Quantitative Methods in Chemistry
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Lecture-34
Analytical Separations-Multistage Extractions Part 01

Hello and welcome back to lecture 1 of week 9 of this course quantitative methods in chemistry. This week our focus will be on understanding analytical separations that include solvent-solvent extraction and a wide variety of chromatographic techniques. So, we will be mainly focusing on the principles of chromatographic techniques and solvent-solvent extraction this week.

And in the subsequent weeks, we will be focusing on how quantitative analysis can be performed on chromatographic data. And finally, we will also understand what are the various theoretical basis for chromatographic separations. This lecture, we will be mainly focusing on solvent-solvent extractions. So, let us get started with this week's lecture 1.

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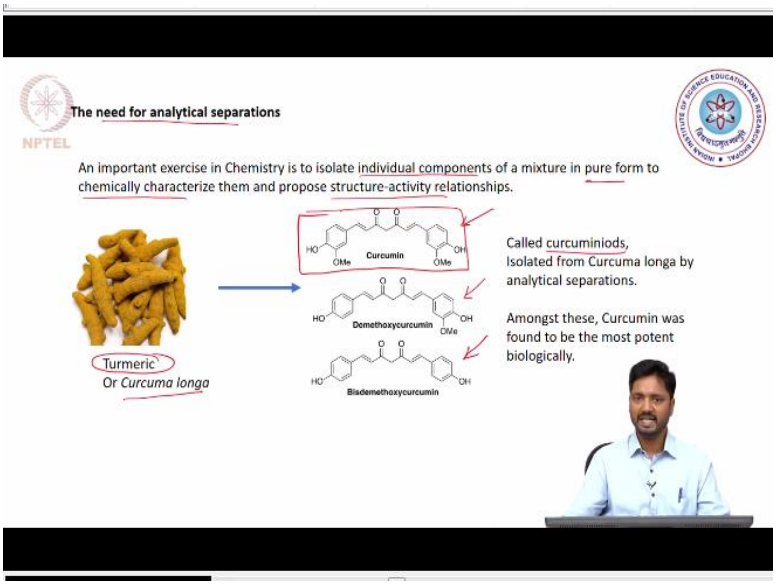

And so, what I have done here is I have included the necessity for undertaking analytical separations in chemistry and as you understand that, in chemistry analytical separations form a very important part of chemistry because the idea here is that we use analytical separations to isolate individual components from a mixture, this mixture can be naturally occurring as part of, say plant material or in petroleum.

And we would want to isolate individual components from this mixture in their pure form. And by pure form we mean that they should be analytically pure, so, that they can be individually characterized and they can be chemically analyzed and based on the chemical analysis and the activity that these compounds generate, we would want to ultimately propose a structure activity relationship.

A very common example that I have taken here is that of our good old turmeric which we all use in Indian cooking and this turmeric when people analyzed the components of turmeric, by analytical separations, they could isolate these 3 molecules from turmeric powder. And these 3 compounds are called curcuminiods collectively, and they are isolated from curcuma longa which is the name of turmeric in scientific parlance.

So, when people isolated these 3 curcuminiods, they undertook the biological activity of these compounds. And ultimately what they figured out is that this first compound was shown here in the box, is supposedly having the most potent biological activity, whether it anticancer or antiinfection activities. So, a lot of research has now focused on utilizing curcumin directly in which is isolated from turmeric powder.

And people have also synthesized various derivatives of curcumin to explore their biological properties. So, this I believe sets a very clear precedents of why analytical separations needs to be undertaken in chemistry.

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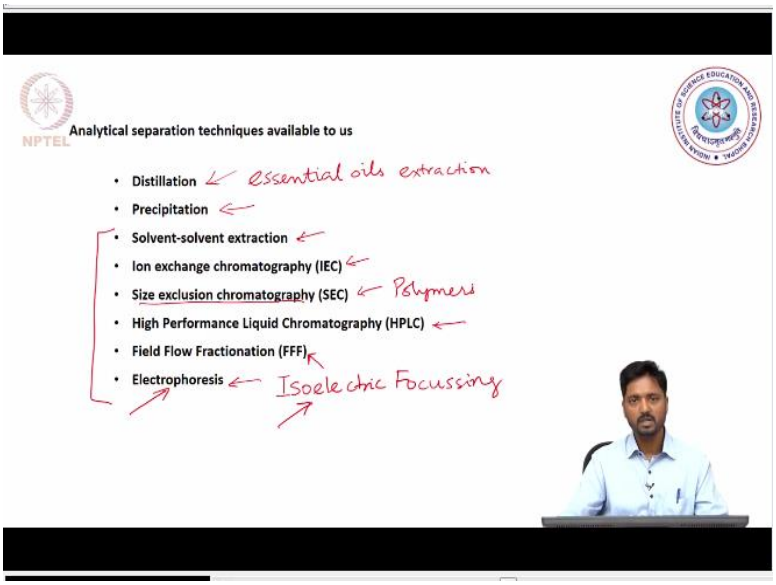

So, what are the techniques that are available for us for analytical separations. What has been traditionally used is the distillation process and you might be aware that many of the plant extracts are distilled. So, that we can isolate what are known as the essential oils from the plants. So, a very common example of that would be the neem oil or eucalyptus oil or the rose oil that we can purchase now.

And most of and even the ether for example, which is made widely in our country, these are remade through the process of distillation mainly steam distillation and these oils are then isolated and sold. Now, we already covered this important technique of precipitation, which is used in analytical separations quite widely and we talked in terms of gravimetric titrations where we can add a precipitating reagent and isolate our analyte of interest.

Now, this precipitation protocol is also used in biology where we can for example, precipitate our nucleic acids under the appropriate conditions and then characterize them properly. Now, this week, we will be focusing on the techniques that are listed afterwards, which is the solvent-solvent extraction, the ion exchange chromatography, the size exclusion chromatography and we will also understand the principle of high performance liquid chromatography.

Finally, we will be focusing on the field flow fractionation and the use of electrophoresis and what is also a subsidiary or aligned technique of isoelectric focusing. So, please be aware that electrophoresis and isoelectric focusing will be employed in the context of proteins and nucleic acids and the rest of the techniques listed above such as the solvent-solvent extraction and the various chromatographic techniques are employed for small molecules as well.

Important point also to be noted here is this concept of size exclusion chromatography and size exclusion chromatography is mainly used for polymers and we will see how this technique helps us in achieving separations based on the size of the polymers. So, let us begin our understanding of solvent-solvent extraction.

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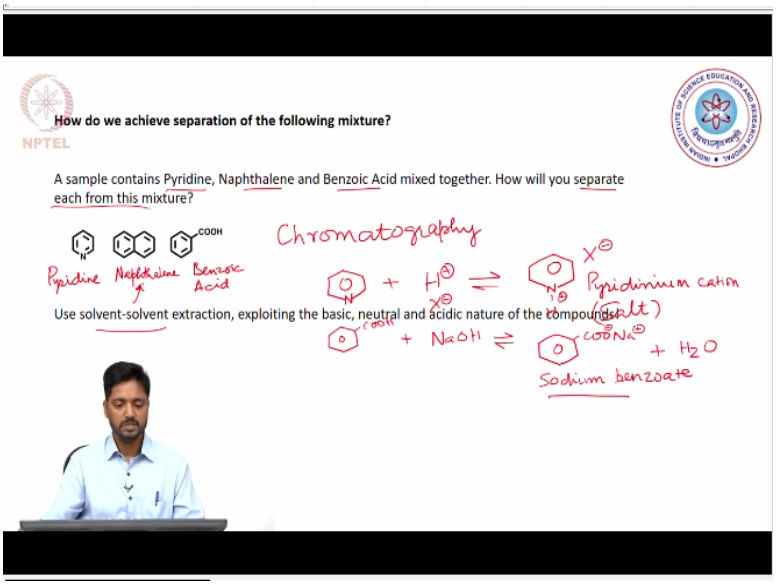

But before that, let me pose to you a quick question. So, suppose we have a mixture which contains pyridine, naphthalene and benzoic acid which is mixed together and the question in front of us is how do we separate each of these compounds from this mixture. So, we need to perform an analytical separation to separate out these 3 compounds from the mixture that we are having.

So, let us look at what are the various techniques that are available and which technique will be best suited for this separation. Now, in my experience, when I pose this question to the students, students come up with a variety of answers, but the most common answer that I have seen is that of using chromatography. While chromatography is of course, one of the techniques that we can employ.

You should also try to look at what is the most simple way through which you can separate these compounds. And for that, you need to look at the chemical structure of these 3 compounds. So, I have given the chemical structure of pyridine, naphthalene and benzoic acid for your reference. And if you see these compounds, what becomes quite clear is that these 3 compounds have inherently different properties.

Now, what you can see is that if you take pyridine you can easily protonated it is a basic compound and can be protonated under the right conditions of pH to generate this pyridinium cation and typically anion will also be associated, so this will become a salt and as we know that these salts are having decent solubility in aqueous medium. So, we can utilize acidic conditions to move pyridine from an organic medium to an aqueous medium.

Similarly, if we take the example of benzoic acid we can treat it with a base and this will undergo the formation of sodium benzoate. Now, this sodium benzoate is also a salt. So, it would be easy for us to separate it or move it in aqueous medium from an organic medium. So, here the take home message for all of you is that we can use solvent-solvent extraction exploiting the basic, neutral and acidic character of these compounds to achieve more straightforward and efficient separations.

So, in a nutshell what we will do is we will dissolve out this mixture in an organic solvent such as ethyl acetate or toluene and then we will extract it first with acidic water which will move the pyridine into the aqueous medium, we will separate out the aqueous medium and next we will extract the residual organic layer with basic water and the basic water will convert the benzoic acid into sodium benzoate.

And the sodium benzoate now, again goes into water. Finally, in our organic medium, we will be left with the neutral compound naphthalene because it will be unaffected by the changes in pH that are happening around it. This example clearly shows how solvent-solvent extraction can be effectively exploited for separating organic compounds into individual components.

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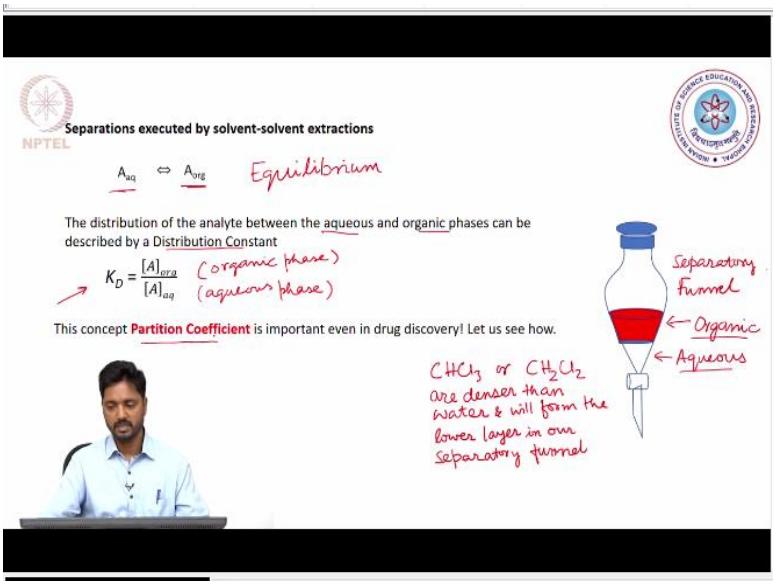

So, basically separations executed by solvent-solvent extractions rely on an equilibrium that happens when the analyte A moves from an aqueous layer to an organic layer and we use this separatory funnel to execute the solvent-solvent extractions. So, what we do is we take for

example, the organic layer that is typically the lighter layer and the aqueous layer is the more dense layer.

So, it forms the lower part of the separated layers, exception to this would be utilize chloroform or dichloromethane, such chlorinated solvents are denser than water and will form the lower layer in our separatory funnel. So, this needs to be kept in mind when you are undertaking solvent-solvent extractions. But basically what we do is we add the organic layer and the aqueous layer and do rapid shake up for a decent amount of time, approximately 2 to 5 minutes.

And that allows an equilibrium to be established where the analyte moves from the aqueous layer to the organic layer. And since it is an equilibrium that is getting established here, we use the concept of a distribution constant for this analyte and it tells us how the analyte is getting distributed between the aqueous and the organic phases. So, this distribution constant or equilibrium constant of distribution of the analyte between the 2 phases is given by this equation, where K D is the distribution constant.

It will be equal to the concentration of the analyte A in the organic phase divided by the concentration of the analyte in the aqueous phase. So, what people have realized is that this distribution constant or what is also known as the partition coefficient is also of high relevance in drug design or drug discovery. And in the next slide, we will be seeing how the concept of partition coefficient is applied in the pharmaceutics.

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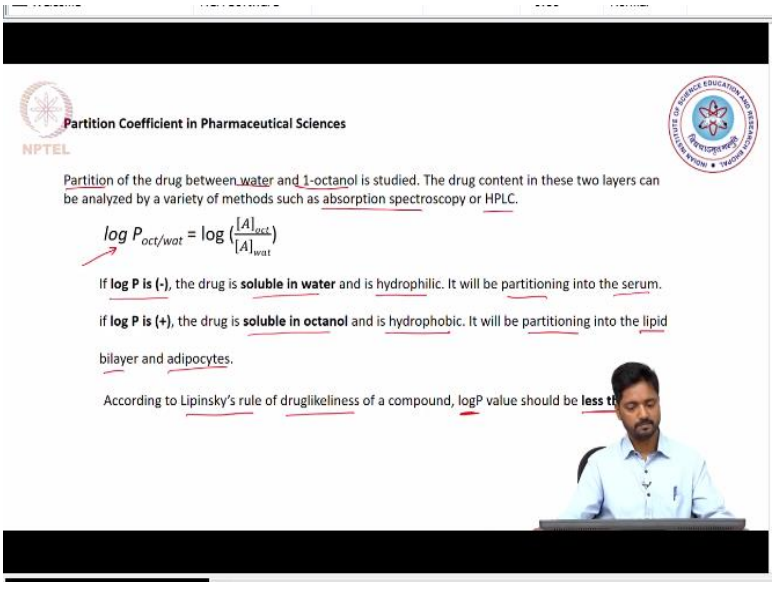

So, in pharmaceutical parlance, the log P value is very commonly used to characterize a drug molecule and in this exercise the partitioning of the drug between water as the aqueous phase and 1-octonol as the organic phases studied. So, 1-octonol in this case becomes the organic phase in which the partitioning of the drug phase studied. Now, the drug content in these 2 layers after the solvent-solvent extraction can be analyzed by a wide variety of analytical techniques.

The most common that are employed are absorption spectroscopy, if our drug molecule is having particular absorption, either in the visible or the ultraviolet range, then we can easily employ absorption spectroscopy because it is a decently sensitive technique. Another sensitive technique, which people commonly use, and which we will also study in the subsequent lecture of this week is the high performance liquid chromatography.

So what we will do here is that we will find out a log P value. So, the partition coefficient is expressed in terms of a log value and this log P value is also reported for the drug molecules rightly. So, this log P value is nothing but the log of the concentration of the analyte or the drug in the octanol phase now, divided by its concentration in the aqueous phase or the water phase. Now, what people have realized is that, if the log P value is negative.

Then more of the drug partitions into the aqueous layer or the drug is considered to be hydrophilic and soluble in water. A very common example of compound that will show a negative log P value will be something like acetamide or acetic acid because it will partition well in the aqueous phase and a biological effect of a negative log P value is that the drug molecule now would like to remain in the aqueous environments or aqueous compartments present in our body and cells.

So, for example, the drug will be partitioning into the serum more effectively and may have difficulty getting into the hydrophobic compartments of the cell. The contrast to that is when the log P value is positive and this indicates that the compound of the drug is having better solubility in the octanol phase. And that means, this compound or the drug that we are dealing with is considered hydrophobic.

And because of it is hydrophobic nature, the drug would like to partition in the hydrophobic compartments such as the liquid bio layers or the adipocytes. So, people have found a very

clear correlation between the log P values and how the drug partitions between the hydrophilic and hydrophobic compartments of our body. For example, even whether the drug will pass the blood brain barrier and be accumulating inside the brain.

So, Lipinski rule is a rule of thumb that was developed some time ago. And it talks about the drug likeliness of a chemical compound. And it is a very famous rule, which for our purpose says that the log P value of drug candidate should be less than 5. That means it should not be too soluble in the hydrophobic or the lipophilic environments of the body. Because under those conditions, it is absorption and distribution in the body will become rate limiting. And if the drug is not absorbed well the effectiveness of the drug will be compromised.