ENZYME SCIENCE AND ENGINEERING

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LECTURE–27

ANALYTICAL APPLICATIONS

Last time we were discussing about the application of the immobilized enzymes and we had mentioned that the enzymes find applications in a variety of situations and we dealt with the applications of immobilized enzymes as industrial biocatalyst in process industries. Two major sectors of applications of immobilized enzymes are analytical applications and also their application in the therapeutics for the treatment of diseases in human beings and both the applications are also particularly important or in contrast to the application as industrial catalyst lies in the effect that you require much more purified systems and the use of high value enzymes in their applications unlike in the case of industrial biocatalyst where mostly we were dealing with relatively lesser valued enzymes and the two applications on analytical applications and therapeutics require relatively high value enzymes and in a higher purified state.

Just to take up the analytical applications the variety of applications that have been reported can be classified into four different types: the first is automated analysis procedures by combining immobilized enzymes with various analytical tools. We come across variety of analytical situations whether in the area of research or even in the area of clinical analysis where the samples are analyzed in large numbers and be required to use very specific reactions that can be catalyzed by enzymes. One way is as you must have done in your laboratory class the use of glucose oxidase for example for assay of glucose. The use of soluble enzyme may be fine if you have to analyze let us say couple of samples three or four. But in situation where the sample to be analyzed are in very large number and very often such instances are met in hospitals and in clinical laboratories where few hundreds of samples everyday are encountered and use of soluble enzyme may be some times uneconomical. Similarly in research also a student might be analyzing the similar sample hundreds of them over a day and just to get his results. So in those cases the analytical procedures can be automated by combining the immobilized enzymes, instead of soluble enzyme, with a standard analytical device.

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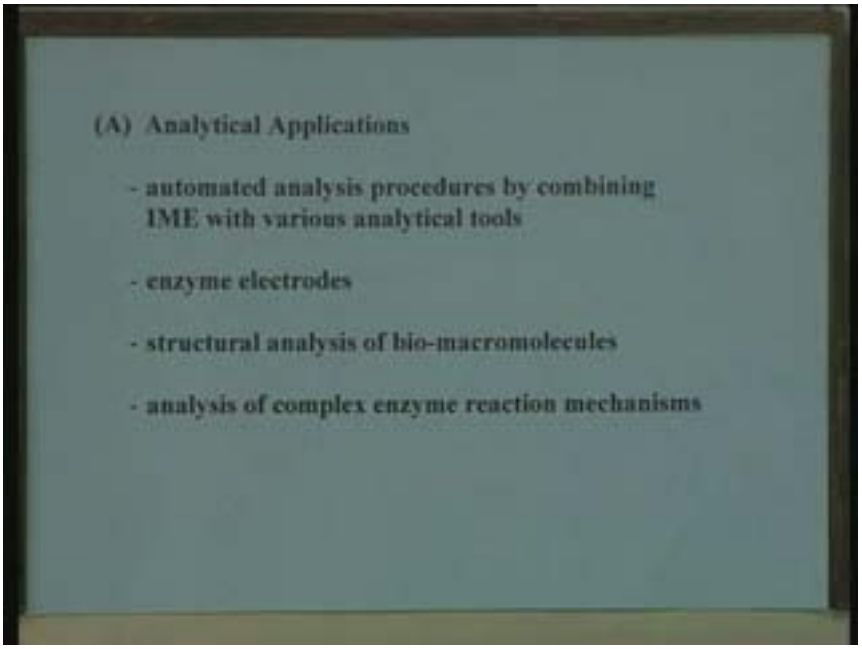

It could be a spectrophotometer, it could be a fluorospectrophotometer, it could be as simple as pH meter. So any analytical simple analytical tool can be combined and used. A very simple example of this approach was reported by Hicks and Updike way back in 66. Another point is the bulk of the analytical applications were the earliest uses of immobilized enzymes. In fact their application in process industry was adopted much later than they were used in analytical applications and that means the cost implications are much more severe in analytical sections. The setup is like this that the given sample in which a chemical species to be analyzed is passed through an immobilized enzyme column a very small column and here in this reference it was glucose or lactic acid determination based on glucose oxidase or lactate dehydrogenase and they were immobilized on polyacrolymide gel and packed in a very small column and the substrate was fed at 0.8 ml per minute and then this is passed through a delay line so that you provide a reaction time and a coloured reagent is added here and after that in a spectrophotometer, a photometric cell which is connected to a system, the reaction passes the and color developed is monitored.

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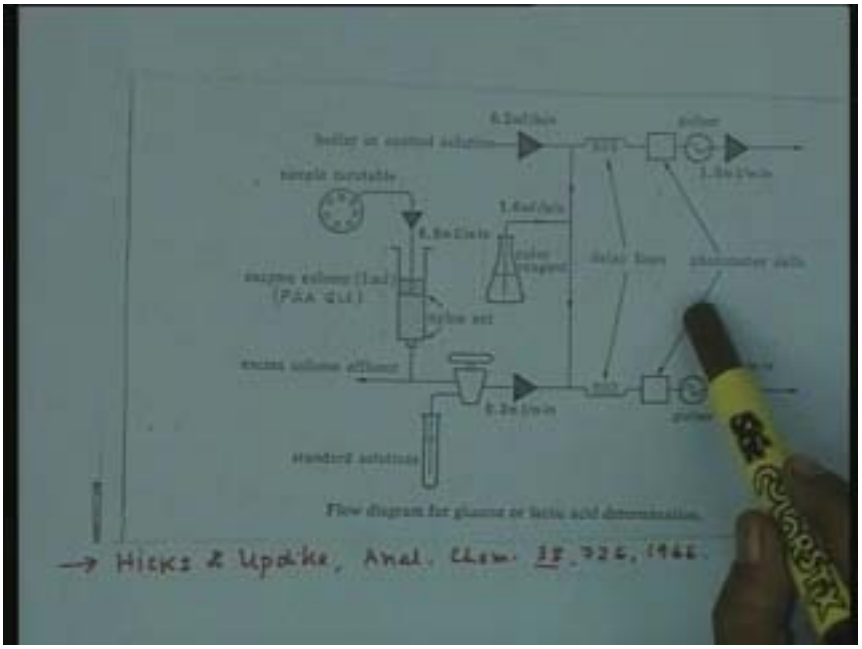

You can also have some standard solutions and this is a two way valve whereby you can either put a standard solution and measure observant in the photometer or you can put the actual cell and on the other side you have a buffer or a control solution which also passes through a delay line and a different photometer of the same wavelength and to empty the photometer tube pulses are used so that in a pulse mode the tubes are filled and emptied while the flow is continuous for the photometer measures in a pulse mode and the materials are removed. In fact two of the systems which were very early reported were glucose by glucose oxidase and lactic acid by lactate dehydrogenase. This is the kind of reaction that takes place in the immobilized enzyme column. The lactic acid sample itself is mixed with NAD solution and pyruvic acid is formed with reduced NAD and reduced NAD is then oxidized with a dye DPI and then the NAD gets regenerated that means that is oxidized and reduced dye is colourless. This DPI is blue and this is colourless. So difference in the absorbance is related to the concentration of the lactic acid. On the other hand, in the case of glucose it is glucose to gluconic acid plus hydrogen peroxide and hydrogen peroxide acts as an oxidizing agent and it oxidizes the DPI to coloured product which is blue in color and is measured.

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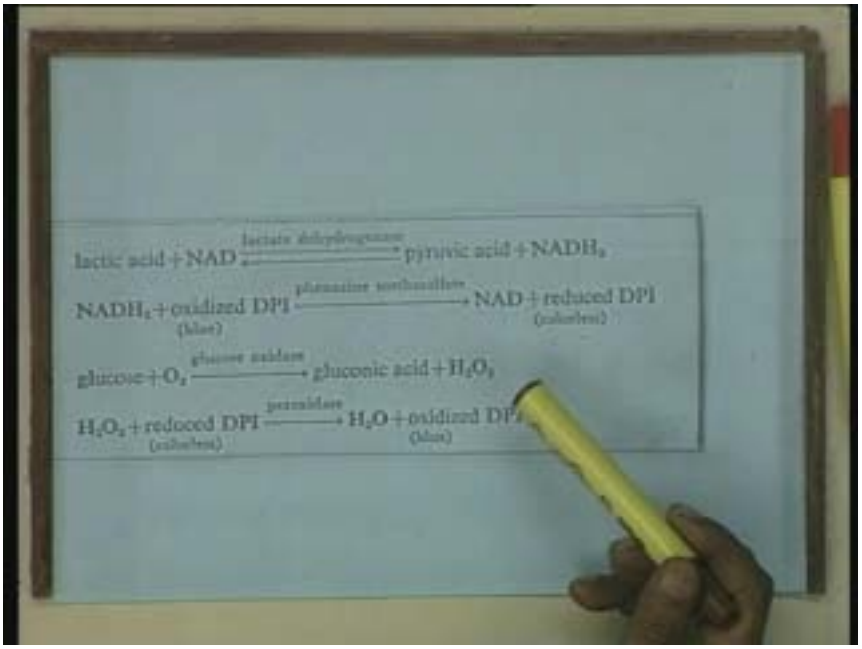

A large number of other dyes also have been reported and this dye is only just to facilitate the measurement of the colour. Two other very interesting examples also came in almost during the same time in 64 and 65. Riesel and Katchalski in Israel reported the use of urease immobilized on diazotized amino acid copolymer used for the determination of urea in urine or serum and also for the removal of urea from body fluids. The urea system in urine samples is used today in many large hospitals on a commercial scale where the requirement is almost thousands samples in a day. So instead of employing number of analysts they have an auto analyzer in which the immobilized column can carry out the job and almost thousand samples can be analyzed using a single cartage of immobilized enzyme and the cost can be saved effectively.

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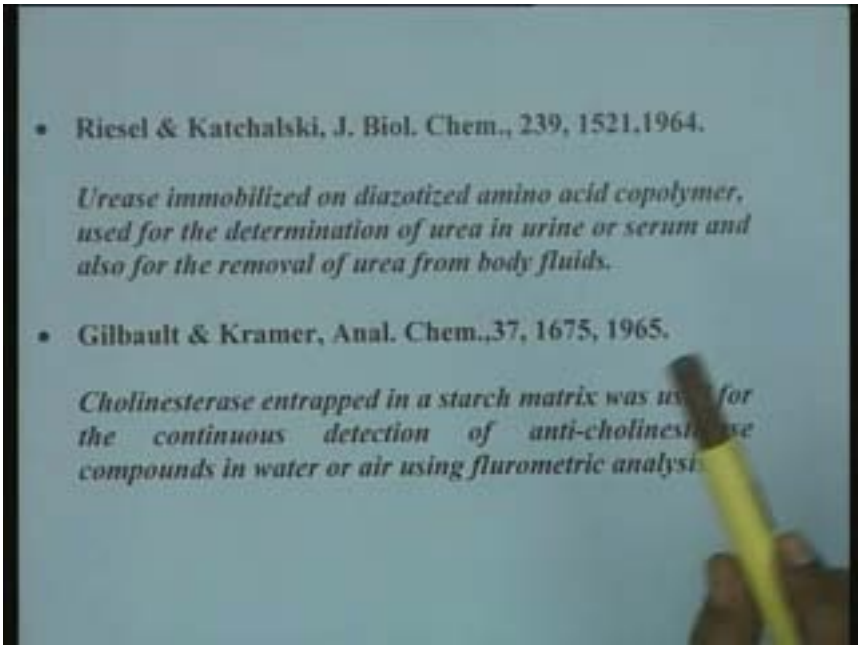

The other report is by Gilbault and Kramer in 65. They did for cholinesterase which was entrapped in a starch matrix and it was used for continuous detection of anti-cholinesterase. This is an example not really of a metabolite or some kind of a substrate or the product but it was just like an environmental analysis where the presence of certain cholinesterase inhibitors present in either effluents or nearby as a result of pollution were monitored continuously using an immobilized cholinesterase entrapped in a starch matrix plus a fluorescence spectrophotometer because the product ultimately gives a fluorescence and the change in fluorescence as a result of inhibitor present. That means florescence decreases and by that one can monitor continuously and the measurement of florescence gives you a direct indication of the concentration of inhibitor in the feed stream.

The second class of analytical applications is enzyme electrodes. Although it is listed as second it has become commercially a very, very important sector of analytical tool.

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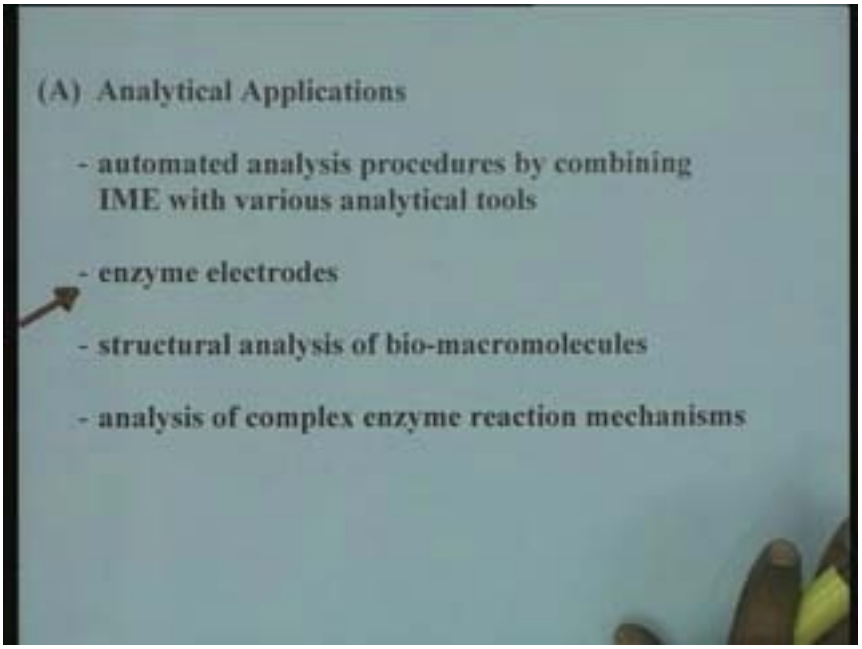

What is an enzyme electrode? In general we call it a biosensor, an analytical device. A very crude definition could include it as a hybrid device where a biological recognition molecule or a cell or cell component is integrated with a physical or chemical detector. We know the transducer to utilize the selective analysis of a biochemical species in a sample. So there are three distinct segments one is the biological unit which is integrated with a transducer and the third is anylate. These three systems, the biological unit, transducer and anylate have to have a linkage. That means if this anylate has to recognize this biological unit there must be an affinity between the two. Secondly the substrate or the product of the reaction which comes out as a result of this interaction must be able to generate a measurable parameter which can be measured by transducer in quantitative terms so that it can be related with either the substrate concentration or some species in the sample.

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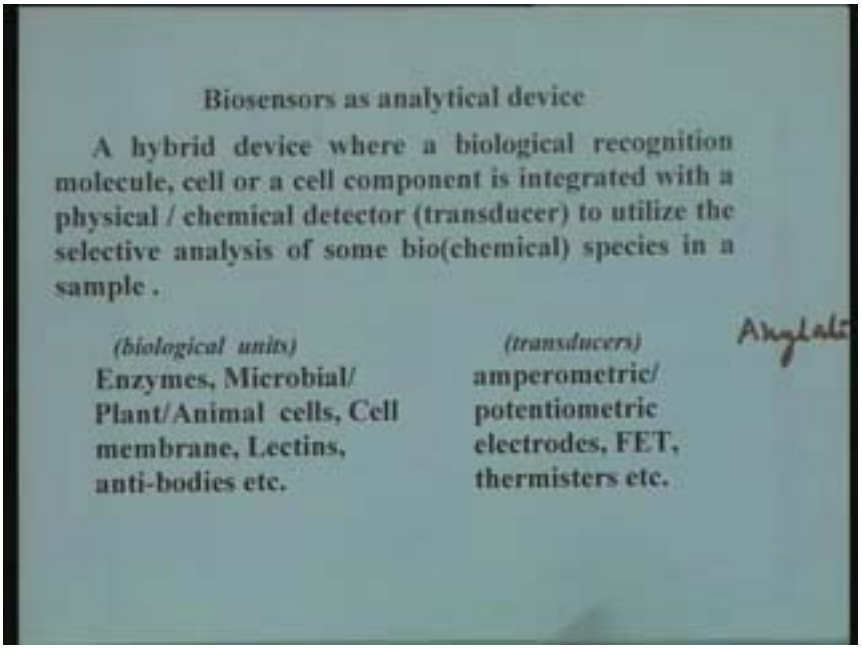

Anylate means that chemical species which is to be analyzed say for example you want to analyze sugar some glucose in blood; glucose is the anylate. The chemical species which needs to be analyzed is anylate.

In other words the concept is very simple and a large variety of biological units have been used to prepare biosensors. As far as we are concerned you will be stressing more on enzymes as biological units but other than enzyme you can also use microbial cells, plant cells, animal cells, cell membrane, Lectins or antibodies depending upon the anylate. The purpose is that the chemical species to be analyzed must have a specific recognition for the biological unit and the type of transducers that are very commonly used are amperometric or potentiometric electrodes and the recent year developments have been in the area of field effect transistors, thermisters. But still on commercial level amperometric and potentiometric transducers are the common tools that are used in bulk. Anylate is the chemical species which is to be monitored in the sample. Say for example if you want to monitor urea in urine samples; urea is the anylate, urine is a sample the fluid to be analyzed and the biological unit which is recognizing that analate is the material either an enzymes or a cell which has the some affinity for that molecule and you need a physical transducer which can sense either the substrate or the product or any other signal which is generated through the reaction. I will come to that later.

To illustrate schematically the same concept of a biosensor I have taken here a substrate molecule to be analyzed in any given system. This is immobilized enzyme. The substrate molecule recognizes the enzyme species present on the immobilized enzyme. There can be a membrane through which the product or any other species which comes out as a result of reaction passes through and approaches transducer, an analytical device which generates a signal that can be monitored. It can be in the form of a current; it can be in the form of potential difference; it can be the form of temperature change and variety of signals can be generated and that depends on the type of …… producing. The basic

concept is you have to choose an appropriate combination of biological and transducing elements that means choose a biological agents; we are emphasizing on immobilized enzymes which will recognize the anylate. Secondly convert the recognition process into a physico-chemical change which either directly or indirectly generates the current.

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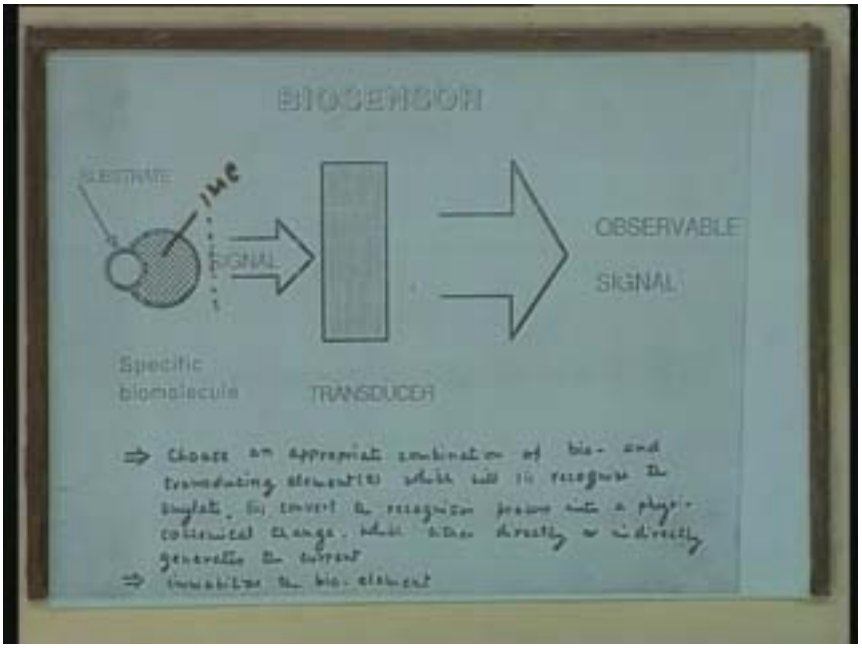

In the electrical circuit current is a very easily measurable parameter and just to make it suitable for repeated use we need to immobilize the bio element. Theoretically the system can be used in a soluble form also but just to make it a continuously usable system the bio element is immobilized.

What kinds of electrodes are used usually? Take for example pH electrode in a laboratory. pH electrode is a transducer, one of the transducers which can be used in the case of an enzyme electrode. What you simply need to do is at the tip of the electrode you need to insert by some mechanism an immobilized enzyme layer so that when you dip that electrode into a sample liquid, the sample comes in contact with immobilized enzyme layer usually in the form of thin film and the reaction takes place; the product goes through and it is sensed by the pH electrode. pH electrode is a potentiometric transducer. It generates the potential and that potential is measured and calibrated in the form of pH change and that can be used simply.

Similarly a number of ion selective electrodes which also function in the same principle of potentiometric transducer can be used as transducing element. In principle the enzyme is bound to ion selective electrode that will detect the presence of a substrate or product. That means if the substrate is ionisable it can be measured or it can measure the product of the enzyme catalyzed reaction.

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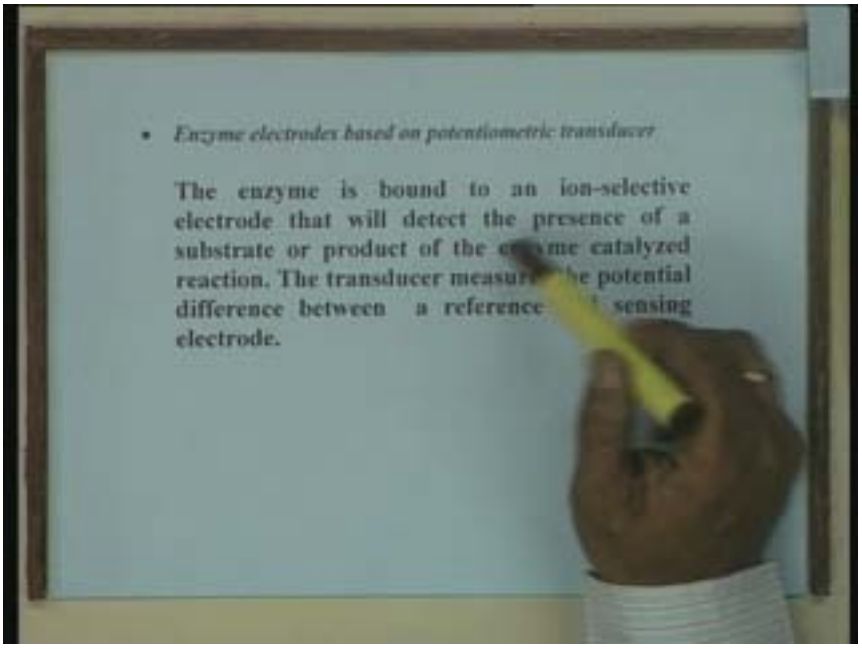

The transducer measures the potential difference between a reference and sensing electrode. We are not doing any manipulation with the transducer; we use the commercially available transducers. It could be a ….. electrode what you use in the fermentor for measurement of dissolved oxygen because that is amperometric transducer; it measures current. On the other hand a simple pH meter or an ammonium ion electrode or carbon dioxide electrode they are potentiometric transducers.

No that mechanism can be developed, but it should be in proximity with the electrode. That means the tip should be next to the sensing site of the electrode. I will illustrate how we do that but the basic principle is that it should be in the proximity of the sensing site of the transducer. One can manipulate and develop number of the strategies like an amperometric ….. type dissolved oxygen electrode what you use in the case of fermentation fluids for measurement of dissolved oxygen. They are based on platinum cathode and a silver anode which is immersed in saturated KCl which is separated from the test solution by a PTFE membrane. That means at the tip of the transducer you have a PTFE membrane so that the sample in which the analysis has to be done for dissolved oxygen is separated from KCl solution because that should not get mixed and a potential of about 0.5 to 0.8 volts, a constant value, is applied because this constant voltage applied will determine the calibration of the current which is generated as a result of dissolved oxygen when it comes in contact with the electrode.

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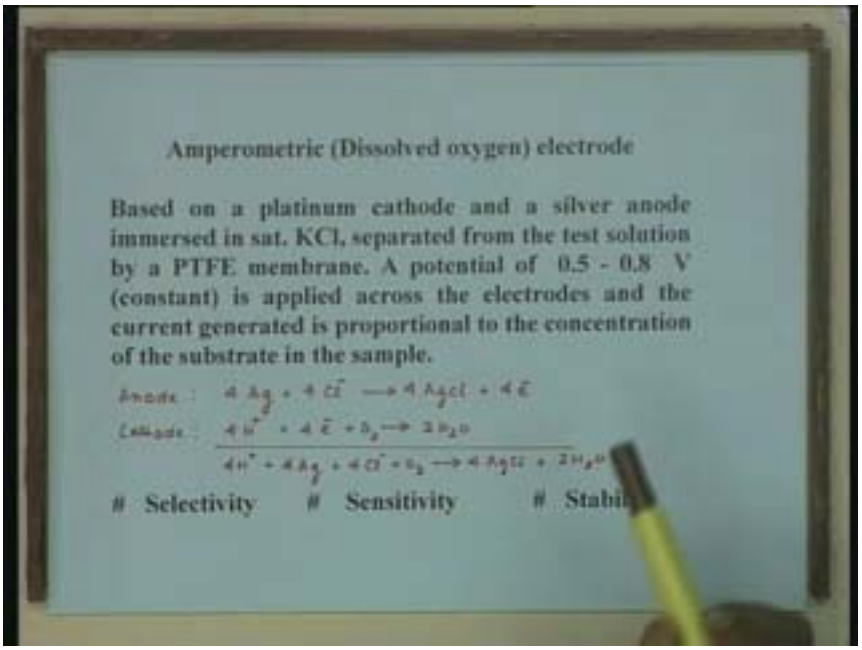

The potential of 0.5 to 0.8 volts is usually applied across the electrode and the current generated is proportional to the concentration of the substrate in the sample. The current generated is reflected by dissolved oxygen concentration because on the cathode it is the oxygen which takes up the protons generated on the anode. This dissolved oxygen is a function or is a parameter which is participating in the reaction. In enzymatic reactions usually there will be oxidoreductases, redox reactions if they consume oxygen or give out oxygen a difference in the dissolved oxygen can be used to measure the substrate.

At the electrode is measured dissolved oxygen but the consumption of dissolved oxygen in the fluid is a result of enzymatic reaction. For example consider glucose. Glucose plus oxygen will give you gluconic acid and in the sample when you bring the glucose sample in proximity of the electrode which has the glucose oxidase as enzyme deposited on it the enzyme will react with the glucose in the presence of oxygen and consume oxygen. There will be a fall in the oxygen concentration, dissolved oxygen concentration which will be monitored by the electrode and this fall in dissolved oxygen concentration under steady state will be proportional to the substrate concentration.

Just like in the earlier automated devices you can have small immobilized enzyme columns and link it to the photometer. That is possible but again to use it in terms of convenience use of pH electrode is very simple. You just dip in the solution and get the result, output whereas if you have to really set up an auto analyzer it will have a full fledged setup with all tubing and you will have a reaction period and all those things and ultimately you will end up in a very cumbersome system. Any analytical device in the form of an electrode, physical device which can be usually calibrated in terms of the concentration of the sample it is much easy to handle. It’s only convenient. You can have either way; also you can put your enzyme into a column and pass it through a spectrophotometer; that can be automated; no doubt about it.

The three parameters which are important in the case of an enzyme electrode are selectivity. The selectivity is a function which is offered to it by enzyme because the enzyme has to be chosen which is highly selective for and that choice is important because if you are not able to choose very selective enzyme the analysis may not be very specific. Then sensitivity; Sensitivity is a function of the K_m value of the enzyme that means the range of analysis that it can perform that will depend on the K_m value of the enzyme. The third and probably, from the operational point of view, very important is stability because we must be able to use the same immobilized enzyme coating for a number of samples so that it is stable over a long period of time plus reproducible. Usually immobilized enzyme is supplied in the form of sandwich between the membranes so that they can be replaced on the tip. This is a picture of a typical enzyme electrode which we used in our own laboratory. It is a dissolved oxygen electrode; simple one showing different components. You have a gold cathode, silver anode and Teflon membrane at the tip; that is the main electrode has a Teflon membrane on the tip.

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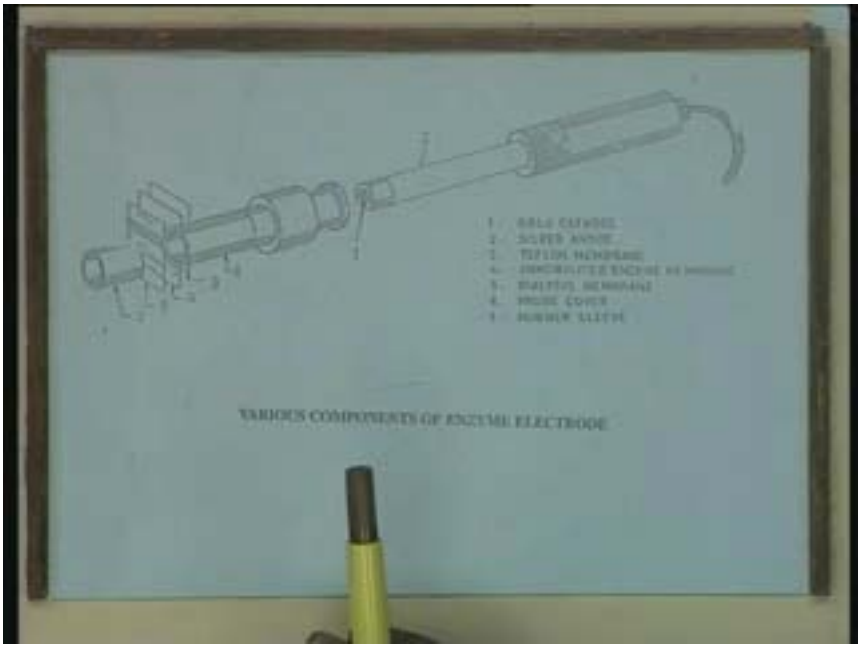

We put another dialysis membrane over this Teflon membrane and this separates the enzyme which is immobilized in the form of a thin film and particularly in this case study which I was referring to, the immobilized enzyme is cellulose acetate membrane. Just to provide a support it is inserted between dialysis tubes which is permeable to small molecular weight compound like glucose freely and which is put on this Teflon membrane which is already present in the dissolved oxygen electrode. The other system what you see is a sleeve. This sleeve is just to insert the enzyme membrane on to the electrode as a as a ……. so that you insert on the tip the enzyme membrane.

Here you see a bigger view. You can have for example glucose measurement. You have a silver anode and platinum cathode, a plastic membrane which is supposed to protect anode and cathode which separates the fluid from the internal fluid and after that we put an enzyme gel layer.

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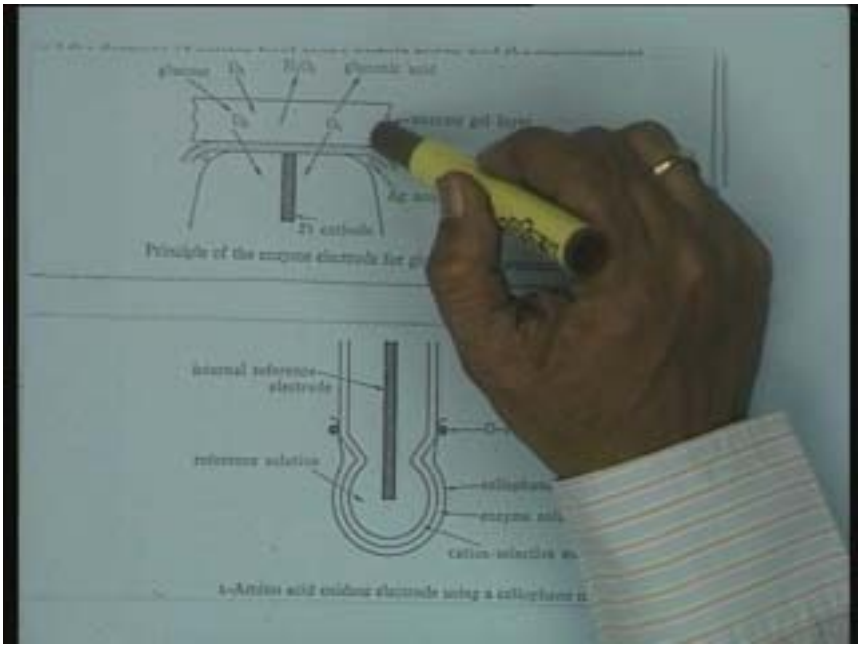

You can have some kind of sleeve which holds this layer. Like in our case we had reverse sleeve which is covered over the tip of the electrode so that the film remains intact on the electrode and it is separate because being inserted in the dialysis bed, it is separated from the PTFE membrane also and it also provides the analytical molecule to permeate through it and come in proximity with the enzyme.

No; enzyme is not leaving direct contact with the anode and cathode. Anode and cathode only come in picture with either the substrate or the product which is a result of the enzyme reaction like for example here you see the second case which is for L-amino acid oxidase you have a simple glass electrode just like pH electrode. They have put an enzyme solution in an ultra filtration membrane or cellophane membrane or you can say dialysis bed which is closed at the bottom and the electrode is dipped into it. That has been supported by 'O' rings just like we have put a sleeve in the earlier case. You put 'O' rings so that the dialysis bed which contains the enzyme is in proximity of the enzyme and it can be used for measurement systems.

We are indirectly measuring glucose via measurement of dissolved oxygen. The dissolved oxygen electrode will measure dissolved oxygen only; it cannot measure glucose directly. But the glucose concentration results in either production or consumption of oxygen as a result of the enzyme reaction and that is the purpose.

Large number of enzyme electrodes have been prepared and listed here are the ones which are commercially available. There are many other experimental electrodes which have been made and reported in the literature. For example glucose is the most commonly used electrode and the immobilization method polyacrylamide gel; oxygen electrode is electrode used and the concentration range it can measure is of the order of ten to the power of minus five to ten to the power of minus four molar. I like you to see the sensitivity of the measurement which is probably much higher than any physical or chemical measurement system at this concentration level. That is we are talking in the micro molar range.

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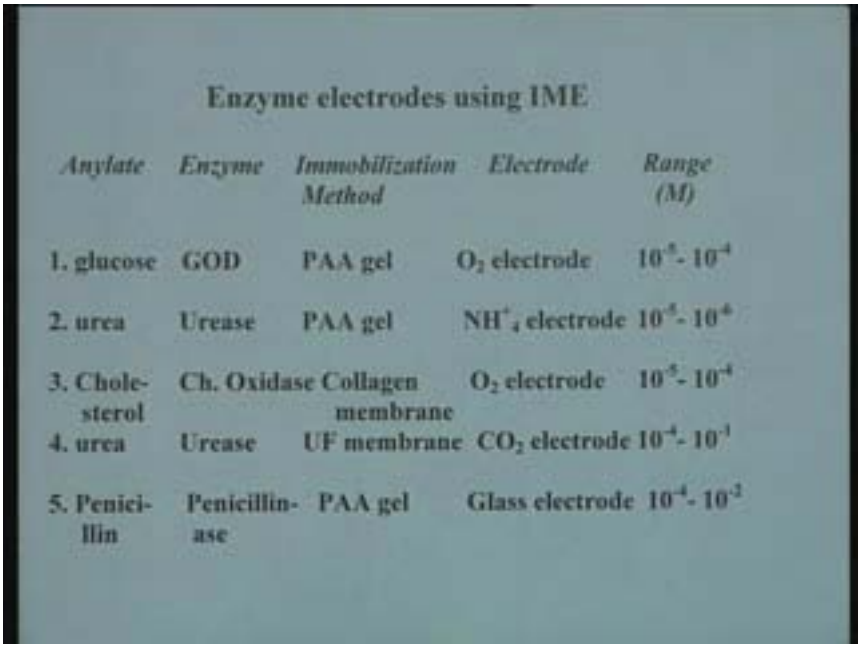

In the case of urea, urease is the enzyme, immobilization method is polyacrylamide gel and ammonium ion electrode is used, ion selective potentiometric electrode and the range is one to ten micro molar. In the case of cholesterol, cholesterol oxidase, collagen membrane and oxygen electrode is the measurement device. In all reactions which are redox reactions oxygen electrodes are used and if any other ion selective measurement has to be done then the potentiometric electrometer is used. For urea, urease is the enzyme, ultra filtration membrane CO two electrode, an ion selective electrode. Then penicillin measurement using an enzyme penicillinase which hydrolyzes penicillin using a glass electrode, this can also be measured.

The advantage is in an industry if they have to monitor the penicillin concentration of their bath on an online basis any chemical measurement will take time. The report may take two hour. Whereas if the plant operator has an electrode in his pocket and he takes a sample and dips into it, the control becomes very easy. He can immediately take a decision to either switch on or switch off or terminate; whatever decision has to be taken on the plan and in those cases such systems are very, very useful.

Another analytical device based on immobilized enzyme is enzyme thermisters. The enzyme thermisters are based on the measurement of temperature difference of the reaction liquid in an exothermic biochemical reaction. Mind it that we are talking of exothermic biochemical reaction.

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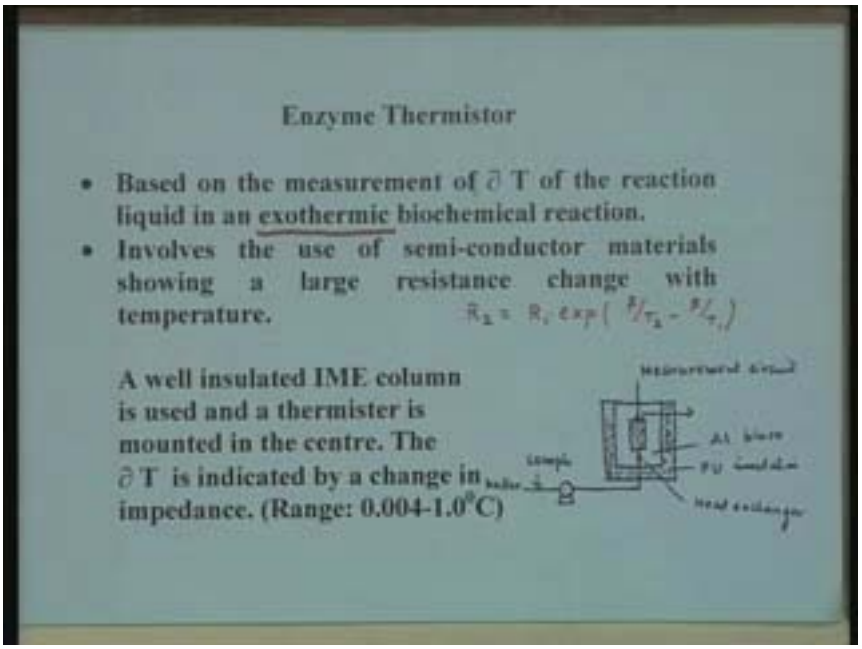

Many of the enzyme reactions which are highly isothermal, I am just comparing the two, although they are known to be in all broad range as isothermal reactions they will have some value of ΔH. So those which have slightly higher ΔH value among enzyme reactions they can be used for enzyme thermisters because they will induce a change in the temperature. Although temperature change as you will notice is much smaller. This involves the use of semi conductor material showing a large resistance change with temperature. So measurement of temperature is based on the change in resistance of a semiconductor material as a function of temperature. In semiconductors, the expression which relates the resistance with reference to temperature T one and T two, resistance changes and all those material and semiconductors are known to have a high change in resistance as a function of temperature and they are used here as the sensing device.

So thermister is nothing else but a semiconductor material which is connected to a circuit which measures impedance and if that is put at the tip of the effluent stream of the immobilized enzyme reaction you can use it for measurement of temperature. In practice a well insulated immobilized enzyme column is used. You take a very fine small column and insulate it. The most difficult part of the enzyme thermister is the very high order of insulation required because the temperature which we are measuring is probably a fraction of the integer; some times as low as 0.1° centigrade, one hundredth of a centigrade; that is the kind of temperature change we are talking about to ensure very

high order of insulation and the effectiveness of this system depends on the quality of insulation one can provide.

The thermister is mounted at the extreme end of the fluid. For example this is the material column and you feed the sample through a pump and you have a small heat exchanger which maintains in a very controlled range the inlet temperature and the outlet temperature is monitored by a thermister which is installed at the outlet end and this is connected to measurement circuit and measures the impedance. The ΔT is indicated by a change in impedance and the range of temperature measurement for different enzymes lies in 0.004 to 1º degree centigrade.

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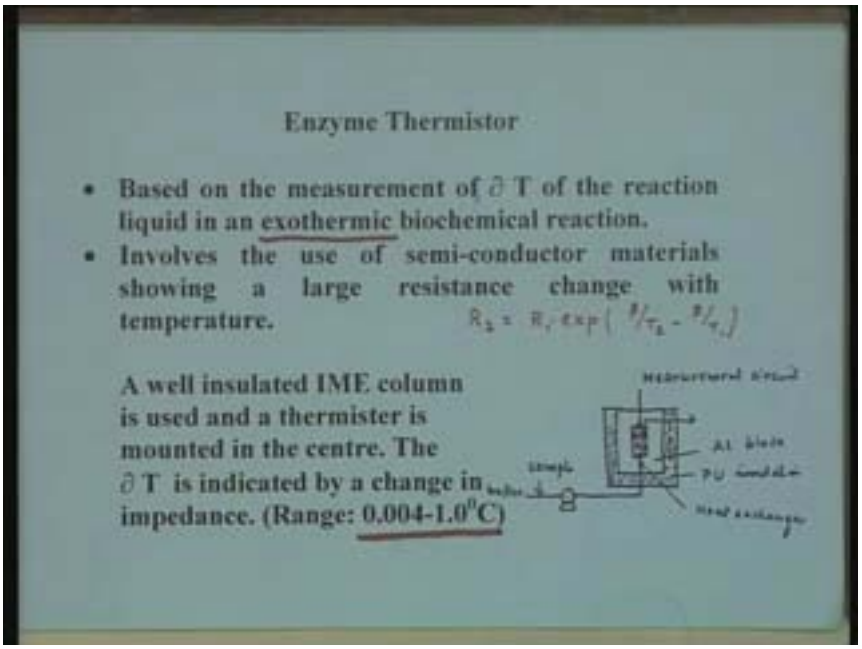

Higher this change is the system can be more effective usually it has been noted that only those enzymes which can generate a temperature change more than 0.1º centigrade are effective. 0.004 is a …… size but you need some reliably measurable amount of temperature change.

The suitability of enzyme thermister depends on the enthalpy changes associated with the biochemical reaction. More exothermic the reaction we are handling, the system can be more effective.

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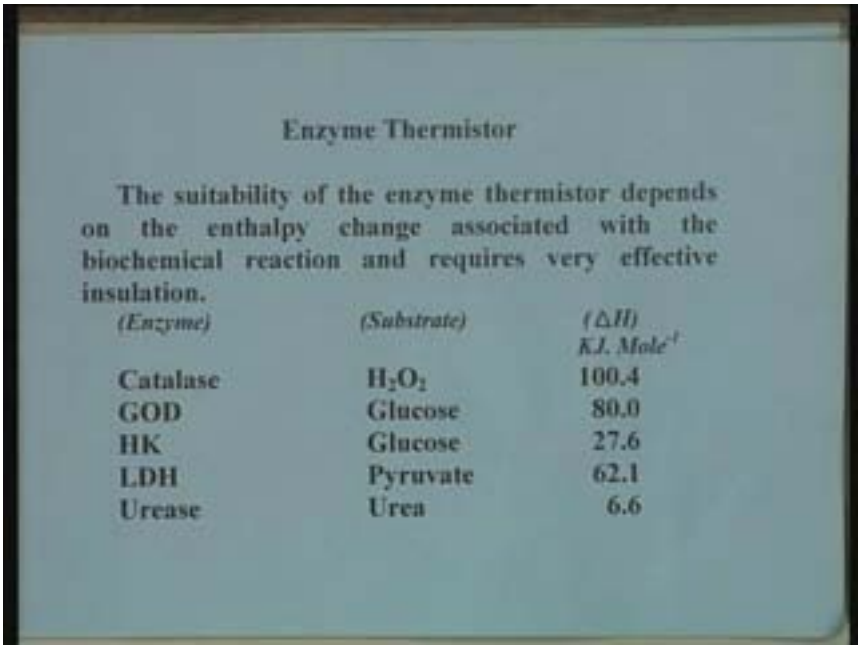

If you look into some values of ΔH in the case of catalase, it is 100.4 kilo joules per mole. This is probably one of the highest ΔH value in the case of enzymatic reactions and this gives almost about a temperature change of 0.2 to 0.3 degree centigrade which can be very easily monitored. Glucose oxidase 80 kilo joules per mole; hexokinase 27.6 kilo joules per mole; lactate dehydroginase another good system 62.1 and urease with the urea only 6.6. As a matter of fact the urease, this system is usually not very effective and the first four ones are considered to be very effective and the system is not commercialized but in many laboratories they have been tested, used and reported. The same anylate whatever substrate concentration, the change of temperature which is measured in terms of change in resistance is related to the change of substrate concentration. This is per mole; per mole of substrate consumed means so much of …….. Just like any chemical reaction you have a ΔH value.

Then the third area of application of enzyme, analytical application I am referring to, is the structural analysis of bio macromolecules.

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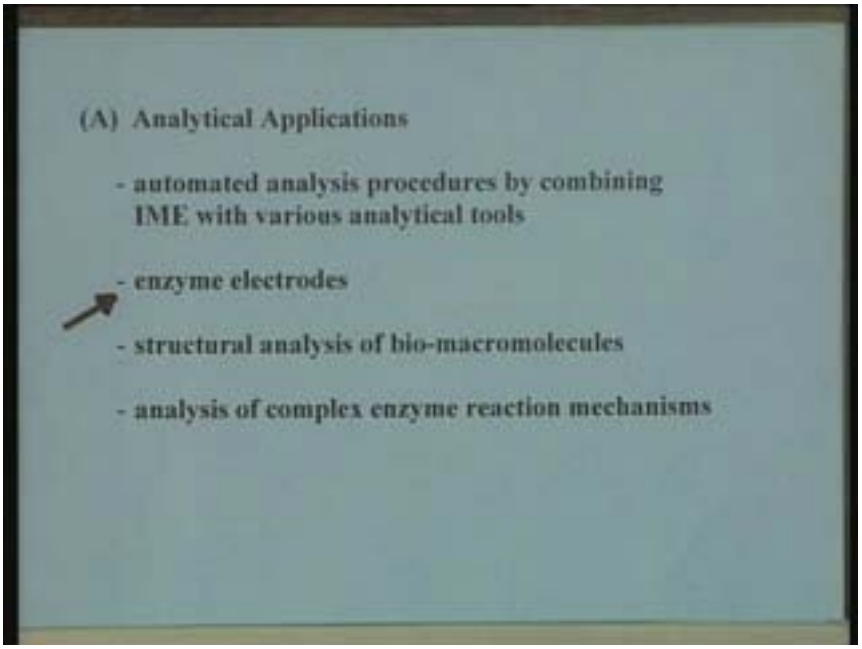

You must be familiar with the primary structural determination of proteins. Usually we take a protein, hydrolyze it by using some enzymes or chemical means by acid or alkali and monitor the different amino acids that are produced. The same job can be done by immobilized enzyme in a more controlled way and you can have controlled hydrolysis. The …… advantage, the kind of problem that is faced in the case of classical analysis is the removal of the hydrolyzing agent that is acid or alkali or even the soluble enzyme.

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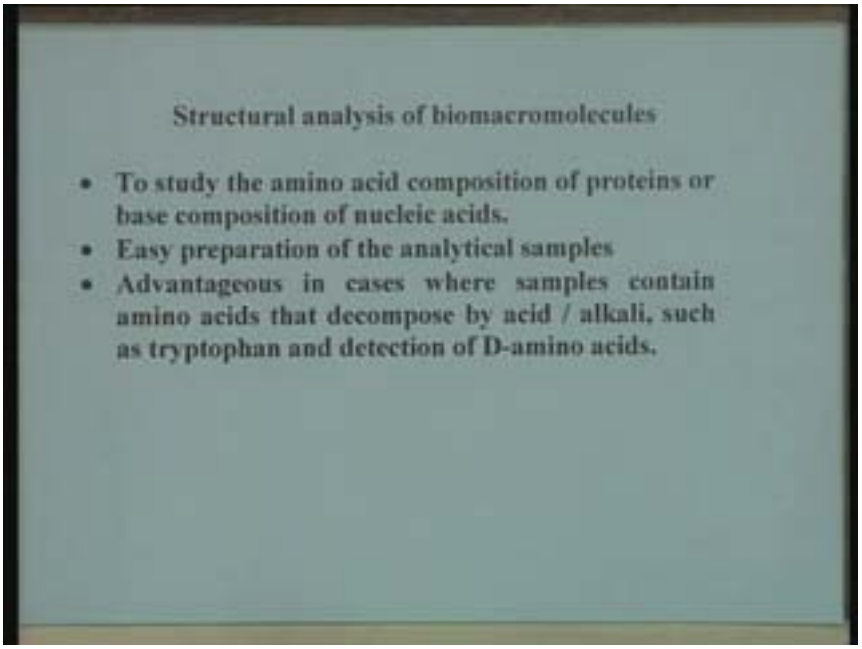

Removal from the sample is very tedious task and also causes in some cases for example tryptophan, it is destroyed by alkali. So alkaline hydrolysis, which is very commonly used for determination of primary structure does not measure tryptophan. But if you use a column of immobilized enzyme for a controlled hydrolysis of a protein sample, first thing no amino acid will be destroyed. They will also liberate the D-amino acids if there are, which are very uncommon occurrences in proteins can also be analyzed. The basic concept is that we can analyze amino acid composition of proteins by using immobilized proteases or base composition of nucleic acids if we use immobilized phosphatases. If you take the phosphoesterases and immobilize them, then you can have the base composition because the hydrolysis will yield and then you can have a controlled hydrolysis and see the sequence.

The principle remains the same as in the case of soluble enzyme but the preparation of the analytical samples becomes very easy because then you don't have to separate the hydrolyzing agents, the catalyst. You can simply either filter it off or centrifuge and your sample is ready for analysis. A major advantage is in cases where sample contains amino acids that decompose by acid or alkali such as tryptophan and detection of D- amino acids. In fact in many cases in the chemical analysis detection of D-amino acid is difficult because it gets destroyed.

The other analytical application sector was as a therapeutic agent and with development in the availability of different enzymes and particularly the understanding of molecular basis of deceases the use of enzymes as therapeutic agents is by and large increasing. The conventional use of enzymes in therapeutic has been the use of proteases. For variety of situations that range from digestion aid to even treatment of certain deceases which involve the availability of one of the enzyme or removal of some metabolite from the body. A very large range of application sectors in the therapy have been known with soluble enzymes. But the use of soluble enzymes has certain very serious problems and the problems are listed here: one is that the enzyme has a higher molecular mass, a very large molecule protein and impermeability through biological membranes. In fact the efficacy of any drug or a therapeutic agent depends on its permeability through biological membranes and even the efficacy of a chemical drug will depend on it's ability to permeate through the biological membranes and reach to the site. So the larger the molecule as the enzymes are it posses serious problem in the permeability and the most toughest biological membrane through which the drug has to permeate is brain membranes. As a matter of fact almost all drugs which are required to be given for action at brain as the site the concentrations are very high almost 4 to eight times more that of the same drug if it is used at some other site in the body. Say for example steroids. If they have to be administered for some inflammation at some site leg or hand, you can give a very small dose; it will reach the site and treat the patient. But if the inflammation is in the brain at least ten fold concentration of the drug is required to be administered because lesser than that will not even permeate to the brain membrane. So giving it will be useless it go to all over the body excepting brain. If you give higher dose you end up in another problem of side effects because there is no drug which is free of side effects. You are taking it to cure the decease but slowly you are also getting exposed to certain side effects.

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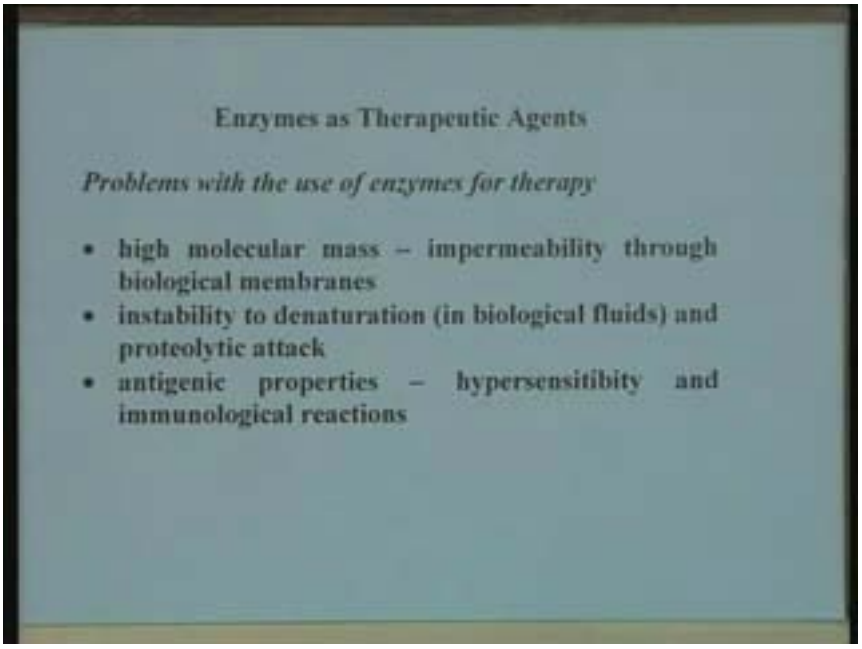

So therefore the high molecular mass which results into impermeability through the biological membranes is a major problem. Then another problem is instability to denaturation in biological fluids and proteolytic attack. Ultimately the drug has to reach into the biological fluids, save blood or let's say it has to reach to stomach. The fluids have very strong conditions for example the fluid in the stomach will have a very low pH. There might be certain inhibitors present for the enzyme which might reduce the rate of reaction or there might be some proteolytic enzymes present and they can inactivate the enzyme protein. Any therapeutic device must take care of these problems if it has to be effective. That means one should ensure what is the site of action required? what is the biological view there? What is the chemical view there? What are the inhibitors present? What is the pH? What is the temperature and then only the enzyme function can be ensured.

Third is antigenic properties hypersensitibity and immunological reactions. Enzyme being a protein or rather a foreign protein to the body, the body is equipped with antibodies to generate immunological reactions and they can also be problematic and infact the administration of any enzyme for therapeutic application must overcome these problems. Number of approaches has been used to overcome this problem and some enzymes had become easily admissible. I like to illustrate only one example which has become quite interesting say for example in the case of patients who suffer from partial kidney failure. That means kidney is probably one of the excellent examples of a filtration system, highly specific filtration system in the body. I don't think any other filtration device is available which is so specific and lasting. Still in some cases the partial failure takes place and the kidney is not able to filter out or remove urea. So the urea concentration in the blood stream increases and it can cause many metabolic problems and it is ultimately fatal.

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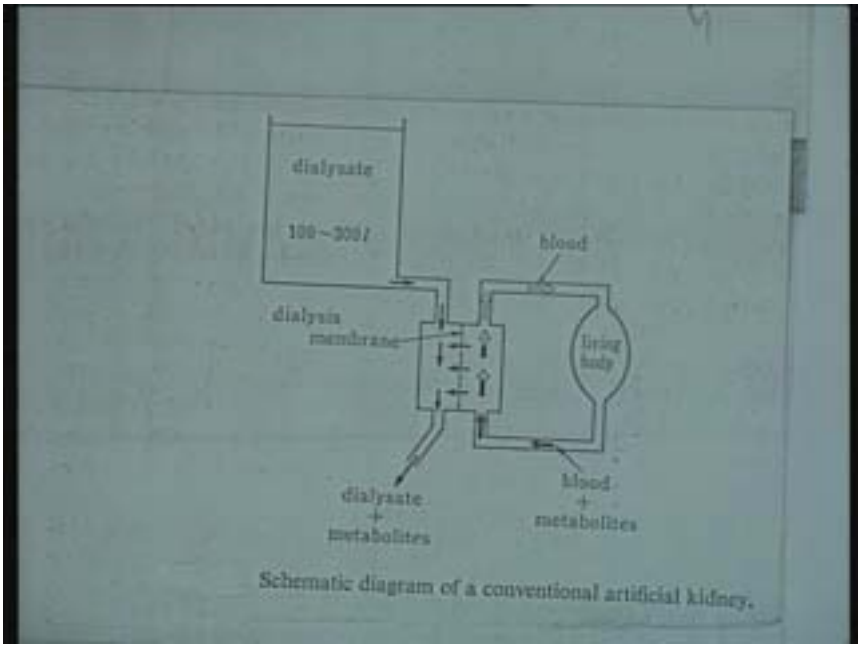

Schematic diagram of a conventional artificial kidney.

There are other chemical treatments but the last resort is to put on a dialysis system. That means dialysis is something like an artificial organ; it's like an artificial kidney that you take out the blood and put it in an extra …….. . This is the living body and the blood passes through the dialysis membrane, a dialysis device in which the blood is dialyzed against a dialysate, usually a buffer. The quantity of buffer that has to be administered or that has to be passed in the conventional dialysis unit is 100 to 300 lts. This is the range. In cases where the concentration of urea is much larger in the blood stream you may have to require more fluid to pass and because of this large volume required for dialysis usually a specific patient has to be hospitalized and the whole setup of a hospital has to be used for dialysis. The dialysate removes the metabolites, urea in this case and the urea level in the blood is brought down, then the conventional way.

This system which has been followed in the past has lot of problems. One is that the patient after sometime fails to respond to dialysis, if the concentration goes very high the volume of the dialysate required is very large. The frequency required is very high that means initially he starts a dialysis once in a month and he may end up in daily dialysis. Putting one daily for dialysis in hospital is also very difficult task because ultimately what you are doing is you are circulating the whole of his body blood through a dialysis system; it's a very tedious system if we look at and you are just putting a secondary circulation system of blood which already exist in the body. The alternate to that is use of immobilized urease and this is an example of an artificial organ which is commercialized which is available commercially and with such a system a patient doesn't have to go hospital because the volume of dialysate they require is only about a liter which he can safely install at the site of his bed in the house and use himself.

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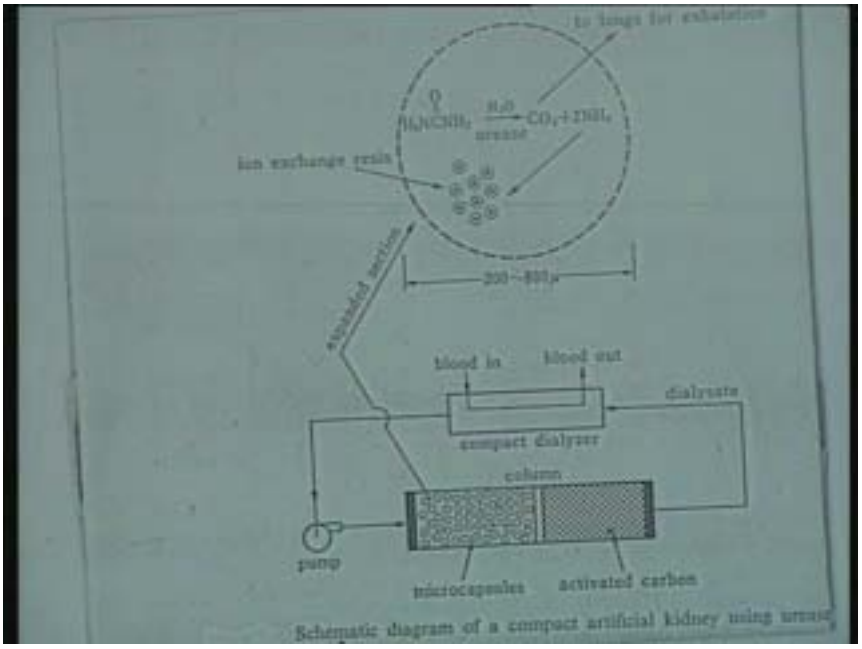

Schematic diagram of a compact artificial kidney using urease

The principle remains the same. You take blood in; the dialyser unit is little compact same dialysis bag only thing is much smaller because as soon as the urea comes in the dialysate instead of being thrown out it is passed through an immobilized enzyme column, the one which is shown here as microcapsule in which ion exchange resin and the enzyme have been immobilized in microcapsules. So the urea which comes out in the dialysate is hydrolyzed to ammonia; ammonia is absorbed by the ion exchange resin. Carbon dioxide is not allowed its not bothered about because carbon dioxide even if it reaches in the blood the body has a capacity to exit out to lungs assuming that the lungs are functioning properly of the patient. So carbon dioxide can be left out; but ammonia cannot be sent back to the body and ammonia is removed by ion exchange resins and other metabolite which are generated if any in the blood sample they are taken care by activated carbon and the body fluid of the blood through a system is then sent back into the patient valve. The advantage here is from the practical point of view instead of dialysate volume using about of the order of three hundred liters we are talking of one or two liters of dialysate and because you are regenerating the dialysate by removing the urea formed in the system and that kind of system is commercially available.

In the box, in an expanded view of the column in which the microcapsule contains the enzyme and the ion exchange resin the reaction takes place; urea gets converted into carbon dioxide and ammonia. Carbon dioxide is led off in the blood stream for the body to take care and the ammonia is taken care by the ion exchange beads and the size approximately of these microcapsules is the order of two hundred to eight hundred microns; very fine microcapsules almost like a tiny set and they are packed and this system is obtainable.

Besides the use of immobilized enzyme as industrial catalyst, the immobilized enzymes has a major role to play even for analytical devices and for therapeutic purposes. I must stress again that at least these two applications have lesser implication as for the economic systems. I think the issue of economics is very, very important in their use in the process industries. For therapeutic purposes economics is secondary because primary is the …… system, the safety of the system. Analytical systems also because the quantity of enzyme required are so low that probably the economics rather is always in favor because you save tremendously in the cost of labour. As I mentioned to you that I have seen myself the analysis in atleast one of the hospitals in Sweden almost about thousand samples in a day done by an auto analyzer using immobilized enzyme and which would have required at least ten persons and you are aware that the hiring of the person is the last thing, at least in the western world. They will try to replace any person with a machine; that is cheaper and the cost of analysis works out to be very cheap if you compare with hiring a person and also more reliable. The human error is by and large avoided. So I will stop here.