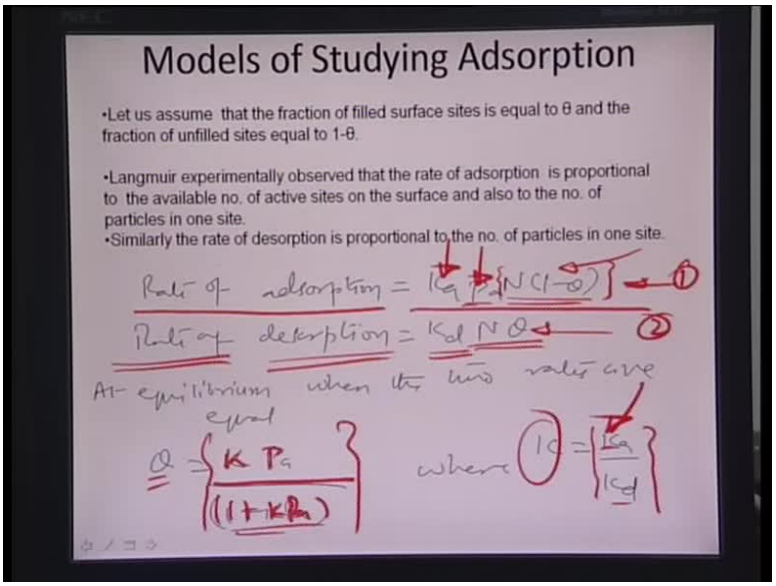

The equilibrium constant here is directly proportional to the concentration of SP and it is inversely proportional to the concentration of the active available sites and the concentration of the particle P, let us call this K. So, equilibrium constant here is the concentration of SP by concentration of S star and P. Let us assume that the fraction of filled surface sites is equal to theta; the fraction of unfilled sites at a particular instant of time is 1 minus theta. Let us say the total number of sites as n and the fraction of those sites is theta - theta n are the number of filled sites and 1 minus theta n are the number of unfilled sites.

Langmuir tried to experimentally observe that how the rate of adsorption would be behaving if theta of the fraction of the active sites or fraction of the filled surface sites is increased with time and he found out that there is a relationship between theta. If theta is decreased and the number of active sites is increased, the adsorption rate would increase. It is a directly proportional relationship. What he found out is if the partial pressure is high, the rate of adsorption would increase. If you put these all together in an equation, you are left with rate of adsorption equal to K a which is the adsorption constant times p a, partial vapor pressure times N of 1 minus theta. This is the number of active sites which are available. 1 minus theta, mind you, the fraction of the sites which are available for binding or they are still not used up and N is the total number of sites which are available.

N times of 1 minus theta is the number of sites which are available onto a certain surface for the binding to happen or the adsorption to take place. So, the rate is proportional to the partial pressure as you can see also proportional to the number of active sites of the surface here. Similarly, the rate of desorption, the ability of a surface to lose a particular adsorped species from it is proportional to the number of filled sites that are on the surface. If the number of filled sites are more, then the tendency of the material to get desorbed from the surface is also automatically increased which makes sense and is more logical. Therefore, let us say, K d is the reaction rate for the desorption and the desorption really is independent of pressure, so, whatever the partial pressure be, desorption would happen at a rate which is totally independent of that particular partial pressure. It is only a function of the number of sites which are filled or bound on a surface and it goes up with the number of sites which are filled or bound.

K d N theta is the rate of desorption. At equilibrium, if we assume that these two rates of adsorption and desorption are equal and that whatever is absorbed after the steady state has been reached, after all the active sites are filled up – would desorb of the surface or typically not bind. Then we can calculate theta by equating these two. Let us say this is 1 and 2. Equate these two equations as K times of Pa by 1 minus KPa where K is the ratio between the adsorption constant and the desorption constant, Ka by Kd and theta, the number of filled sites on a particular surface is nothing but the ratio of the adsorption desorption constant times of the partial pressure of the particular medium divided by 1 plus K times of Pa. K is the ratio of the adsorption and desorption constant as you can see here in this particular expression.

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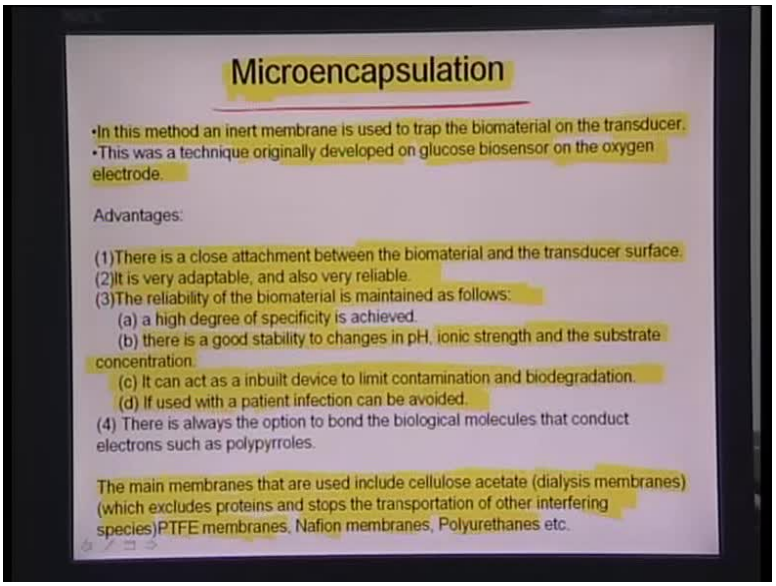

That is what would happen. Typically in a gas based adsorption process, there is an equilibrium which is achieved or reached. This gives you some idea of how you could study adsorption, especially physics option on a particular surface. Let us look at a different technique, the micro encapsulation as I had been talking before. It is really the trapping of a biological moiety in between two membranes or two membranous structures. In this particular method, an inert membrane is prepared and is used to trap the bio material of interest on the transducer surface. For the very first time, this technique was developed for a glucose biosensor and this was developed on an oxygen electrode. It is essentially developed on an oxygen electrode originally for glucose biosensor.

There are several advantages of the micro encapsulation process. One definite advantage can be illustrated here is that there is a close attachment between the biomaterial and the transducer surface. You can think about this particular membrane set to hold together the biological entity very close to a certain surface and therefore, this provides a firm binding mechanism of closeness of the entity to the particular sensor surface that is being used. Definitely it is much closer as an attachment than adsorption where it may just randomly adsorb on a surface without taking care of any textural issues of the particular surface.

This process is very adaptable; also very reliable because typically you do not have to modify the sensor surface. That is an advantage you are trapping using external membrane without any modification, chemical or physical whatsoever on the sensor surface. The reliability of the biomaterial is maintained, particularly the enzyme or whatever you are immobilizing, because you not chemically reacting. You are keeping the biological entity in its original state as it is supposed to be.

This results in high degree of specificity almost always, because you are not modifying chemically the bio material that you want to replace onto surface. There is a good stability to changes in pH because since the bio material is in its own nascent state, no chemical change whatsoever. Therefore, pH change would typically only induce very small changes to the parent molecule; whatever those changes are would get registered as opposed to a case where you would have modified the biomaterial already. So, the changes would be much more drastic in nature. It also stabilizes the material against different ionic strengths of the solutions and different substrate concentrations. It can act as an inbuilt device to limit contamination and biodegradation because again you are not handling or you are not playing with biomaterials chemical property in general. It can be used to prevent infection because you are trying to guard the biomaterial using the membrane and trying to guard it from getting in touch or getting contaminated with the patients' fluid samples which we are trying to measure with this.

So, you could have an option of binding of the biological molecules that conduct electrons such as polypyrrole etcetera to make the membrane so that you could have an electron transparent path from the biological moiety onto the sensor surface. The membranes that are used in this kind of micro encapsulation are cellulose acetate. Remember dialysis membranes are made up of cellulose acetate and they are put external to the body where the blood is flown and there is always some kind of a separation of essentially urea and other harmful salts in the blood which excludes proteins and stops the transportation of other interfering species. This particular cellulose ascertain membrane due to its hydrophobic nature, prevents the proteins or some other interfering species to get filtered across it. Then you have other kind of structures like PTFE politatra polyurethanes membranes, Nafion, a very good material, and polyurethane etcetera which can be used successfully for micro encapsulating the biological moieties.

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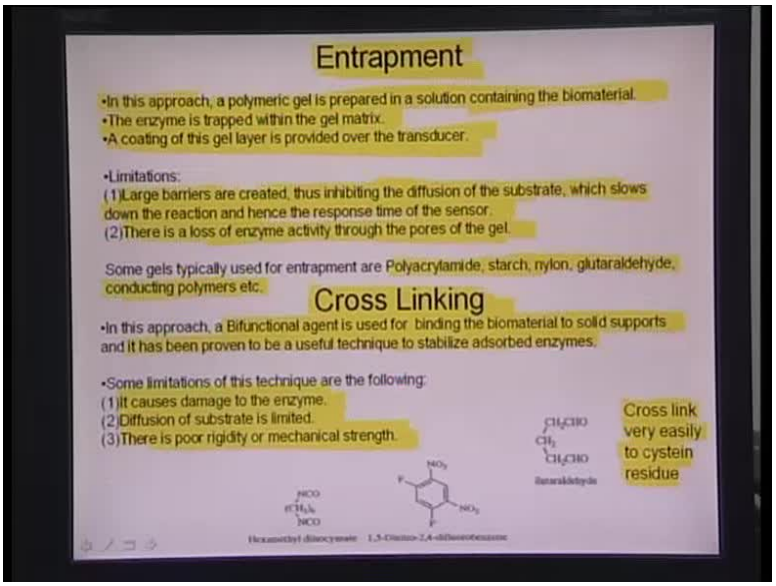

The next in line is entrapment. That is another very important mechanism here. You are entrapping the material in a gel kind of matrix, so, instead of binding it to gather within the membrane or between two membranes, you are trying to bind it by entrapping it into a polymeric gel kind of matrix in this particular approach. For that, you first prepare a solution and then gillette it later at suitable conditions after the enzyme or whatever biological moiety we are talking about is trapped within the gel matrix and then finally, a coating of this gel layer is provided over the transducer surface. This process also has some limitations; one is that a large barrier is created. You have no control on the distance of the biological moiety from the sensor surface as there is a thick gel which is entrapping the moiety in its thickness. The thickness of the gel can be made thinner and thinner but still there is no active control on where the entity is present- is it towards the sensor surface or is it away from the sensor surface onto the other surface of the gel, we do not have control on that aspect while preparing.

Therefore, sometimes large barriers are created and they can do all sort of things like heavy diffusion of substrates, it can slow down the reaction in general. Sometimes this is all at the cost of the response time of the sensor; it gets drastically changed because of fat or thick gel layers entrapping the enzymes. There is a loss sometimes of enzyme activity because the gel surrounding is natural is really not the natural surrounding of a certain enzyme and therefore, whatever chemical properties the gel has would definitely have an

influence on the enzyme itself. Of course there is a loss in enzyme activity particularly through the pores of the particular gel.

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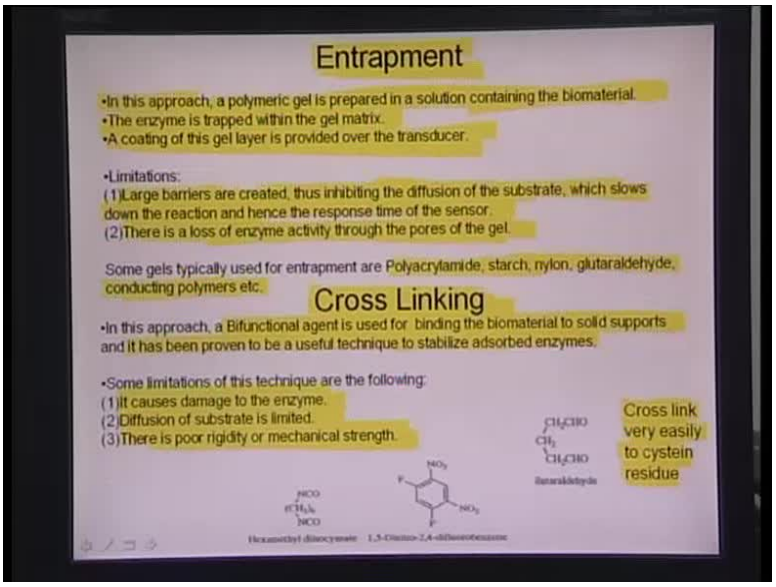

Some typically used gels are Polyacrylamide, starch, and nylon glutaraldehyde and some of the conducting polymers etcetera. Other technique which is important to be understood is crosslinking. This is an approach where the biological moiety is bound to a biosensor with a cross linker molecule; you have some kind of a ligen molecule which is one side bonded to the biological entity, another side bonded to the particular surface in question. We call it a bifunctional agent. This kind of cross linker molecule is used for binding the biomaterial solid supports. It has been proven to be a very useful technique particularly to stabilize the adsorbed enzymes and this technique also has some limitations. It sometimes causes damage to the enzymes because you are playing around with the chemical nature of the enzyme itself. You are trying to modify the enzyme to kind of cross link it to the linker molecule or the ligen molecule so it causes some damage to the normal functionality of the enzyme because its structure is changed. Proteins, as you know are extremely sensitive to this change in conformation. Their whole properties change because of a slight change in the conformation in the molecule. One more interesting factor here is that the diffusion of the substrate sometimes limited because cross links are typically formed- a dense brush like material onto the surface of the sensor which can inhibit the flow of the substrate material very close to the electrode resulting in loss in information and loss in signal connection and of course there is a very

poor rigidity or mechanical strength because these are all just the sometimes chemically cross linked; they are themselves not very strong as molecular bonds and therefore, we have to largely depend or rely on the nature of the material.

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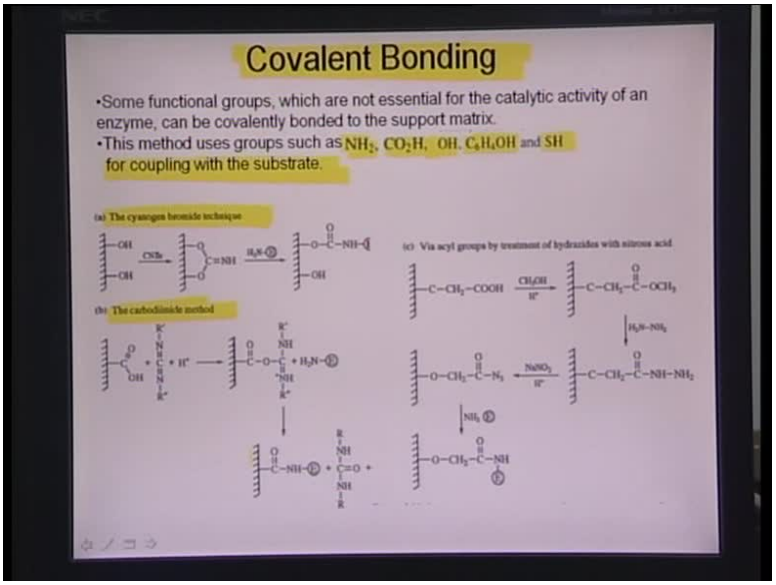

Some examples are typically - the cross links that are formulated to system residues in proteins etcetera. The other mechanism that I would like to talk about is covalent bonding wherein as you can see here -some of these functional groups present within the biological moiety is directly bonded onto the surface and it can be covalently bonded to the support matrix to just be a part of the surface and do its job. So, the method can use the presence of groups such as NH 2, COOH, OH, C 6 H 4 OH, SH etcetera and develop charge on it, so that the charge could ionically bond or attract or do some kind of a direct covalent bonding with groups onto the substrate surface. These for example, are some of the illustrations where it talks about how different moieties can be covalently bonded onto the substrate surface. With this, I would like to end this particular lecture and will cover some stuff related to things like cyclic voltammetry and some related to electrode chemistry on an electrode surface in the next lecture. Thank you!