BioMEMS and Microfluidics
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Lecture – 24

Hello and welcome back to this twenty fourth lecture of BioMEMS – bio electromechanical systems.

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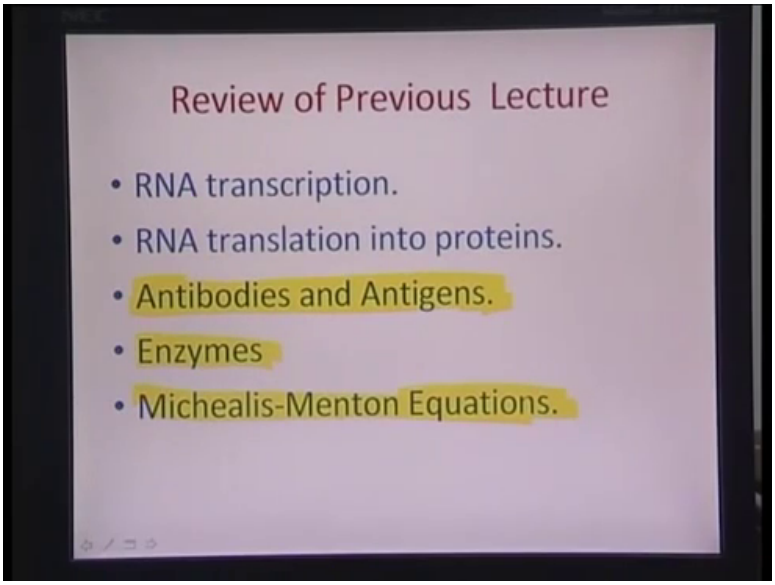

I would like to just have a brief review of the precious lecture. We talked about RNA transcription and translation processes. Transcription again is basically conversion of a double-stranded DNA in the chromatin region of the nucleus into a messenger RNA. And, translation again essentially is the change of language; that means, from the RNA into proteins or a sequence of amino acids. And, we saw how beautifully this nano-machinery inside the living cell works especially in the ribosome which kind of assembles all the different messenger RNAs and coordinates that with the transfer RNA, which is essentially around in the cytoplasm. And, these transfer RNAs again have aminoacyl groups, which kind of conjugate with the messenger RNA on a base pair or a base triplet – base triplet bases. And thus, there is a sequence of amino acid, which is generated. So, this is a very very interesting process because this coding essentially is also responsible for the physiological state of health of a living cell.

So, we also described about antibodies and antigens and would be doing this a little bit later in more details. And then, we briefly talked about enzymes where we were just about to begin what we know as the Michealis-Menton equations of the formulation of enzyme substrate complex. So, today, we will actually 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. So, to begin with, let us look at why all these is important really. I mean we are talking about enzymes; we are talking about proteins; we are talking about antibodies. From a BioMEMS perspective, it is almost always essential that, we immobilization mechanisms, wherein all these groups, all these different moieties are used for capture essentially of targets in the analytes.

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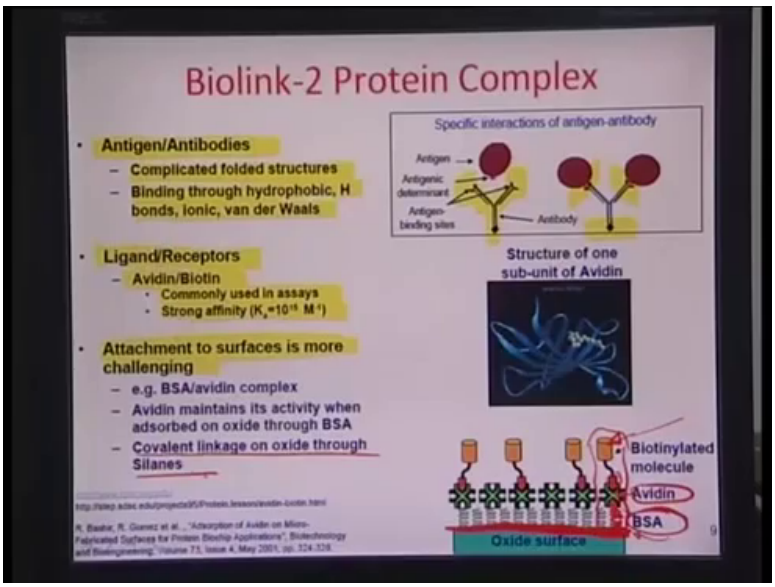

And for sensor design point of view, it is a very very important aspect that these antigens, antibodies, etcetera, which are specific to capture of a certain reagent or certain analyte is immobilized on to the surface of biochips or bio systems. So, some of these immobilization mechanisms are for examples antigens and antibodies as you can see here; they are complicated folded structures; and, their binding mechanisms are through hydrophobic-hydrophobic interactions, hydrogen bonds, ionic and Van der Waals interactions. Typically it is almost all antibodies are like these – Y-shaped molecules. And, their proteins essentially with two heavy chains and one light chain; the light chain is at the base here and these two are really the heavy chains of the antibodies. And, 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 a little bit detail.

So, the idea is that, these sites correspond to certain constitution, certain molecular constitution, which can bind to flowing specimens like cells – bacterial cells, mammalian cells different capture aid and so on, so forth. And so, this is one – definitely, one of the mechanisms of immobilization or capture of a certain specimen over a surface. Assuming that, you can somehow localize this antibody on to 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 kind of collect flowing things in a medium. Then, we also have these ligands and receptors essentially the very famous biological lock as we popularly know this as, is called the avidin-biotin lock. So, essentially, these are two moieties.

Avidin essentially a protein and biotin is a vitamin. And, there is a strong; there is a strong bondage between the two. They have a very high affinity constant of the order of about by 10 to the power of 15 mole inverse. And, essentially, whenever these two species are together, they almost always bind with each other. So, assuming that you have a biotin end group biotin moiety put into one of these biological entities like let us say 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. So, you flow the biotinylated antibody in question. And then, you put another avidin molecule in between and then you have another biotinylated antibody. So, it can kind of form into a sandwich mechanism of two antibodies together. So, these molecular locks of this concepts of ligands and receptors can be very effectively used for immobilization of some of these biological moieties on to biochip surfaces. They are very 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 again.

Another very interesting mechanism is how you can really attach these antibodies, etcetera to surfaces using the BSA avidin complex. So, this is very interesting… This is very important to describe as you can see here in this particular figure. So, essentially, there is a silicon dioxide surface and this right here is essentially the surface as you can see is the oxide surface. And, what you do is you take this BSA molecule with a biotinylation done on to this molecule. So, BSA – 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 on to the surface of a BSA molecule. So, you can

actually pass it through a protocol where you keep on changing pH, etcetera; adsorb that physically on to a silicon membrane and then put this biotin end group under a certain pH, so that it can protonate very near to this BSA. So, they have a kind of ionic attraction. Although there is not a covalent bond; but, then there is a tendency of this biotin to bind to the BSA molecule. And then, essentially…

So, you have a biotin here as you can see in this end particularly – bound to this spring-like BSA molecules. So, this spring-like molecules on the surface are essentially what is describing BSA or Bovine Serum Albumin. And then, you can use a similar mechanism to bind an antibody with about an end group. So, you take an antibody and you pass it through the same set of filters and use the variation in pH, etcetera, so that there is a – there is a ionic attraction developed between the modified biotin moiety and the antibody. So, there is a some kind of an affinity between the two; and, they kind of bind ionically. And so, you have a biotinylated antibody. So, you have a biotinylated BSA molecule on one end.You have a biotinylated antibody on another end. All you need to do is to simply put an avidin moiety inside here. As you can look at in this particular figure, this right here is really avidin; the green area that you are seeing here is the avidin. And, you have a biotinylated antibody on one end and other biotinylated BSA.

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 kind of hydrogenated amine group NH3 plus and the negatively charged oxidized – the surface SiO minus. So, therefore, there is always an ionic attraction between the BSA's 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 this surface. And, again a ph based mechanism is done in order for the BSA, which is biotinylated to bind on to the surface using similar chemistry or similar mechanism. And, you are putting an avidin in between and then a biotinylated antibody. So, this essentially – this whole structure is nothing but a molecular hook, where what you get is the biotinylated molecule; it could be an antibody, it could be a DNA, it could be a protein. And so, therefore, this is a very good mechanism of binding. So, essentially, it is kind of a covalent linkage, which is developed through this BSA, avidin, biotin, etcetera, where you can trap a hook molecules to oxide surfaces.

So, having said this, let us really look into some of the binding characteristics and especially this again holds true for enzymes. And, definitionally, really enzymes are large complex macromolecules consisting largely proteins and one prosthetic group, which may be a protein or may be some kind of a non-amino acid – organic or inorganic group. And, the enzymes play a very vital role by behaving like catalysts essentially to move forward a reaction. So, we will try to investigate the reaction kinetics in such a case and we can kind of extend that to even the antibody-antigen binding or in solution or over a surface pretty much in a similar manner. So, let us look at some of these basic kinetic characteristics and equations.

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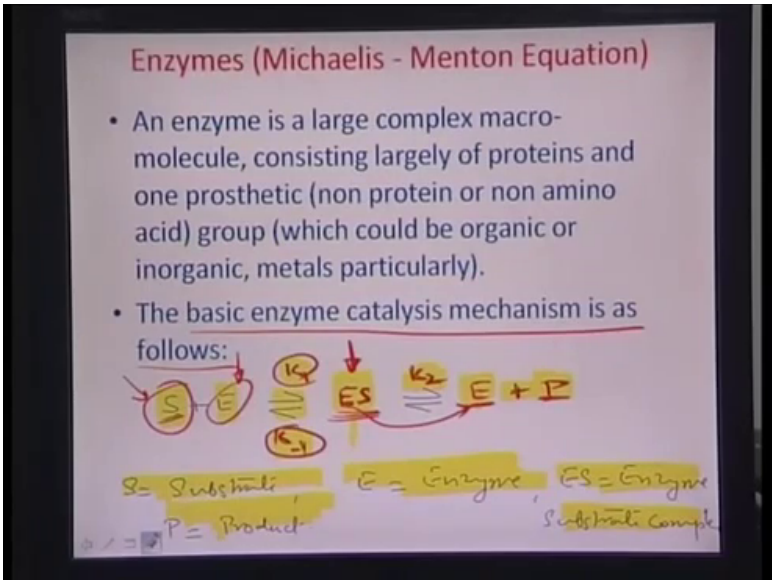

So, if you look at the basic enzyme catalysis process really – mechanism really; what it follows is the following. So, you have a substrate here mind you; substrate essentially again is the molecule of the moiety in which we are trying to bind something or convert into some other moiety. So, here substrate does not really mean the physical meaning of it like a wafer; substrate essentially means something to which the particular biological moiety – be it an antibody or an enzyme would bind. So, you have an enzyme E, which is binding to a substrate S here. And, there is essentially an equilibrium established between these two. There is an intermediate complex, which is formulated as you can see here. ES enzyme substrate complex, which is essentially ephemeral nature; it lasts very very short and breaks down into an enzyme itself and the products. Enzyme really comes out as it is. So, therefore, it is just a catalyst; it does not… Although it participates physically into the reaction, it is not a part of the product; and, it can retrieve itself back normally. So, you have substrate and

enzyme on one side; you are actually doing a reaction here; add a certain equilibrium state, where may be the forward reaction has a rate constant k1; the reverse constant reaction is a rate constant k minus 1 let us say. It converts into an enzyme substrate complex and it again breaks down into the enzyme and the product at a forward reaction rate let us say k2. So, in this kind of a situation, let us actually see an example what kind of enzyme can do what kind of behavior to certain molecules.

$$S + E \underset{\Leftarrow}{K_1} ES \underset{\Leftarrow}{K_2} E + P$$

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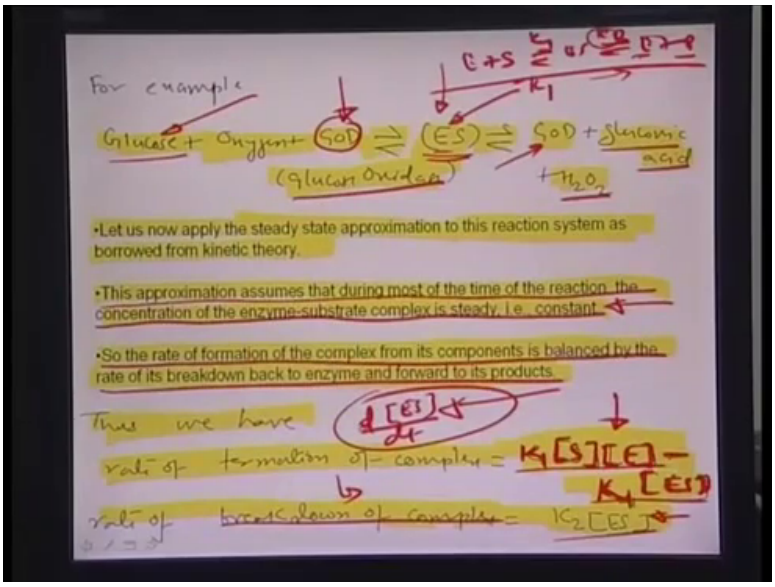

So, we have many times before discussed about this famous glucose-detection reaction. So, glucose here is oxidized in the presence of this enzyme GOD – glucose oxidase in order to convert again into an enzyme substrate complex. And then, essentially, the GOD separates out and you have gluconic acid and H2O, which gets generated. So, 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 a kind of rate kinetics placed method to a certain whether what kind of times do we need to hold these solutions on a certain substrate on maybe on certain wafer for the moiety to bind to that wafer. Or, indirectly, if you want to perform a sandwich between two molecules and a solution, what kind of time delays should you do between different steps of the chemical processes for the binding to occur. So, you have to have a good mathematical idea about the rate constants, etcetera. And so, the enzyme – the model that we have taken here for describing that is really the enzyme substrate reaction.

$$Glucose + O_2 + GOD \rightleftarrows (ES) \rightleftarrows GOD + Gluconic\ acid + H_2O_2$$

So, if we apply a 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 really is borrowed from the 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. And, the rate of formations of the complex from its components is balanced by the rate of its breakdown back to enzyme and forward to its products. So, essentially, there is some kind of an equilibrium between the enzyme substrate – enzyme substrate complex and enzyme product. Let us say we have the rate of formulation of the substrate $\frac{d[ES]}{dt}$ given by this particular equation. So, you have an enzyme E reacting with the substrate; and, the forward rate is k1; reverse rate is k_{-1} ; and it is formulating ES. Again you have another combination with the forward rate is k2 . let us say and you are converting into enzyme and product.

So, here if you really look at the way it is formulated or the amount of the substrate consumed is $k_1[S][E]$ from first of the kinetics. And, the amount of the reverse reaction; that means the breakdown of enzyme substrate complex into the enzymes of substrate is at the rate of k_{-1} times of concentration of enzyme substrate $[ES]$. And, the rate of formation or the rate of breakdown of this complex in the forward direction is really k2 times of the concentration of the enzyme substrate complex i.e. $k_2[ES]$. So, therefore, because k2 is the forward rate reaction in which the enzyme substrate complex is broken down into enzyme and product as it can be seen here. So, that is really the overall formation breakdown rate of the enzyme substrate complex.

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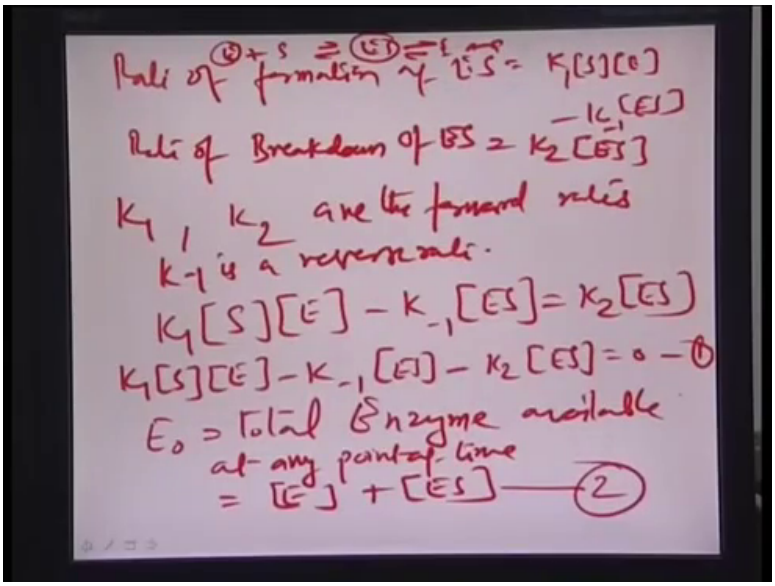

So, the rate of formation of ES – enzyme-substrate complex is given by $k_1[S][E]-k_{-1}[ES]$. And, the rate of breakdown of ES is $k_2[ES]$ . . Now, this k_1 , k2 – k1 and k_2 are the forward rates; k_{-1} is a reverse state. So, assuming this to happen, we can say that, if there is a steady-state behavior of this reaction and the way that the enzyme substrate is formulated or enzyme-substrate complex is formulated same as the rate at which the enzyme products are formulated and the rate… is same as the rate at which the 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. And, if we take that into picture, then we can say $k_1[S][E]-k_{-1}[ES]=k_2[ES]$ .And therefore, basically, the different rates all put together $k_1[S][E]-k_{-1}[ES]-k_2[ES]=0$. Let us call this equation 1 at this time.

So, we describe the enzyme concentration really in terms of the total concentration E_0 of the enzyme as the concentration available free with the substrate and the concentration available in the complex state with the substrate. So, essentially, if you do that, then we can write very safely that, E_0 – the total enzyme available at any given point of time, can really be represented as the $[E]+[ES]$. You see here in the reaction really, 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 really the

sum of enzyme substrate and enzyme. So, assuming that to happen here in the whole reaction system and if we substitute this, we call equation 2.

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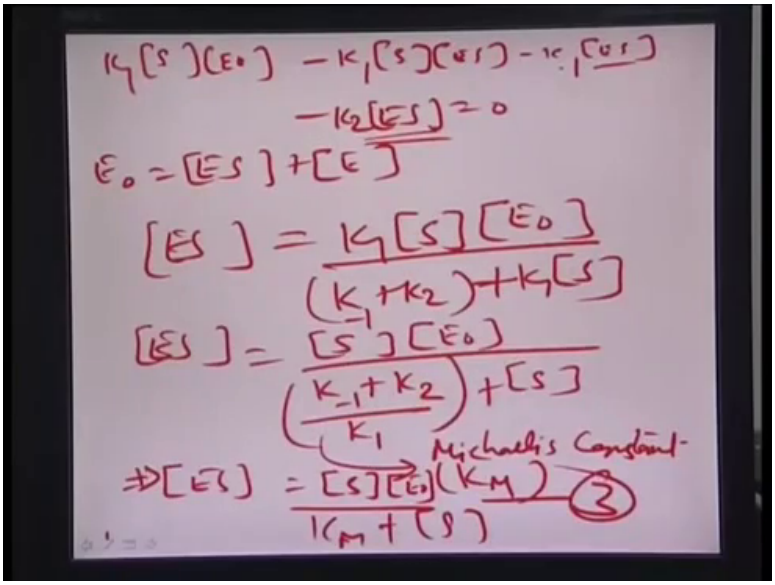

Substitute this 2 back in 1; we are left with the equation as $k_1[S][E0]$; where, E0 is the total enzyme concentration at any given point of time, $-k_1[S][ES]-k_{-1}[ES]-k_2[ES]=0$. So, this is by just substitution of E0 value, which is equal to the $[E]+[ES]$ at certain point of time in a reaction – equation 2. So, assuming this to be true, so, we are able to solve from this really the concentration of the enzyme-substrate complex at any point of time, which is equal to $[ES]=\frac{k_1[S][E0]}{(k_{-1}+k_2)+k_1[S]}$. So, if we divide by k1 on the numerator and denominator, we are really left with enzyme substrate concentration as $[ES]=\frac{[S][E0]}{\frac{(k_{-1}+k_2)}{k_1}+[S]}$. So, this is really known as the Michealis constant upon the name of its inventor essentially. And, the set of equations are called the Michaelis-Menton equations. So, let us call it K_M – Michaelis constant. So, in this particular case then, we can represent the [ES] value as really $[ES]=\frac{[S][E0]}{K_M+[S]}$. Let us call this equation 3.

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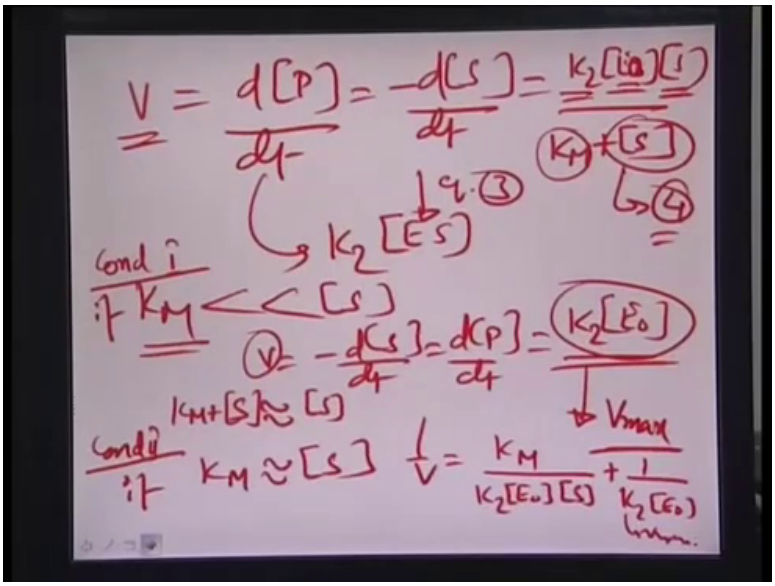

So, then really the overall reaction rate or rate velocity, rate of formation products any of them is given by the equation velocity V is equal to $V=\frac{d[P]}{dt}$ at which the rate of formulation or product or the rate of formulation or the rate of degradation of the substrate $\frac{-d[S]}{dt}$ is given by Michaelis-Menton equation as $k_2[E0][S]/(K_M+S)$ because essentially this is nothing but $k_2[ES]$, which was derived from equation 3 before; all right. So, therefore, the reaction velocity V here really can be represented by $k_2[E0]$ – the total enzyme concentration times of concentration of substrate S divided by Michaelis constant plus the concentration of the substrate. So, there are several conditions here that, this reaction or this equation can be imposed. And, this is… Let us call it 4.

So, in condition 1, let suppose that, this Michaelis constant K_M somehow is very very small in comparison to S. So, let us assume that, in condition 1, just give me a minute here. So, you have the first condition here called condition 1; where, we assume the Michaelis constant K_M to be very very less than S. So, equation 4 really changes into V equals $V=\frac{d[P]}{dt}=\frac{-d[S]}{dt}=k_2[E0]$, because essentially, K_M plus S can be approximated as S itself. So, therefore, it is really a case, where the velocity of the reaction V is proportional to the concentration of the total enzyme [E0] that have been given at the very beginning of the

start of the reaction when the enzyme-substrate complex was just about to get formulated. So, therefore, this is also known as the maximum concentration or the maximum velocity V max. So, 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 of V is always going to go down. So, therefore, mathematically also, this can be represented as the V max or maximum rate constant or rate of 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 Michaelis constant. And, if I… I will show you later a plot – how these different points are very very significant for understanding the enzyme-substrate formulation reaction. So, let us say that, in condition 2 here, we assume that, this Michaelis constant K_M is approximately equal to the substrate concentration S. So, in this particular case, what would happen is that, you have 1 by V from this equation 4; it can be also written down as $\frac{K_M}{k_2[E0][S]}+\frac{1}{k_2[E0]}$, which is nothing but V max as we just saw little bit before.

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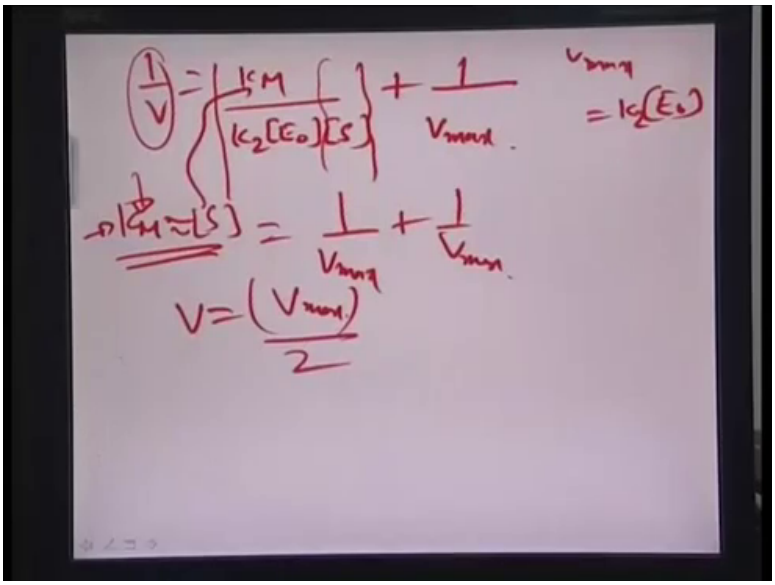

So, we are left with an equation of the form concentration of S equal to… 1 by V equal to $\frac{1}{V}=\frac{K_M}{k_2[E0][S]}+\frac{1}{V_{max}}$; where, V max is as you know already, $k_2[E0]$. This here right here if you assume K_M and S to be equal can be represented as again 1 by V max plus 1 by V max. So, if K_M is same as S here; if you substitute this, the concentration of S really goes off

and you have 1 by k 2 E 0, which is nothing but 1 by V max. So, in this case really, when the concentration of S is equal to the Michaelis 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. So, let us plot now; I mean if you look at really the curve here; the plot is between… Let us say if you plot between 1 by V and 1 by S here; this is going to be a linear plot with a slope and an intercept. The slope is essentially V max.

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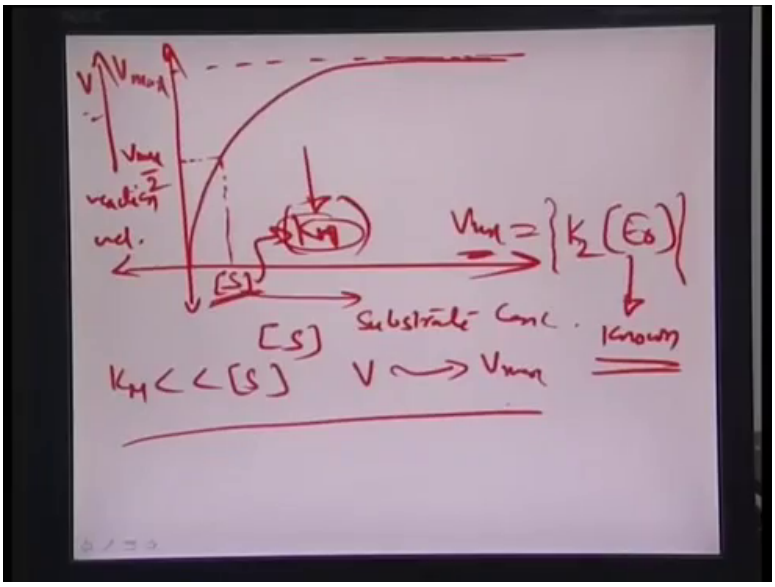

On the other hand, if you plot between the V max and the substrate concentration S, the way this plot would go is something like you have let us say V max or V as the y-axis here – reaction velocity and substrate concentration S in the x-axis. So, in this particular case, as we can see that, as K_M is very very smaller than S and the V really approaches V max. So, let us say we have a straight line here describing what we know as V max; then, the reaction rate really should be asymptotically approaching this V max line. And, this is what the relationship between V and S would really look like. So, a 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 Michaelis constant K_M. So, this is essentially an experimental method of determining from reaction chemistry, what the Michaelis constant K_M would really mean at a certain substrate concentration.

So, if you know what V max is based on your initial enzyme concentration, which is known; mind you, in a particular reaction, this is really 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. So, if you know these two, which would give you an indication of V max; then, you could really find out what the Michaelis constant K_M is by looking at substrate concentration at a point of intercept on the curve corresponding to V max by 2. So, this is really how the rate kinetics of the enzyme-substrate reactions happen. And, it is a matter of fact, any antibody-antigen reaction also kind of governed by the same set of equations. Only thing is that, in this case probably, 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.

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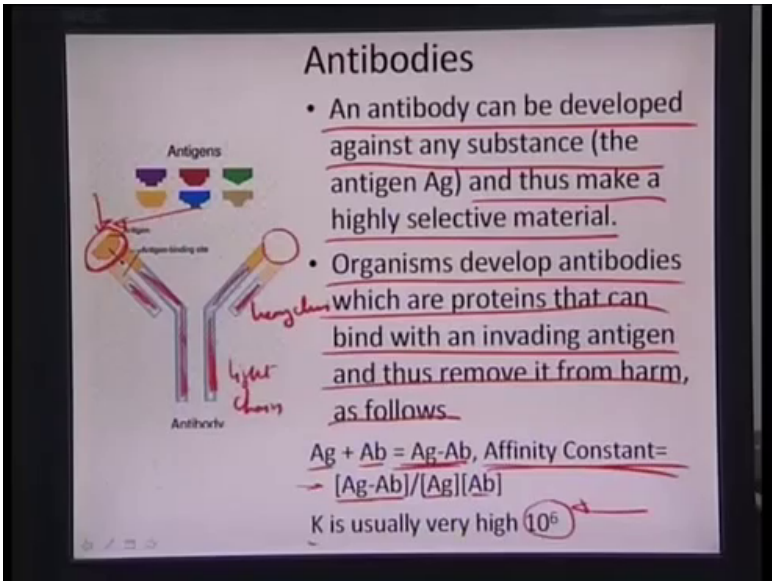

So, let us look at a little more details of what really the antibody is or what their structures are in order to understand more. So, number 1 – the very important fact is that, an antibody can be developed against any substrate, any substance popularly known as antigen. And, it can be raised essentially. And so, the idea is that, the antibody so developed would be highly selective to the substrate. So, essentially, an antibody can be developed against any substrate and can be made a very highly 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 so-called D cells within a certain living organism. And essentially, the organisms can be used to develop antibodies, which can be later on used for the purpose of capture or specific recognition – in vitro.

So, therefore, organisms develop antibodies, which are proteins and they can bind with an invading antigen and remove it from harm as follows. So, the antigen, an antibody binds together to form this particular complex. And, the affinity constant in this particular case is actually the formulation of or the concentration of complex divided by concentration of independent antigen-antibody. And usually, it is very very high; not as high as the evident biotin link; but, again K is usually about 10 to the power of 6 mole inverse in case of antigen-antibody binding, which is a pretty high affinity constant. So, if you really look at antibodies, they are Y-shaped mechanisms like you can see here. So, you have these sections here at the stem of the Y which are really light chains and on the two upper sections here which are the heavy chains.

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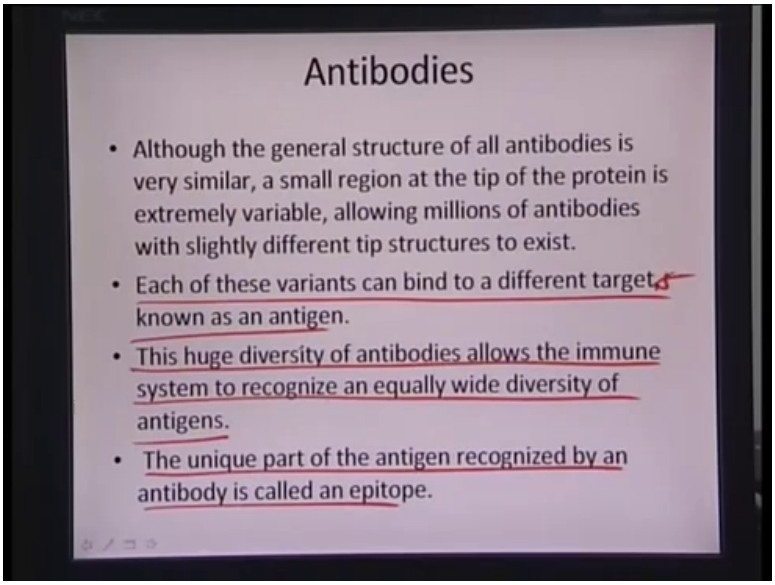

And, essentially, the overall structure of the antibody by enlarge remains constant; there is no much variation except the fact that, there are certain subtypes based on what these chains are really in mammalian cells or in mammalian organisms typically; they are about five different kind of such subunits, which can be categorized as this heavy chain, light chain structures or antibodies. What is interesting though is that, the portion here on an antibody, which is actually known as an epitope site is the only variation in the whole antibody. By enlarge, the other subtypes of the antibodies, which are available are only 5 in number. But, if you look at the amount or the amount of variation that these particular sites would have or the epitope sites would have; they can be different – million different antibodies of corresponding to binding; or, which can be capable of binding to million different biological entities. So,

essentially, this keeps on changing, the epitope site keeps on changing; whereas, the other part of the Y, that means, these light chains and these heavy chains by enlarge remain similar except categorization into a few subtypes. So, the different subtypes are also known as isotypes.

Isotypes are essentially based on which heavy chain the antibody would possess. So, as I indicated before, there are five different antibody isotypes known in mammals so far. That is all the range that the antibody could have except the fact that, the epitopes are varied and numerous in region. So, an antibody essentially made up of this epitope with the heavy and light chain isotype can bind to different targets. And, the targets that they typically bind to are known as antigens. Also, there is 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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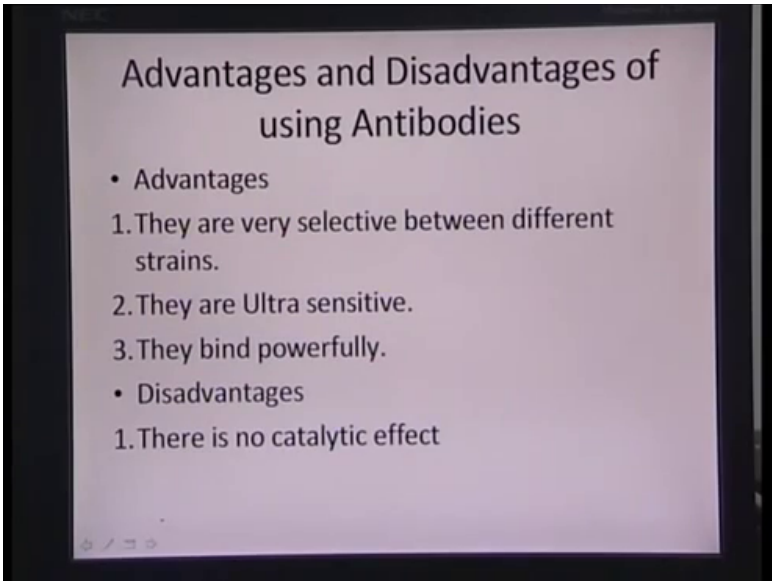

So, there are several advantages and disadvantages of using antibodies. One of the most prominent advantages are the selectiveness or the selectivity that these antibodies would normally possess between the different strains. Another very important advantages that these antibodies are ultra sensitive to any kind of small variation in the analyte of interest, I mean the binding or unbinding, is typically independent on what is the characteristic of the particular analyte. If there is a slight variation and also 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 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 an antigen and antibody as happens in the case of enzyme. So, here whatever is bound is bound; I mean it cannot be reconverted back into a proper, I mean a pure antibody again or a pure antigen again. It is an irreversible reaction of formation of the antibody-antigen complex. So, by enlarge, these are some of the advantages and disadvantages of antibody-antigen systems.

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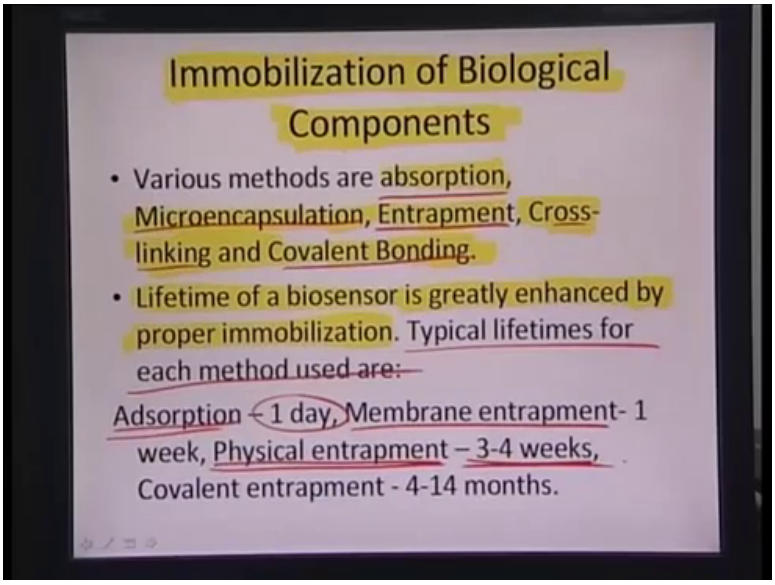

Let us now turn into a little bit different component; how we can immobilize the different biological components. We have done a little bit of this while looking at sensors; but, essentially, as is obvious, the next question, which comes into being is utter way of kind of understood the reaction kinetics and also try to find out more details of the structure of these different biological moieties. How do we really use them in sensors? And there, immobilization protocols are very very important for that. So, there are various methods for immobilizing; can be absorption, it can be microencapsulate, can be entrapped on a particular electrode or a substrate; you can cross link or covalently bond a certain biological moiety or entity. So, really, the lifetime of a biosensor is greatly greatly enhanced by proper immobilization technique. If you can choose the right immobilization technique, you can use the biosensors many times reusably without really very many changes on to the sensor surface.

So, based on the absorption method that, based on the particular immobilization method from these different range of methods that I have stated before just about a minute back – absorption, microencapsulation, entrapment cross-linking or covalent bonding. The typical lifetime of a biosensor also vary a lot. Like for example, in case of adsorption, this is only about a day or so. Absorbed substance is really do not stay very long on to a surface and they are kind of prone to de-adsorbing based on changing the partial pressure of that atmosphere in which such a surface would be kept. The membrane entrapment that we have talked about here is typically about 1-week life time; it is pretty stable, because membranes are essentially thin perforated structures, which would be able to hold these biological moieties very very close to a particular substrate. For physically entrapment cases, it is about 3 to 4 weeks; the revised life time is greatly greatly entrapped – enhanced because of the schematic wherein this entrapment is in a gel kind of a network. And, for a covalent entrapment again, it is very high, because there is a covalent bond now between a particular moieties; it is about 4 to 14 months.

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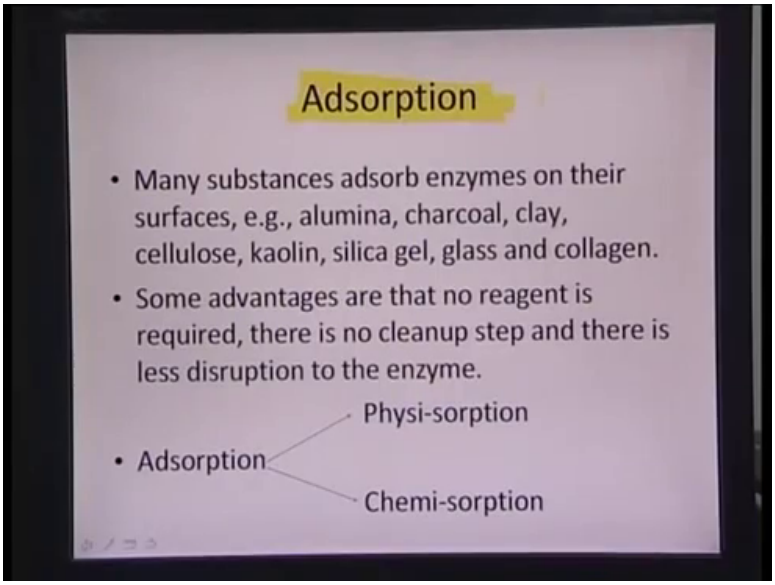

Details of the different immobilization aspects and schemes; the first aspect which comes into picture is adsorption. What really adsorption is, is really, I mean it is kind of some kind of either physical or chemical attachment over a surface physical – by physical attachment over a surface, what we mean is that, if there is a size-based selection of a certain moiety on a surface, that could be set as physically absorbed on to the surface. Chemical adsorption on the other hand could be because of very many reasons one could be just ionic attraction

between the 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 Waal forces, there may be the tendency of a particular chemical to adsorb on to the surfaces it is a weak interactions between the moiety on the surface.

So, many substances really absorb enzymes particularly on their surfaces. Some of the examples could be alumina, charcoal, clay, cellulose, kaolin, silica gels, glass, collagen, etcetera. One of the reasons why if you look at really the microstructural aspect of these surfaces, they are all very highly porous in nature; and, essentially, more surface area is involved. So, therefore, if the area is more, there is a tendency of the adsorption of a particular moiety to be more. So, absorption is a dependent phenomenon on typically the number of binding sites that a surface has to offer. If you have more surface area, more binding sites, you have more absorption. So, some of the advantages of this process are that, typically, no reagent is required. That is a biggest advantage that adsorption has to provide that, there is absolutely no reagent required. Also, there is no clean up step and there is less disruption to something like a biological moiety, an enzyme or an antibody just because as less as possible, the involvement of chemical steps are as less as possible essentially.

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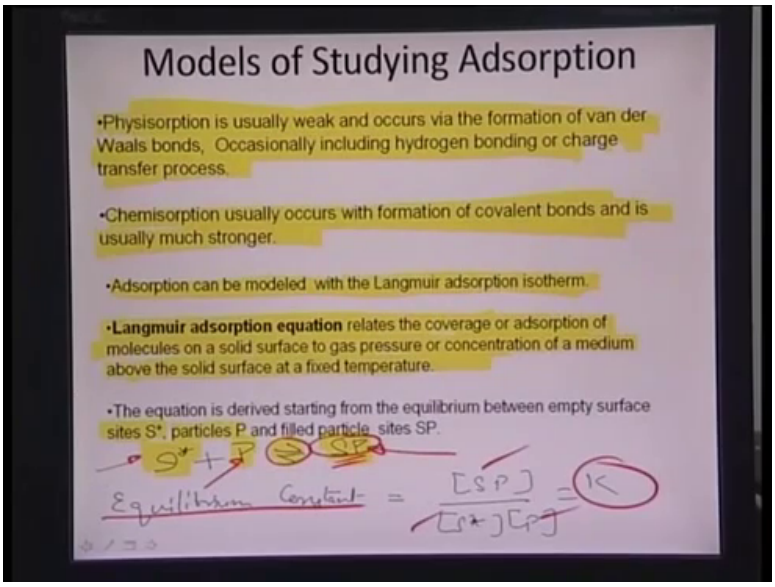

So, adsorption can be classified into physisorption and chemisorption. This is physical adsorption; this is chemical adsorption. And, people have been really studying this adsorption on a very modular manner as I have already indicated physisorption typically is called by Van

der Waals bonds, which are usually weak. And, occasionally, hydrophobic-hydrophobic interactions or a charge transfer process are hydrogen bonding. That is how physisorption would typically occur. Chemisorption on the other hand would be usually by the formation covalent bonds; and, it is a much much more, much much, usually much stronger process of adsorption on to the surface. So, the adsorption phenomenon can be model as you can see here by the Langmuir adsorption isotherm. What this equation really relates is that, it relates 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. So, essentially, it is also known as the Langmuir adsorption equation.

So, 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 very critical. Another is especially in gas phase of absorption; the vapor pressure, which is over the surface is very very critical to determine what is the adsorption if the pressure is little higher than that adsorption rate automatically increases, because you can think about it as some kind of a forcing mechanism for the molecules to seep through the different vias and trenches on a particular surface. And so, therefore, the partial pressure is high in the atmosphere; the P is high and the adsorption rate is manually increased.

So, adsorption is typically given or described by this equation here. Let us say S dashes the number of sites, which are available on a particular surface. Your adsorbing particles flee on to this S dash or star sites on to that surface. And, the filled particle sites are represented as S – SP. So, typically, there is an equilibrium between these two. So, you have S tap, which is the number of active available sites; and, P – number of particles. And then, this is the filled site or number of sites, which are fully filled – SP or there is an equilibrium between these as you can see. So, the equilibrium constant here is really 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.

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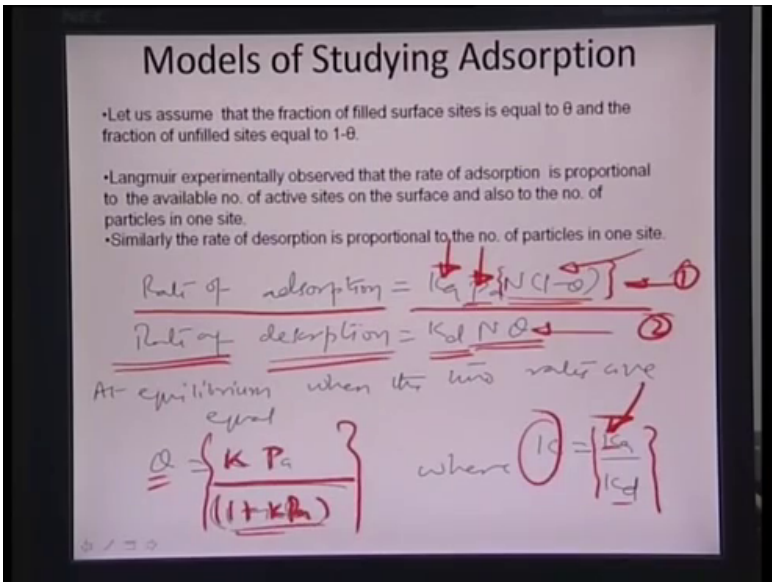

So, let us assume that, the fraction of filled surface sites is equal to theta; the fraction of unfilled sites on a particular point instant of time is 1 minus theta. So, you assume that, you have total number of let us say the total number of sites as n. And, the fraction of those sites is theta means theta N are the number of filled sites. And, one minus theta N are the number of unfilled sites. So, Langmuir what he did is he kind of tried to experimentally observe that, how the rate of adsorption would be behaving if theta is increased. The fraction of the active sites or fraction of the filled surface sites are increased with time. We found out that, there is a relationship between theta. So, if theta is decreased and the number of active sites are increased, adsorption rate would increase, it is a directly proportional relationship.

Also, what you found out is if the partial pressure is high, the rate of adsorption would increase. So, if you put this altogether in an equation, you are left with the rate of adsorption equal to k_a, which is the adsorption constant times Pa – partial vapor pressure times N of 1 minus theta i.e. $P_a\left[N\left(1-\theta\right)\right]$. So, this essentially is the number of active sites, which are available. 1 minus theta mind you is 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. So, $N\left(1-\theta\right)$ is really the number of sites, which are available on to a certain surface for the binding to happen at the adsorption to take place. So, the rate is proportional to the partial pressure as you can see and also proportional to the number of active sites on the surface area.

Similarly, the rate of desorption – the ability of a surface to lose a particular adsorb species from it is proportional to the number of filled sites that are on the surface. So, if the number of filled sites are more, the tendency of the material to get desorbed from the surface is also automatically increased, which makes sense and is more logical. So, therefore, let us say the k_d is the reaction rate for the desorption. And, desorption really is independent of pressure. So, whatever the partial pressure be, desorption would appear to be or would happen at a rate, which is totally 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. So, $k_d N\theta$ is the rate of desorption.

So, at equilibrium, if we assume that, these two rates of adsorption and desorption are equal; then, whatever is adsorbed after a while, after the steady state has been reached would after all the active sites have been kind of filled up, would typically 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; they equate – these two equate these two equations as $\theta = \left\{ \frac{k P_a}{1 - k P_a} \right\}$; where, k is the ratio between the adsorption constant and the desorption constant $k = \frac{k_a}{k_d}$. 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. So, that is what would happen typically when in a gas phase adsorption process, there is an equilibrium, which is achieved or reached. Now, this gives you some idea of how you could study adsorption especially physisorption on a particular surface.

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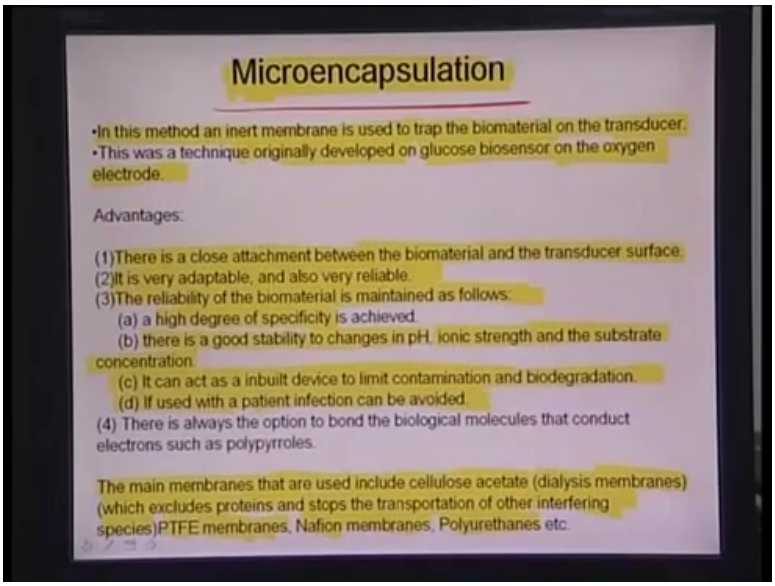

Let us look at a different technique now – the microencapsulation as I have been talking about before. So, it is really the trapping of moiety or a biological moiety in between two membranes or two membrane of structures. So, in this particular method, an inert membrane is prepared and it is used to trap the biomaterial of interest on a transducer surface. And, essentially, for the very first time, this technique was developed for a glucose biosensor. And, this was developed on an oxygen-level electrode. So, it is essentially developed on an oxygen electrode originally for a glucose biosensor. So, there are several advantages of the microencapsulation process. One definite advantage as 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 very close to a certain surface. And therefore, this kind of provides a firm binding mechanism of closeness of the entity to the particular sensor surface that is being used. So, definitely, it is much more closer as an attachment than let us an adsorption, where it may just randomly adsorb on a surface without taking care of any textural issues of the particular surface.

So, there is also… This process is also very very adaptable; also, very reliable, because typically, you do not have to modify the sensor surface. That is an advantage. You are trapping using an 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 are not chemically again reacting; you are keeping the biological entity in its original state as it is supposed to

be. So, therefore, this results in a high degree of specificity almost always, because you cannot – you are not modifying chemically the biomaterial that you want to place on to a surface. And also, there is a good stability to changes in pH, because essentially, since the biomaterial is in its own state, it 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 supposed 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 the biomaterials chemical property in general. And, it can be used to prevent infection, because you are trying to guard the biomaterial using a membrane. I am trying to guard it from getting in touch or getting contaminated with the patients' fluid samples, which you are trying to measure with this. So, you could always of course, have an option of binding the biological molecules that conduct electrons such as polypyrroles, etcetera to make the membrane, so that you could actually have an electron transparent path from the biological moiety on to the sensor surface.

Some of the membranes that are used in this kind of microencapsulation are cellulose acetate; remember – dialysis membranes are made up of cellulose acetate; and, they are put external to the body wherein the blood is flown and there is always some kind of separation of essentially urea and other harmful salts in the blood. This can… This which excludes proteins and stops the transportation of other interfering species. This particular cellular acetate membrane is kind of due to its hydrophobic nature; kind of prevents the proteins or some other interfering species to get filtered across it. Then, you have other kind of structures like PTFE – polytetrafluoroethylene membranes; Nafion is a very good material, polyurethane, etcetera, which can be used successfully as for microencapsulating the biological moieties.

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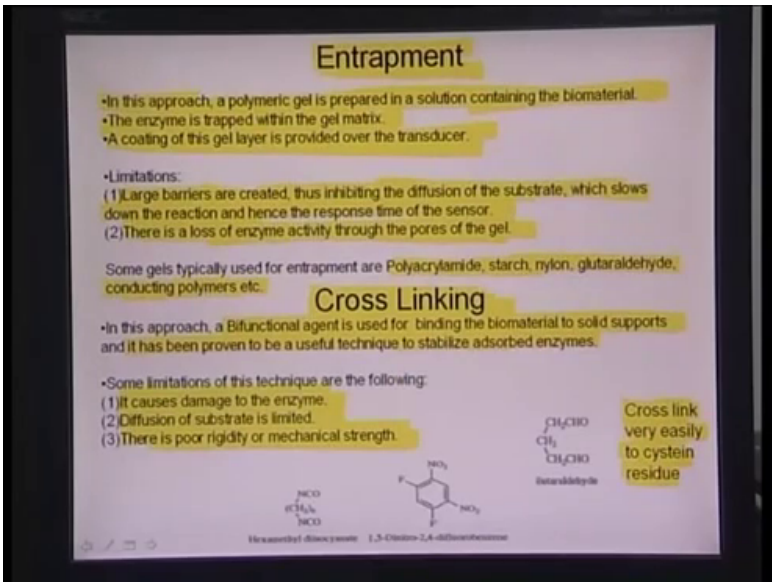

The next line is entrapment. That is another very important mechanism here; you are really entrapping the material in a gel kind of matrix. So, instead of binding it together within a membrane or between two membranes, we are now trying to bind it by entrapping it into a polymeric gel kind of matrix in this particular approach. And, for that, you first prepare a solution and then agitate it later in suitable conditions after the enzyme or whatever biological moiety you are talking about is trapped within the gel matrix. And then, finally, a coating of this gel layer is provided over the transducer surface. So, this process also has some limitations.

One is that a large barrier is created. Essentially, you have no control on the distance of the biological moiety from the sensor surface. There is a thick gel, which is entrapping the moiety in its thickness. And, the thickness of the gel can be made thinner and thinner; but, still there is no active control on where the entity is present. It is towards the sensor surface or is it away from the sensor surface on to the other surface of the gel; we do not have control on that aspect while preparing. And so, therefore, sometimes, large barriers are created. And, they can do all sort of things like diffusion of substrates; it can slow down the reaction in general. And therefore, sometimes this is all at a cost of the response time of the sensor; it gets drastically change because of fact or thick gel layers in trapping the enzymes.

Also, there is a loss of sometimes the enzyme activity, because the gel surrounding is natural, is really not the natural surroundings of certain enzymes. And therefore, whatever chemical

properties the gel has would definitely have an influence on the enzyme itself. So, there is of course a loss of enzyme activity particularly for the pores of the particular gel. So, some typically used gels are polyacrylamide, starch, nylon, glutaraldehyde and some of the conducting polymers, etcetera. The other technique which is important to be understood is cross-linking. This is an approach essentially, where a biological moiety is kind of bound to a biosensor with the cross-linker molecule. So, you have some kind of a ligand molecule, which is one side bonded to the biological entity and another side bonded to the particular surface in question. So, we call it a bifunctional agent. This kind of cross-linker molecule; and, it is used for binding the biomaterial solid supports. It has been of course proven to be very useful technique particularly to stabilize the adsorbed enzymes. And of course, this technique also has some limitations.

Some of them is that, it sometimes causes damage to the enzymes, because we 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 ligand molecule. So, it causes some damage to the normal functionality of the enzyme, because its structure is changed and proteins as you know are extremely sensitive to this change in confirmation. Their whole properties change because of a slight change in the confirmation of the molecule.

Also, one more interesting fact of here is that, the diffusion of the substrate is sometimes limited, because cross-linkers typically form a dense brush-like material on to 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 sometimes chemically cross-linked and they themselves not very strong as molecular bonds. And so, therefore, we have to largely depend and rely on the nature of firm or the material. So, some examples are typically the cross-links that are formulated to system residues and proteins, etcetera.

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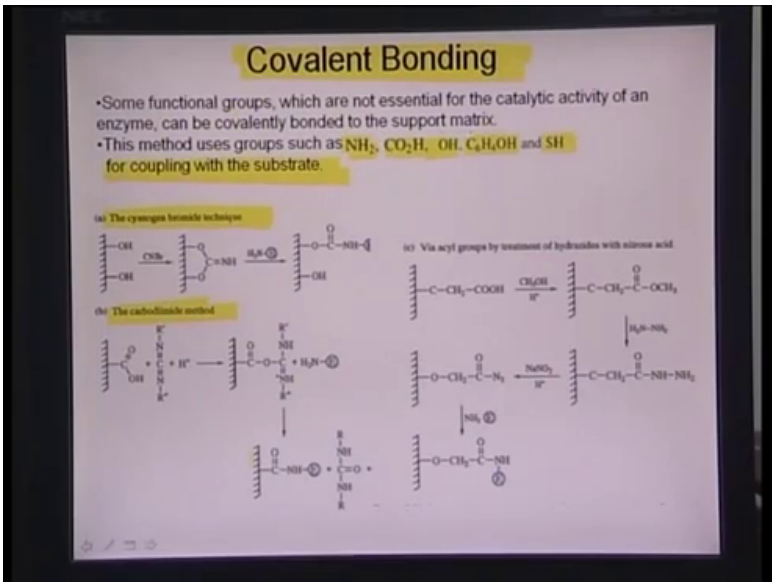

The other mechanism that I would like to talk about is really covalent bonding wherein as you can see here, some of these functional groups present within the biological moieties directly bonded on to the surface. And, essentially, it can be covalently bonded to the support matrix to just be a part of the surface and do its job. So, the method can use the presence of group such as NH2, COH – OH - C6 H4 OH – SH, etcetera develop charge on it, so that the charge could ionically bond or attract or do some kind of a direct covalent bonding with groups on to the substrate surface. These for example, are some of the illustrations, where it talks about how different moieties can be covalently bonded on to the substrate surface. So, with this, I would like to end this particular lecture. And, we will cover some stuff related to things like cyclic voltammetry and some related to electrochemistry on an electrode surface in the next lecture.

Thank you.

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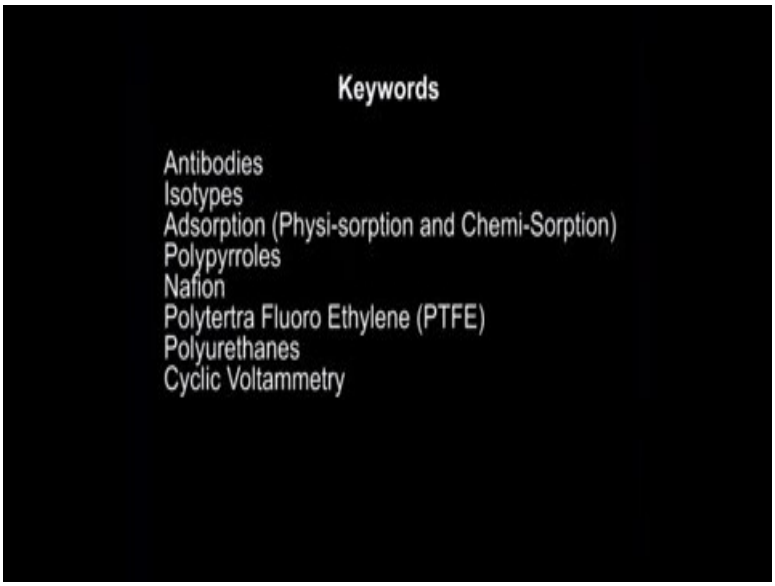

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