

**Bio-electrochemistry**  
**Prof. Mainak Das**  
**Department of Biological Sciences and Bioengineering and Design Programme**  
**Indian Institute of Technology, Kanpur**

**Lecture – 18**  
**pH Measurement Ion Selective Electrode**

Welcome back to the lecture series in bio electrochemistry. In the last class, I highlighted the different kind of analytes which could be evaluated or which could be measured in the biological platform especially in a critically care unit.

(Refer Slide Time: 00:37)

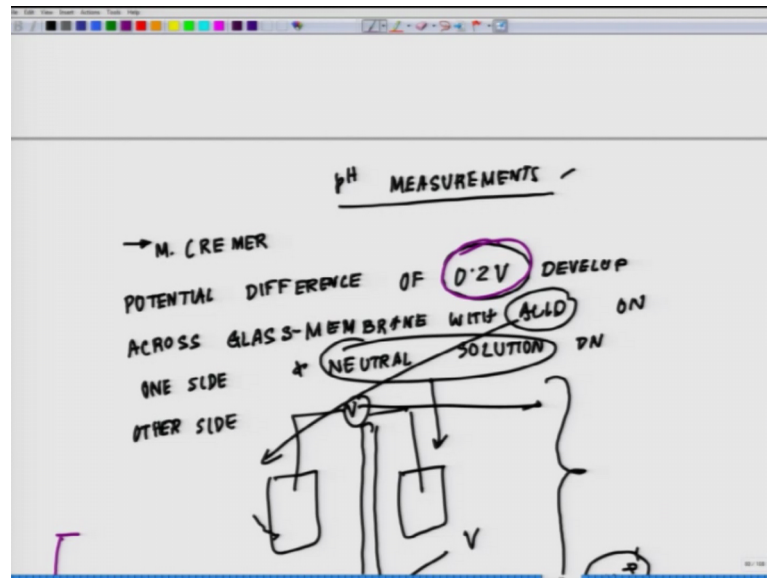
FUNCTION	ANALYTE
CONDUCTION	$\text{Na}^+$ , $\text{Ca}^{2+}$ , $\text{K}^+$
CONTRACTION	$\text{Ca}^{2+}$ , $\text{Mg}^{2+}$
ENERGY LEVEL	GLUCOSE, $\text{PO}_2$ , LACTATE, HEMATO CRIT
ACID-BASE	$\text{pH}$ , $\text{PCO}_2$ , $\text{HCO}_3^-$
OSMOLARITY	$\text{Na}^+$ , GLUCOSE
ELECTROLYTE BALANCE	$\text{Na}^+$ , $\text{K}^+$ , $\text{Ca}^{2+}$ , $\text{Mg}^{2+}$
RENAL FAILURE	BLOOD, URFB, $\text{N}_2$ , CREATININX

And we talked about analytes like. Sodium, calcium, potassium, magnesium, oxygen, p H, glucose.

So, today, we will start off with the p H sensor, which is a ion selective system. And it is very interesting, if you look at the historically and here, what I will do? In the last class we talked about the metal electrode and today with the ion selective electrodes. So, what I am going to highlight is, the basic concept.

I am not going to go into the in-depth detail of it, but the basic concept which I believe will help you. So, talking about the p H.

(Refer Slide Time: 01:09)



What really pH measurement using an electrode is all about. All of you use pH right measurements in your lab.

So, we know that, pH is essentially hydrogen ion concentration  $H^+$ . So, we have a neutral pH which is at 7. As  $H^+$  ion concentration proton concentration increases, it is called as acidic. So, you go to 6 5 4 3 2 1 0 and if it is an alkaline, it goes to 8 9 10 11 12 13 14 ok.

So,  $OH^-$  ion concentration increases. But, where was the genesis? Where it all started? It all started in Germany, in the year 1902, around 1902 or 1906. What was observed?

So, here is the observation which is the most fundamental thing. So, around 1906,. There was a student, Cremer at instead of physiology at Munich, who discovered in 1906. So, that came from Cremer (Refer Time: 02:19); Cremer who attains to have physiology in Munich discovered that.

A potential difference. So, this is the important part a potential difference. Potential difference of 0.2 volt developed across glass, across glass membrane, develop across glass membrane with acid on one side and neutral solution on other side.

So, the student working with Fritz Haber, in calt shop of 1908, improved the glass electrode and carried out the first acid base titration to be monitored with a glass electrode.

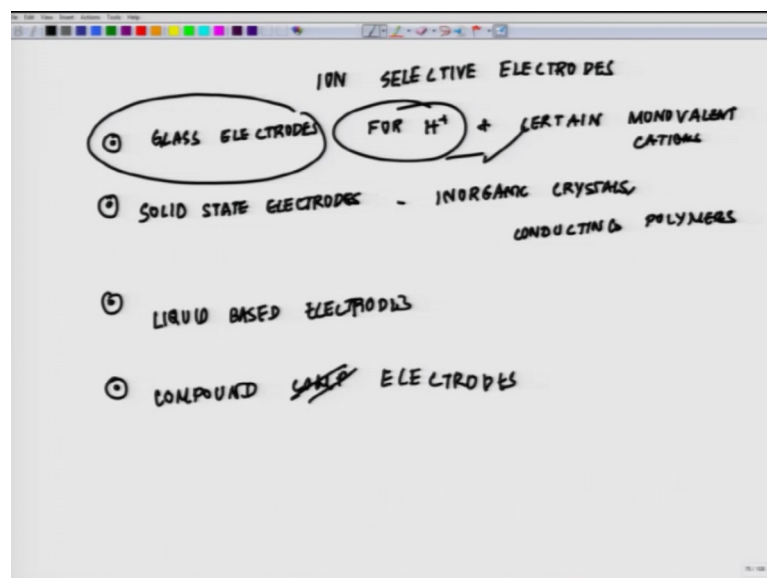
So, essentially, what you are observing is that, suppose, there is a glass membrane like this, ok? And on one side, you have acid on one side you have a neutral solution on the other side.

Now, across it, you are seeing a; you read a voltage. So, if you connect it like this, you will be reading a voltage. Now, if you know that voltage and if you know the molarity and molarity of whatever concentration of this. So, if you change the acid concentration, then you will see a change in the voltage.

So, you can really use this system to say, if this is the strength of the acid this is the value of the voltage. If this is the strength of the acid or vice versa. If this is the strength of the neutral solution, then this is what I am going to see.

So, this is the catch, which led to what we call as the glass p H electrode. So, if you remember, when I showed you the classification of the different kind of electrode, I talked to you about glass electrodes for H plus ion concentration.

(Refer Slide Time: 04:55)

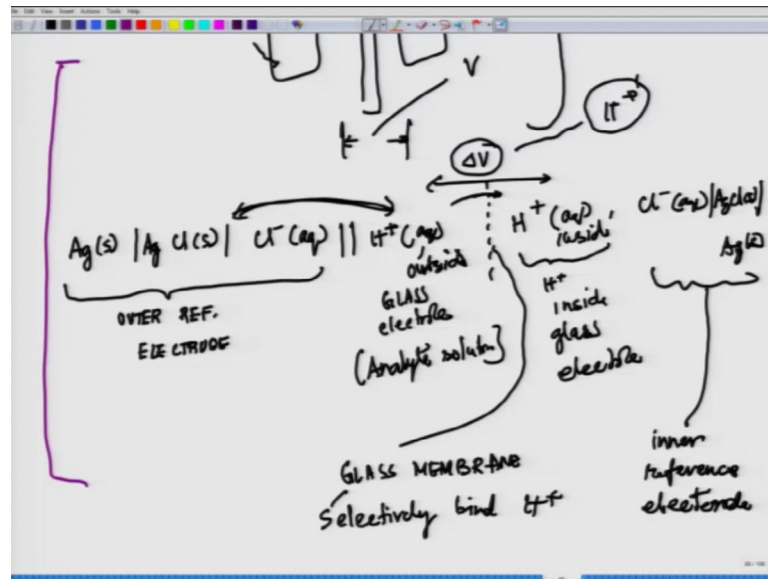


So, this is that glass electrode, what I am trying to discuss here, is out here.

So, essentially, a glass electrode used to measure p H is the most common ion selective electrode. A typical p H combination electrode incorporating both glass and reference electrode whose line diagram is something like this. Now, put the line diagram on it.

And the line diagram of this cell can be written as.

(Refer Slide Time: 05:27)



So, you are using Ag Ag Cl as the reference electrode solid slash Ag Cl solid slash Cl aqueous. This is your outer reference electrode. Outer reference electrode. Now, with H plus aqueous outside.

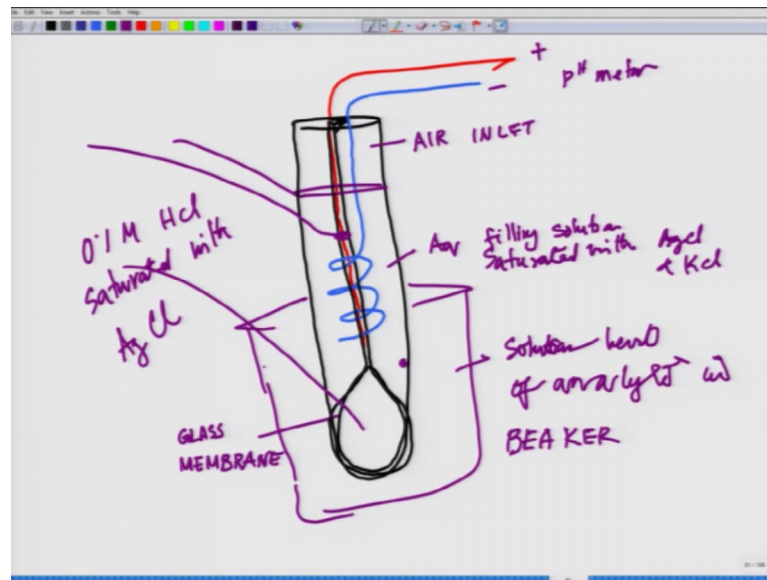
Now, you have the glass membrane selectively bind bound to H plus outside glass electrode. This is your inish essentially this is your analyte solution. This is what you are going to measure, ok; with respect to this. Now here, you have the glass membrane. And this glass membrane has a property to selectively bind H plus ions.

Now, with respect to this, you have H plus aqueous inside. Once I will draw the picture, it will kind of make sense with Cl minus aqueous. So, this is what we have as H plus inside glass electrode, H plus inside glass electrode. And then, you have the inner reference which is Cl Ag Cl solid Ag solid.

So, this is your inner reference electrode, inner reference electrode. So, you have 2 reference electrode; inner reference electrode and the outer reference electrode.

With these 2-inner reference electrode, whatever shift which is happening, which selectively binds. So, there is a there is a change which you are seeing here as  $\Delta V$  and that  $\Delta V$  is calibrated with H plus ion concentration. Based on that, we measure all our p H. And if you look at the picture of this thing, try to draw it for you.

(Refer Slide Time: 08:26)



It looks something like this, with another inner chamber going through and here, you have a wire which is coming like this.

Another and this wire is going all the way down, you have another wire coming like from outside, this is ok. Now, this is the glass membrane is here. This is the glass membrane. This is the positive lead. This is the negative lead leads to the p H meter. And here is the air inlet. This is the aqueous filling solution saturated with Ag Cl and K Cl.

Here, you have beakers sitting there, which is the solution level of analyte in beaker level, analyte in beaker. And there are porous plugs, which are a sight kind of a problem. And out here, you have 0.1 molar of HCl saturated with Ag Cl.

And, the liquid level of the outer reference electrode is somewhere out here. And liquid level of the inner reference electrode is somewhere out here. These are the different liquid level and Ag Cl paste suspended between the 2 sides of the phone

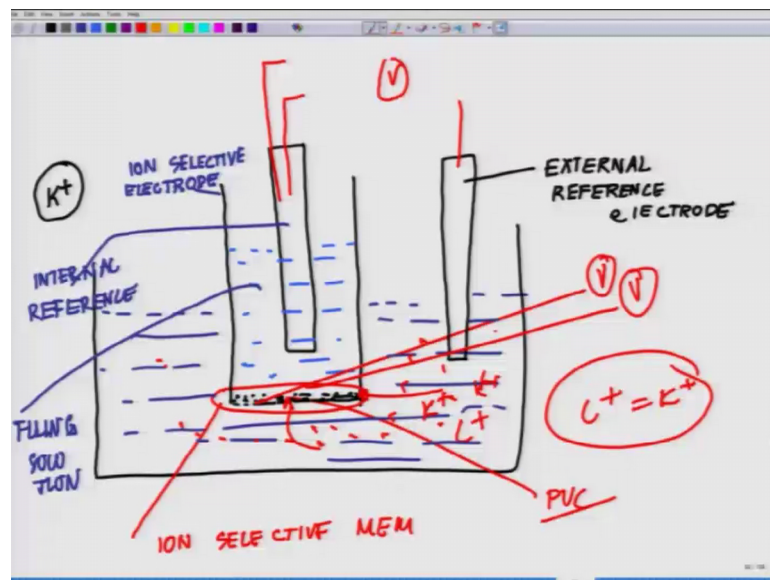
So, essentially, what you see, it is a; it is kind of complex geometry. But as long as you understand this basic reactions, it should not be a problem for you to understand how the

basic pH electrode works. So, these are the kind of glass electrodes which are used over the years for measuring the pH figuring out the proton concentration.

And this all starts with a very simple observation with dates back to 1906 this value; potential difference of 0.2 volt develop across glass membrane with acid on one side and neutral solution on the other side.

Now, from here, I will move on to another ion selective electrode today, which is a slightly complex one though. So, I will draw the electrode first this time. Before I, so, the ion selective electrode out here something like this.

(Refer Slide Time: 12:14)



Here, we have, ok. This is an ion selective electrode, which is for potassium ions, ok. Selectively binding of potassium ions which is one of the analytes. And out here, you have a reference electrode. So, this is your external reference electrode and this is outside.

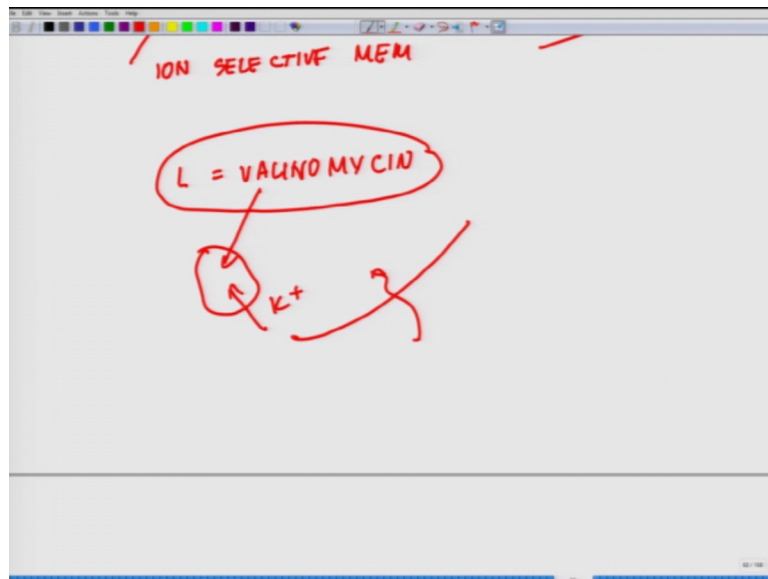
Now, that is external reference electrode. This is ion selective electrode, ion selective electrode. You have internal reference which is out here. So, you realize there are different kind of references you have to put internal reference out there and your; this is the filling solution inside and outside what you have is, where you have the analyte present.

So, this is where your analyte will change its concentration and this part, which is the most critical part, this is your ion selective membrane. Ion selective membrane. These ion selective membranes, are immersed in aqueous solution containing analyte cations, which is say, we say C plus. In this case, C plus is equal to K plus, what we are trying to measure.

Typically, the membrane is made up of polyvinyl chloride. So, this is P V C, polyvinyl chloride. It is impregnated with plasticizer, which is diacetyl sebacic, a non-polar liquid that softens the membrane and dissolves the ion selective ionophores.

So, what is the ion selective ionophore here which are used? The ions for potassium measurement, there is something called valinomycin.

(Refer Slide Time: 15:11)



Valinomycin is an ion selective material, which is an ion selective ionophore and ion selective. This valinomycin is essentially, is the ligand which is soluble inside the membrane and selectively binds the analyte ion in a potassium ion selective electrode.

For example, we could use valinomycin. It is a natural antibiotic secreted by certain microorganisms and valinomycin what it does is, valinomycin binds to potassium ions. If this is the valinomycin ion, it binds to the potassium ions and carries it inside the cell membrane.

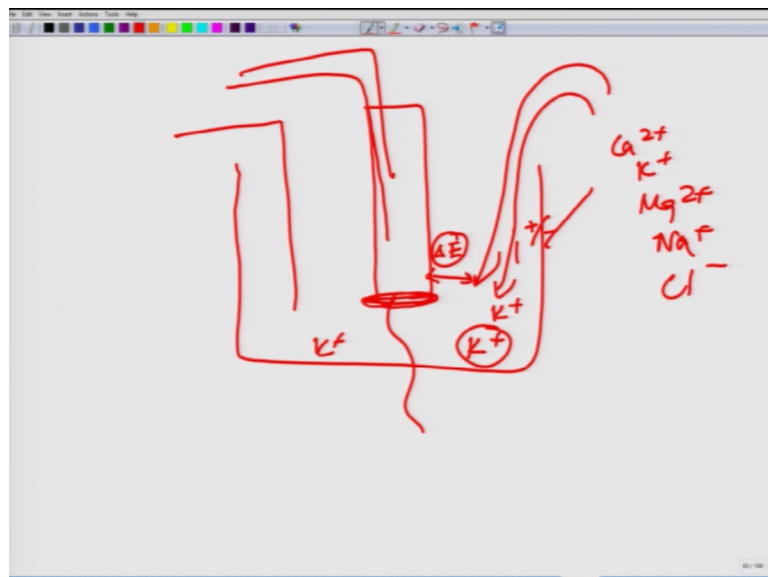
The ligand which has to be chosen has to have a very higher binding to the x y z analyte. So, what you are absorbing here is, say for example, I know the binding. So, I have external reference here. I have a iron selective electrode here and I have an internal reference which is present there.

So, with respect to the internal and external, I have a potential which could be measured. Now, if I know out here, I have lot of valinomycin which are sitting there as analyte.

Now, here, if I change the potassium ion concentration, so, automatically the binding of potassium ion concentration of the valinomycin is going to shift. Once that shift, the potential across this membrane is going to shift because, there is a mobility of the ions which is happening. So, if you remember, I told you there is no redox reaction as per say is taking place here.

There is absolutely no redox reaction, but this is happening in a slightly different way. So, as long as you have something, so, if you think of it logically, so, what is happening is that, here you have a beaker where, you place your analyte.

(Refer Slide Time: 17:09)



Here, you have a reference electrode, along with the; your indicated electrode with respect to it is reference.

You have a external reference and you have an internal reference. Your multiple references there. Now, you have a membrane out here. So, across this across the



electrode and outside there is a potential which is maintained,  $\Delta E$ . Now, that  $\Delta E$  is a function of the ion concentration outside. It could be I ok.

I/ could be plus or negative ok. It could be calcium, it could be potassium, it could be magnesium, it could be sodium, it could be chloride, what so ever you wanted to. With respect to outside, there is a potential difference. But now, if you add more of that ion, then the potential drop is going to shift and if the potential drop shifts, if you already have a standard curve, you know, but for this much ions.

So, for that what you need on this membrane, a selective binder to a particular ion and that is the key. So, in this case I gave you an example of valinomycin, which is produced. It is a kind of a cyclic peptide which is there, which binds to the potassium and carries ferries it inside the cell.

So, if you coat that membrane with valinomycin, what it will do? It will pull out the potassium ion and it will take it inside and it will maintain an equilibrium. Now, if you add more, it will ferry more. If it ferries more, so automatically across this, there will be a concentration difference.

Now, if you remember the concentration cell and all what I have taught, based on that, you can always calculate; what is the concentration with the analyte. So, put the logic. I can give you example after examples. But that is not important. Important is that, you have to put the logics in place what essentially is happening in this situation. So, this is the classic case of ion selective electrodes, ok.

From here, we will move on to, so, as of now, if you realize, we are only talking about the change in the potential. But there are situations, where you can utilize this technique by measuring the current and that we will see once we talk about the oxygen and the glucose electrode. So, I will closing here. We will catch up in the next class.

Thank you.