Environmental Quality: Monitoring and Analysis
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Lecture – 13
Environmental Analysis: Quality Control – Part 1

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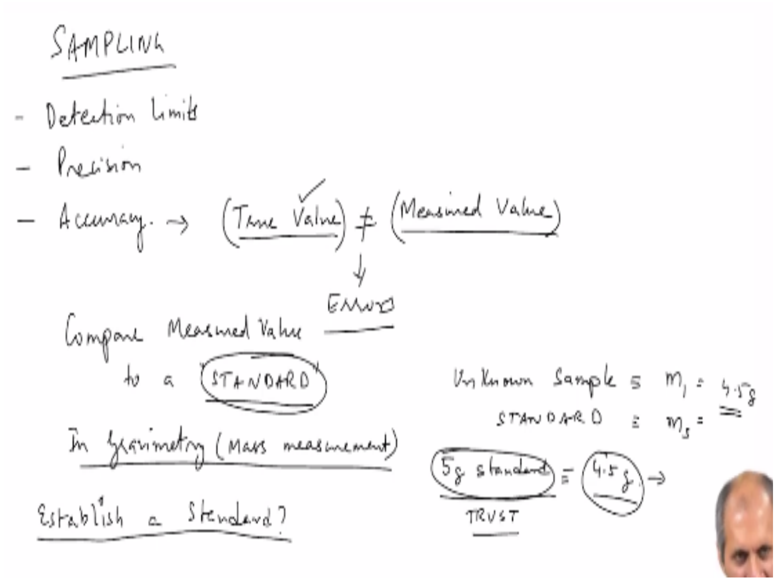

So, yesterday we had started discussing about some aspects of sampling and the measurement. We talked about detection limits, we talked about precision, we started talking about accuracy, okay. So, the question about accuracy that we discussed last class was true value versus measured value. This true value means whatever you are measuring its actual value that supposed to be and you are measuring something else. Usually, the idea is to bring the measured value as close as possible to the true value, that's the goal.

This true value and measured value are not equal, which implies that there are errors associated with the measurement itself and the errors can arise from different sources for various reasons, this we'll talk about this in a little bit of detail. But to start with, how do you even know what is the true value? So if you measure something, so if I measure a mass, I need to be able to check if whatever I am measuring is how close it is to true value okay.

So, in order to do this, we compare the measured value to what is called as a standard. Standard by definition is a very strict thing. You know it is used fairly loosely, but what we mean by standard is something that is known to be of a certain value. What do you mean by it is known to be of certain value? Who will decide that? So, for example if you take gravimetric analysis, gravimetry is mass measurement okay or weight measurement. How do we know what is true value? So if you place something on a balance it shows 5 grams or it shows 4.5 grams or 5 grams. How do you know it is 5 grams? You have to check the same thing with what you think is actually 5 grams, okay. So we have an unknown sample you measure it, it gives you some reading m1. If you put a standard, it will give you some reading m standard okay. The assumption here is that if the reading that is shown by the instrument is not what the standard is.

For example, if a 5-gram standard is placed on a balance and the balance shows 4.5 grams right, from this you can infer that something is wrong with the instrument. The instrument is showing some error. The instrument is supposed to show 5 grams and it's showing 4.5 grams, there is a .5-gram error in the measurement from this. Why do you say that? We say that because we have we trust the standard because standard itself cannot be wrong and we trust the standard.

So, the definition of the standard is absolute, the standard is proven to be true and therefore if you see a difference in an unknown sample say in an unknown sample is showing 4.5 grams and if you want to check whether this is correct or wrong, you put a standard on the balance and standard for 5 grams is also showing 4.5 grams which is very likely that this unown sample could be 5 grams. So, you get the error based on that.

So whatever is the standard and whatever is the value the standard is showing you check the error on the basis of a true value and measured value. For a standard you know the true value, the measured value is what the instrument is showing, and the difference between this is the error and then you go and try to investigate where the error is coming from, from the instrument or something else in the system and so on. So, who decides standards? Standards are a very strict business. So, usually it is a global association. You have heard of international standards organization ISO and all that. Before that, there were other versions of it. India has a standard organization, the US has one, Europe has one, but worldwide you have organizations of standards, it is very straightforward, you know. What is 1 meter for example, 1 meter, 1 centimeter, these are all international units. There is a definition of this and there is a standard somewhere, somebody makes 1 meter ruler and if there is 1 meter you have to go and check it with whatever the standard is there, wherever it is in the world.

So, this is from very simple measurements like length or mass and all it can go all the way up to very complicated concentration measurements in sophisticated instruments. So, the way a standard is usually prepared, suppose tomorrow I claim that I have prepared a standard for 5 grams and I show you that this something I show you a weight, a cylinder or something I say this is 5 grams, will you believe me? I say its 5 grams; how will you believe that it is 5 grams?

It is a very tough question to answer, this is why standards definition is not very easy. So, somebody if they said 5 grams, how will I establish this as a standard? How can you establish standards? I propose a standard, I said this is 5 grams' standard, for the rest of the society or rest of the people who are using balances have to accept it, what should they do? Common sense, this is all most of this is common sense, you don't need to be an engineer to understand all of this. This is what people do in general.

How do you establish a standard? If I give you a standard 5 gram sample and I say please follow it. Before following it, what will you like to do? Never thought of this? From a position of power somebody gives you something it is established. How would you establish a standard? What would you do? Forget about standard, if I claim something if I claim phenomenon, scientific phenomenon, if I say I can make electricity from dust, let's assume that I have made that claim. What will you do? Or even I will say that I give you a medicine and I say it treats some disease X, ok, simple for you. Before taking it, would some people want to believe whatever is the claim made by a particular thing? I think that is a bad example because one can always do trial and error, but this is not same example okay? This is a different example. This is quantitative because I am saying it is 5 grams, it may not be 5 grams, it may be 4.5, it may be 4.8, it may be 5.2. So how does one establish a standard again?

Those examples of scientific discoveries and all that are analogies for this, but even scientific discovery, somebody makes a discovery, how is it established as a fact?
Student: When everyone agrees.
Professor: When everyone, why will everyone agree?
Student: Proofs.
Professor: Ah? Proof and proof is enough, one minute, why will anyone agree? You show a proof, but why will they accept the proof. So, I have discovered some phenomenon, something say I have discovered a method of making nanoparticles in certain shape okay and I say that this is a methodology to make the nanoparticles and this is possible this, this material has this

kind of properties. People will agree to it? People won't just accept whatever you say and move on, what will they do?

Student: Disprove,

Professor: They will?

Student: Disprove

Professor: No no, they will not try to disprove.

Student: Verify,

Professor: They will?

Student: verify it, reproduce it

Professor: they will verify it. They will reproduce it. They will also verify, everybody has to verify, everybody will repeat it again, they will see if this is the same. So what they will do is you are saying this is 5 gram standard, what they should do?

The simplest thing they can do is they will take it and put it on their balance and see if it is showing 5 grams. You are saying it is showing 5 grams in your balance, my balance I will see if its showing 5 grams, I can tell you that if you say 4.8, I said your balance is wrong. So then you will give it to another person, they will check, they will say no, it is showing 5 in my instrument and this person will challenge that third person saying yours is also wrong, then it goes to third, fourth, fifth, sixth. So, it can only happen by consensus, right?

As many people verify it and it becomes a standard. Sometimes all of them, I say 5 grams, but the majority of them are showing 4.9, this 5 gram standard become 4.9, that's all. So there is no absolute standard, there is absolute value of this thing. There is some definition for it, yes, but there are always errors associated with the way you are measuring it, yeah, and the sensitivity of your measurement. Now, I will say it is 5.00001, somebody will say it is 5.004. So, these kinds of issues are all there.

So, depending on the sensitivity of the way you are measuring it and all this, the standard will change a little bit. So, this is all to be taken with a little bit of uncertainty that is associated with all of this, but to the best extent possible, if all people agree it more or less that this is a standard with a certain amount of error associated with it, that is the standard, we will accept the standard and move on.

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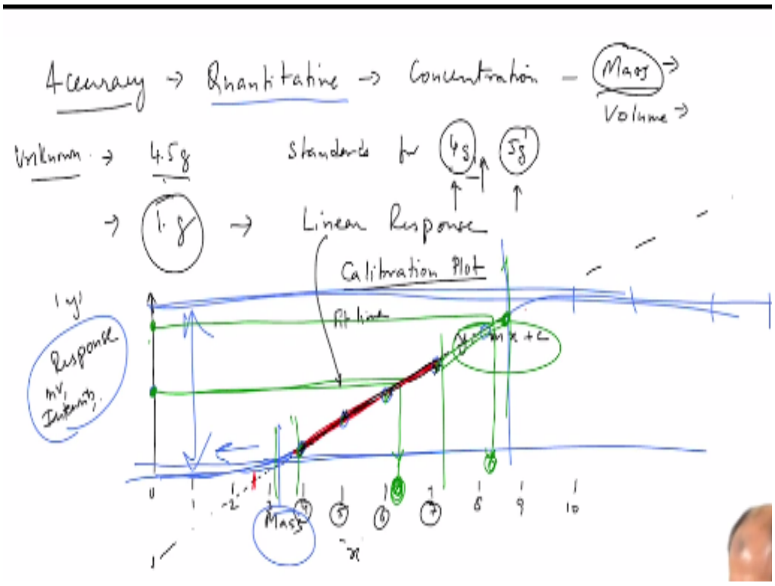

So accuracy, now here we are talking about quantitative accuracy, because our goal here in this discussion primarily is to measure concentration, which means it is mass by volume. So we are looking at the accuracy of mass and accuracy of volume that you are measuring, okay. So, a lot of these things boils down to the accuracy of measuring mass. How accurate are you able to measure mass and at how low you are able to do it? Okay. So, in a weighing balance for example, I check, I get 4.5 grams, an unknown sample shows 4.5 grams and I have standards 4 grams and 5 grams. So I know the instrument, I put 4 grams on an instrument it is showing 4 grams, I put 5 grams on the instrument it is showing 5 grams, which means that I know the instrument is working well for 4 grams, it is working well for 5 grams, I know this, I establish. If unknown sample is showing 4.5, I have a reasonable amount of belief that the sample is 4.5 because it works well for both 4 and 5. Suppose I have an unknown sample that shows 1 gram. Now I have standards for 4 and 5, I really don't know if the instrument is measuring 1 gram because I am extrapolating it okay.

So, for this reason, see the instrument is measuring mass by some principle. Yesterday we talked about balances doing pressure-based measurement and all that. So, what happens to the pressure device when you put 1 gram or when you put 15 grams on it? We do not know. So that has to be established. If what is called as if there is a proportional increase in the response what we would like instruments to have is what is known generally as a linear response. So, here is where we come up with this term what we call as a calibration plot or calibration data.

So, what we are doing is, this is the response of an instrument. So usually instruments have an internal response, say it is in terms of millivolts or it is in terms of signal intensity, light intensity, or something okay or any such there are all kinds of responses that an instrument can give and here we have mass, actual mass that we are putting. So, if I have 1, 2, 3, 4, 5, 6, 10 let us say I have this many and the instrument gives a response. So, I have say 4, 5, 6, and 7. The instrument gives a response.

I measure this, instrument gives a response like this. I measure this, instrument gives a response like this. I measure this, instrument gives a response like this, instrument gives a response like this. What I am seeing is that instrument is giving an increasing response to when I am increasing my mass on the x axis, okay. So, this data is good, as I see it, it is good in this range, between 4, 5, and 7, I know it works. How do I know it works because if I plot a response through this, if I fit a line through this, linear response means I must be able to fit a straight line through this, okay.

I will have to fit a straight line. So, they will not be all on the straight line, they will be somewhere close to the straight line. So, we fit a linear curve. So, I will get an equation which says response with this is y and this is x. We have an equation as $y = mx + c$, y is the response and x is the mass that we are adding. So, there is a slope of this line and then there is an intercept. So, if I extend this, ideally speaking this is the only line I can fit because these are the only 4 point, but I can extrapolate it.

I can pull it down and it will give it gives me a 0 value somewhere there and I can pull it up here. So, essentially I am doing this, this is the only region in which I am actually measuring and I have a lot of confidence in this. This region I do not know, I am guessing, but straight away from this equation I can tell one thing that below this point I will not see any response right, which means that this seems to be some kind of a minimum amount of mass that this instrument will show some response, below this it will not show anything, okay, and above this I don’t know.

Have you seen balances, this old type of balance, you put something on the right side, you put something on the left side. At some point if you put 1 kilo, it will go all the way down. If you put 2 kilos also, it will not change position. If you put 10 kilos also, it will not change position, it will stay there. So what will happen to this curve when it does that? What we are saying is the response there is this marker, for the old balance you have this thing that moves and that has to come to the center position.

It goes all the way to one side, no matter what I do on this side, it will not move, it will keep staying there okay. I cannot balance it beyond a certain point and you can see that in your regular pressure balances also. If you put 5 grams, if it says 5 grams, beyond 5 grams whatever you keep adding, it will not show any change, it will stay there okay. So, this response will then become, what it means is there is no change in the response beyond a certain point. So, this curve will become something like this saturate.

So, there is a region in which you can trust this equation, this calibration equation okay. Similarly, on the other side also you can see, instead of doing this, the instrument may do this, it may be not go to 0 directly. It may do this kind of a behavior; it is a non-linear behavior. So definitely, I cannot use the instrument beyond this because I don't know what's going on. I have no idea whether it is, I don't know if it is here, here, here because all of them are giving the same response, I don't know where it is. So, I cannot trust this. And here, it is nonlinear beyond this point, also I cannot trust it because it is nonlinear. It is very close to its 0 and because of the reasons we talked about yesterday, I cannot really trust that because it may be very close to noise value okay. So, there may be a response above which only you can trust it, so there is a range in which this instrument will work between these two values. So, this calibration has to be done. You have to find out what is the range in which you have to plot this entire calibration curve to know this range in which I can find out what is the quantitative response of this instrument, which means I need to have standards that help me construct this calibration curve, okay. For every instrument, you can obtain standards. When people make an instrument, they will also make standards, they will arrange to make standards or standards will develop over a period of time for that particular instrument by consensus again.

So, people will make and they will propose this as a standard, others will use it and so on and so forth. Sometimes, there is no standard established worldwide, you have to prepare your own standards and then that will be of course be questioned by other people because it is not accepted worldwide will come to that, right. So, this is the calibration plot. The calibration is a check. First of all, it is a method in which you link your response to the actual measurement unit that we are using.

Because say in in pressure devices, pressure transducer based devices like a balance, instrument is not measuring mass, instrument is measuring something else that has to be calibrated to the mass okay, and almost all instrument it's like this, none of these are measuring, a pH for example, measuring hydrogen ion concentration but it is actually measuring some current voltage that is changing in the electrochemical system so on. So, these are all things that you have to check, so, if by the way we use this calibration curves now if I have this calibration curve, if I have an unknown sample, I take an unknown sample put it on a balance, I will get some response. Let us say this response is green in color. I can calculate what is the mass by using this calibration plot and going this is the mass of my unknown sample, that is how a calibration curve is used, which means you need this the relationship the relationship is used in order to calculate response within the limits of calibration, okay. So strictly speaking, when you extrapolate, you are only using 4 standards, I really don't know what is happening beyond this.

So, if you are reporting my calibration limit is done here. So, in this case if my response if my sample is here, I am able to say very confidently that this is true, okay. If my sample is here, I am not really sure if this is the value, it could be, but I am extrapolating it, I am not exactly, I don't know whether it is true because I have not run a standard here. If I have a standard here and if it is also falling on this line, I can say with great certainty that this is this value, it is not. So, therefore, when we are doing this, you have to report this, you have to say that it is outside my calibration range.

So, you can extrapolate it, but it could be wrong okay. So, when you report a value, you have to do that because it has repercussions to it. So, if you yourself realize that it is beyond your calibration, you have to make comments for that okay.

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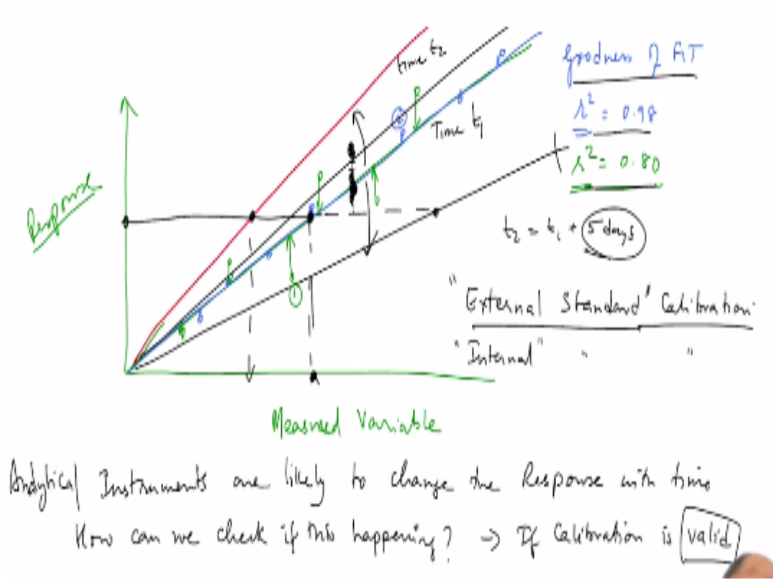

So, in this calibration, if you get a calibration like this, you have a response versus this measured variable, whatever it may be mass, volume, concentration, anything you want. This is the response what the instrument is reporting okay. So, you will see as we will see examples of different instrument, they all report different things okay. Sometimes, they will report the actual concentration here also, the measured variable will also be reported here, internally you don't know there is some equation there.

So let us say you have calibration that looks like this. Let us say I try to fit an equation that is going through 0, the fit looks like this (green). Then I have another calibration where the fit looks this (blue), so here again I draw another line through this. Among these two, calibration 1 which is represented by the green this thing and calibration 2 which is represented by the blue points. Two people have done calibration and one of them does this, another one does this or two different instruments have shown this calibration of the same standards.

One of them showing the green, one of them showing the blue. We generally tend to trust the blue one closer, because there is a very close linear relationship, okay. If I am fitting a straight line, the difference between the straight line and the points not very high, so we look for some statistics of goodness of fit. A lot of times there are different variables for doing it, lot of time there is a correlation coefficient or what we call as the r square. You have to be careful when you use these things, okay.

Simple common sense point of view if I have an equation, if I have a calibration curve that looks like this, which is very far away, it will still give me the same equation, right. The blue line and the green line are almost similar, but the r square values are very different. So the r square values for the blue may be close to 0.98 something. So the r square value for the green may be 0.8 close to that okay. So there is a significant amount of difference here okay. So there is this, when we do these kind of analysis, the distance between your fit and your experimental data becomes larger, then you have lesser confidence in that data.

You are not really sure, there is all uncertainties creeping into these things. I am not sure if which of this data point is incorrect, okay. So, that will change my actual value a little bit. So, in general, people try to keep this calibration curve as tight as possible, we would like to keep it like the blue points. If you have green these things, then usually we don't like it, unless that is the limit because if you cannot get better than this, then nobody will be able to get any better than this, then that is fine. That's the maximum you can get and so you have to work with it, there is no other way of doing it okay, that is one. The second is this response curve itself might change over a period of time. So sometimes this may happen, sometimes this may happen (red). This is at time t1; I have done a calibration today. This is at time t2, t2 is t1 plus say 5 days, after 5 days or after a week or 10 days. This is the calibration I am getting. So it is a big difference as you are going from here to here because if I take an unknown sample, I am following this calibration curve, but the calibration has changed internally and I am not noticing it, I follow this older calibration which means that I may be overestimating the concentration based on my older curve, but the new calibration curve is showing this smaller value or worse it may be like this (black), where I am estimating a lower concentration than what it actually is ok. The calibration changes which means that something is wrong, the instruments response to standard.

So this happens in lot of instruments with time, 5 days is too short, but this can happen in some instrument where over a period of 1 or 2 days it will change. Because the way the instrument measure something and lot of these artifacts appear in this thing. So, the standards are a very good way of checking. Let us say I have established this blue line as a calibration equation. How can I check if the calibration still holds good after 5 days? So instruments are analytical instruments, they change the response with time.

So, how can we check if this is happening? In other words, how can we check the calibration is valid?
Student: By measuring at regular timings.

Professor: What, measuring what?

Student: Standard.

Professor: Measuring a standard, you have to always check with the standard, that the only way to check it. If calibration curve exists and you have those standards, you go and check again after one day. So if it is moving, so if you check the standard, it is supposed to give you this mass.

This is standard here, I am checking this mass. So, this is a standard, I am looking at the standard for example. Every time I run the instrument, I will check the standard. If the response is going up or down, it may go up or down a little bit, right? I can allow it to go up or down to some extent because as we discussed yesterday it is always not going to be the same, a little bit of a precision difference is going to be there in the instrument. So I am okay with it going up and down a little bit, but you decide when is it that you think it is not acceptable anymore.

So if the standard that what I am looking at day 1, I measure this and the day2 I measure this and so on and I reach a point where it is no longer acceptable. So this could mean that the calibration has shifted significantly, okay. At that point, I stop and I redo my entire calibration and I get a new calibration curve which may look like this and I follow it until my standards satisfy that. So the calibration is not a permanent thing. It depends on the state of the instrument and type of samples you are using. Sometimes calibration will change during your run sometimes, okay. Some instruments do that depending on your sample. So it is a very sensitive thing. So, there is a lot of statistics in what we are doing. So, starting from this goodness of fit itself because this one, the value of the r square here and the goodness of fit determines how good your calibration is and which will determine how much error that is there, how far are you are from your true value, okay. So, these are all inbuilt into this, statistics is inbuilt into all analytical sciences and you cannot you cannot go away from it.

You have to I think keep that in mind whenever you are reporting any values, the statistics of the measurement will determine what is the utility of that value you are giving, number that you are giving. Any questions on this? So, normally what people do when we are doing this calibration, you obtain standards and you calibrate for the response, this is called as the external calibration. There is something called an internal standard calibration, we will talk about it when we get to one of those instruments where it is used later, it is not important right now.

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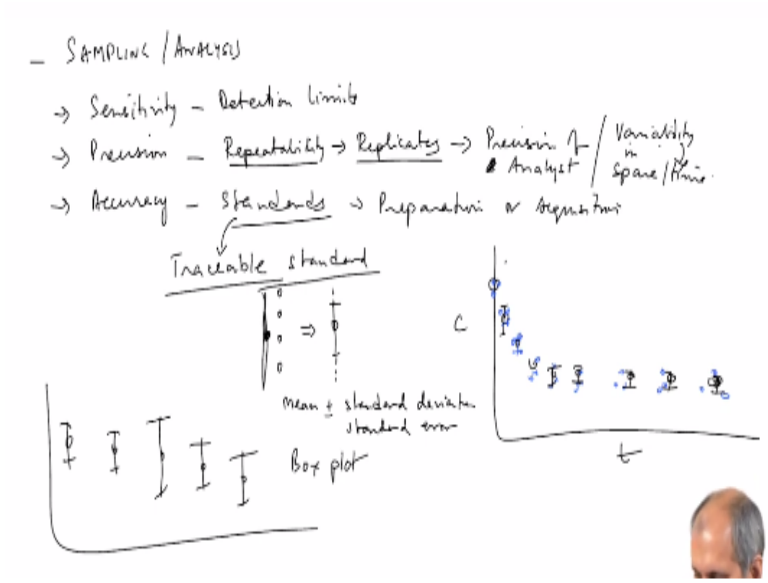

Okay, so we have talked about sampling, analysis. We have talked about instrument detection, we have talked about sensitivity, we have talked about precision, and talked about accuracy, okay. This is the detection limit. We establish sensitivity by looking at detection limits. We establish precision by repeatability, which means we do multiple replicates of the sample. Now, this precision is not one precision is for the instrument, when you say repeatability, instrument repeatability is one thing, but it could also mean that there may be variation in the way you are doing your sample.

So, if I take a sample now, and then after 5 minutes I take a sample again, I go through the entire process of analysis, there may be changes in the way I am reporting this thing. So, this repeatability replicates this precision, strictly speaking the term precision applies to the instrument, but we are talking about repeatability precision in terms of the measurement itself, the sampling and the measurement. The sampling precision and the sampling repeatability and repeatability is indicative of different things, okay.

Repeatability as we discussed yesterday, repeatability you repeat it multiple times, so this precision repeatability replicate means multiple samples. Duplicate is 2 replicate, triplicate is 3 replicates and so on. So, replicate is the general word for how often do you repeat the sample. Now, the replicate is indicative of several things, it is indicative of the precision of the person who is doing it and how the same procedure is being done every time without mistake, okay.

It is also indicative of spatial or temporal differences in the system, you can look at variability in the system. So replicates indicate precision of the analyst, you are the analyst, okay. It also indicates variability in space and time. So you can design your replicates based on that. If you are interested in looking at whether it is changing with time, I do not expect sample to be different spatially, but you think that something is happening with time. So for example, let me give an example of the data.

Suppose you are doing something, concentration versus time measurement. I am measuring sample at some times. So I measure sample like this. So I will go in and measure every half an hour. Now, if I am measuring at half an hour, within each and every half an hour, if there is a significant change, how do I know that it is the same as is the timescale of whatever happening is half an hour or 4 days or 10 seconds, 20 seconds, we don’t know.

So you must be able to differentiate whether it is variability in space time or if something that has to do with the sample itself. So this will give you that kind of information. So for example, you are doing a reaction you know the initial concentration, after 1 hour you go and you will see all data like this. Now, this is a very fast system. So, you must be able to take samples quickly enough so that you are able to fill in the blanks, full data looks like this, that is one part.

The other part is if you take replicates, then you must be able to figure out if the replicates all are very close to each other. So this is a very fast process, if you take a replicates it will it will include these 3. So, you must be very careful whether you consider that as a replicate or if you consider that as a process itself. So, these are all things that one cannot priori give you any idea, you have to design for that with some goal in mind, and then you have to redo the entire thing based on that initial set of value.

So, the first set of readings that you take usually will give you some idea of how fast the system is happening process is happening and then you go back and refine your sampling process so that you get more confidence out of it, okay. So normally when you do replicates, your your data is typically your for say for every time I have taken 3 replicates, I can represent the replicates as an error bar. So if the replicates I take, so if I in the actual replicates are say the blue spots here, there are here 3 spots here. This is actual data I am collecting, which means that all of these 3 represents this this particular time, this particular time, and so on.

So I have to be able to represent it and I am usually capable of representing this as an error bar. So I will put some error bar here and some error bar here, some error bar here, some error bar here, some error bar here. So if you have multiple data that looks like this, so this is not a good example. For at the same time if I am collecting the data looks like this, this is the range in which this can happen. So, this is equivalent to having a mean and having some variation. So, there are different ways in which this can be denoted, ok.

So, we look at it as mean plus minus standard deviation or a standard error or different other types of statistical markers that has a percentile this. If I do this measurement 10 times, 20% of the values are above this value, 20% values are below this value and the rest of them are in the middle. So, you have what is called as a box plot that you can make in order to get this, all these possibilities exist. So, it will give you the full representation of the data. One point does not represent all of these. If I give you one point here, I have no idea, this I am saying this average, but I have also measured these other values. So, I need to be able to represent this entire range in some way. The statistical plots allow you to do that, will give you full information. So, what it will mean is this so if you get data like this, the average is going down, but the error bars are huge, big error bars. Then this question will come whether these points are all different or the same. So, which should help you to make a determination whether some processes happening or not, okay.

So this is very important, this is a statistical analysis of this okay. So you have to do the statistical analysis for that. To allow you to do statistical analysis, you have to have multiple replicates, at least 3 or more below that you can't do anything, okay. You must have at least trip triplicates, more the better, the more samples you have more statistical analysis you can do and the statistical analysis will give you a basis for which you can make decisions whether something is happening or not.

A lot of questions will be asked based on the statistical analysis; you can answer all of them using this. So, the precision, the repeatability as replicates all these things can be demonstrated by this error. So if I have a procedure for doing an analysis, but if 10 different people are doing it on the same sample, they are all doing it differently, there is error, you can also get that error, sampler error, how variable is each analyst's operation, okay.

So, all this is there and this is also the basis for which people automate analytical instruments, lot of sampling and analysis. What we mean by automation is they use an instrument, a machine to do all of it. So, a machine can be programmed to do the same thing again and again and again, with a little bit of error, okay. Humans, depending on the skill may be susceptible to more error. So, the automation of these things is done based on this.

So, accuracy of course we talked about the use of standards and the preparation of standards or acquisition. Some people will sell the standards. So, for example if i have a weighing balance it is difficult for me to make a standard, somebody will sell me a standard, standard organization will sell standards. So it says it is one 5 gram standard, you have to buy it because they have done all the hard work, they have compared it with 20 other labs across the world and it is traceable.

So, what we call it as when you look at standards, it gives a traceable standard as the highest value. Traceable standard means you know who has done it, where has all it been measured. If I give a standard, it is not traceable, you can trace it to me, but then nobody will believe that I did this. So it is traceable to an organization which will stand behind it, they will say that they will take responsibility for it. There are a lot of standard organizations. So India has one and but there are a lot of worldwide standard organizations which people use in order to so whenever an instrument analytical method comes up, it has to adhere to some of these standard methods. Okay, so I will stop here for today. Tomorrow, we will look at methods of analysis for different media and some of these questions will come up there, okay. So, will look at the analysis of samples in water, air, soil, sediment, all of that, organic. We will we’ll start with general methods and then we will move on to specific methods for chemical analysis that we will do probably after the quiz.