Advance Analytical Course

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Lecture No. # 12

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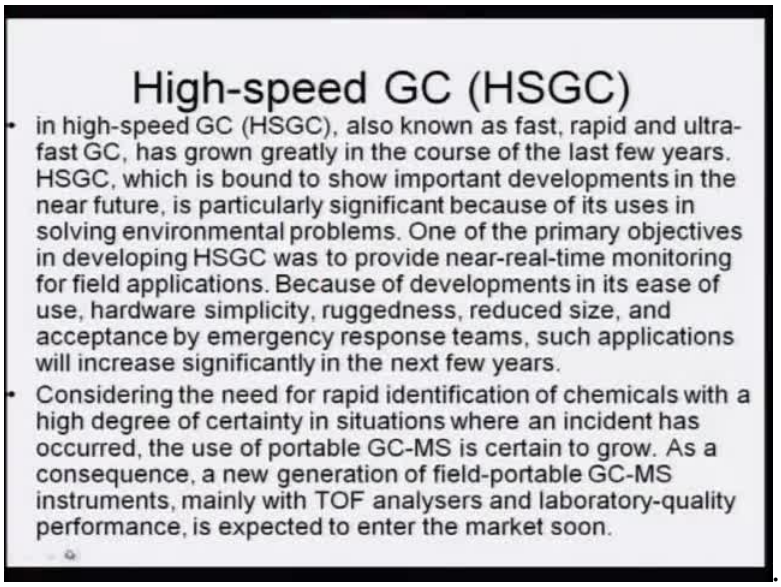

High speed GC - in high speed GC, also known as fast rapid and ultra-fast GC, has grown greatly in the course of last few years. High speed GC, which is bound to show important developments in near future, is particularly significant because of its uses in solving environmental problems. One of the primary objectives in developing high speed GC was to provide near real time monitoring for field applications. Because of developments in its ease of use, hardware simplicity, ruggedness, size to be reduced and acceptance by emergency response teams, such applications will increase significantly in the next few years.

Considering the need for rapid identification of chemicals with a high degree of uncertainty in solutions and situations where an incident has occurred, the use of portable GC-MS is certain to grow. As a consequence, a new generation of field portable GC-MS instruments, mainly with TOF, that is, time of flight analysers, and laboratory quality performance, is expected to enter the market very soon.

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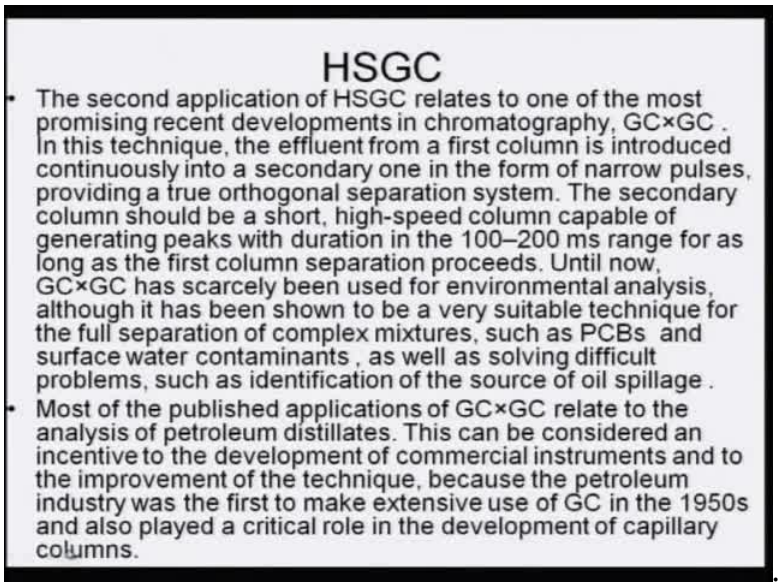

High speed GC - the second application for high speed GC relates to one of the most promising recent developments in chromatography, that is, GC coupled with GC. In this technique, the effluent from a first column is introduced continuously into a secondary one in the form of narrow pulses, providing a true orthogonal separation system. The secondary column should be of short, high-speed column capable of generating peaks with duration in the 100 to 200 mass range for as long as first column separation proceeds.

Until now, GC coupled with GC has scarcely been used for environmental analysis, although it has been shown to be a very suitable technique for the full separation of complex mixtures, such as PCBs and surface water contaminants, as well as solving difficult problems such as identification of source of oil spillage.

Now, I will try to explain this a little bit, because GC and GC, when they are coupled, the separation that has taken place in one GC is then transferred to another GC. Now, there are wider separations, and more and more number of contaminants can be looked into and can be identified. That is an advantage of high speed GC. Most of the published applications of GC into GC relate to the analysis of petroleum distillates. This can be considered an incentive to the development of commercial instruments and to the improvement of the technique, because the petroleum industry was the first to make

extensive use of GC in the year 1950s and also played a critical role in the development of capillary columns.

Now, you would know that in petroleum product, the higher homolog is just CH 2 away. That means, if you try to recall the ethane, methane, and propane, and butane, you see that they are just CH 2, CH 2, CH 2 away from each other and as we go along the higher homologues series also, they are very narrowly different; that is only difference that takes place between them is the atomic weight difference of CH 2, which is 14 and such small differences bring the peaks very close to each other. When the separation is done in one GC, they are partly separated and then the same effluent is transferred into the second GC and they get separated further more. So, that is where the application comes very handy, for such petroleum distillates.

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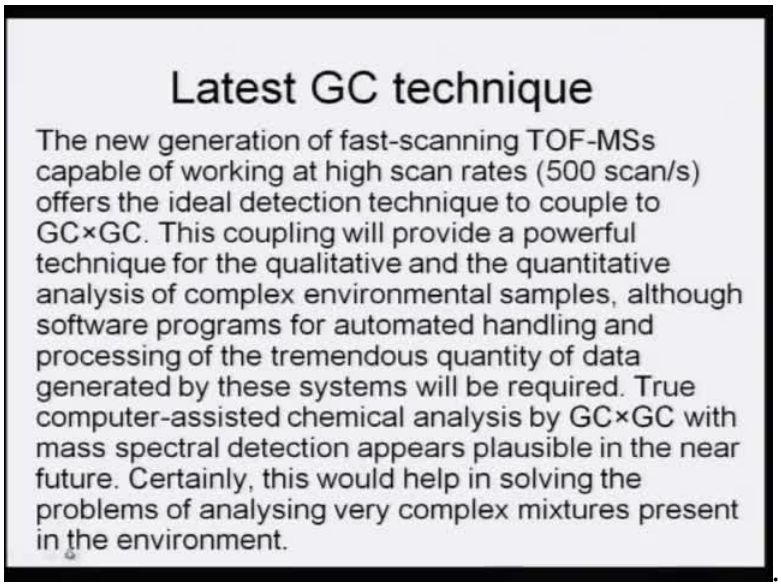

Latest GC technique - the new generation of fast scanning TOF-MS capable of working at high scan rates, that is, 500 scans per second offers the ideal detection technique to couple to GC and GC.

So, with this you need to have the time of flight MS analyzer; otherwise, it will not solve the situation. So, the adaptation of GC into GC with a TOF analyzer is an ideal situation for such very closely connected compounds.

This coupling will provide a powerful technique for the qualitative and quantitative analysis of complex environmental samples, although software programs for automated handling and processing of the tremendous quantity of data generated by these systems will be required.

True computer assisted chemical analysis by GC into GC with mass spectral detection appears plausible in the near future. Certainly, this would help in solving the problems of analysis very complex mixtures present in the environment. So, with this we have come to an end of a very important topic, where we had tried to understand the significance of these particular sampling process and high speed GCs.

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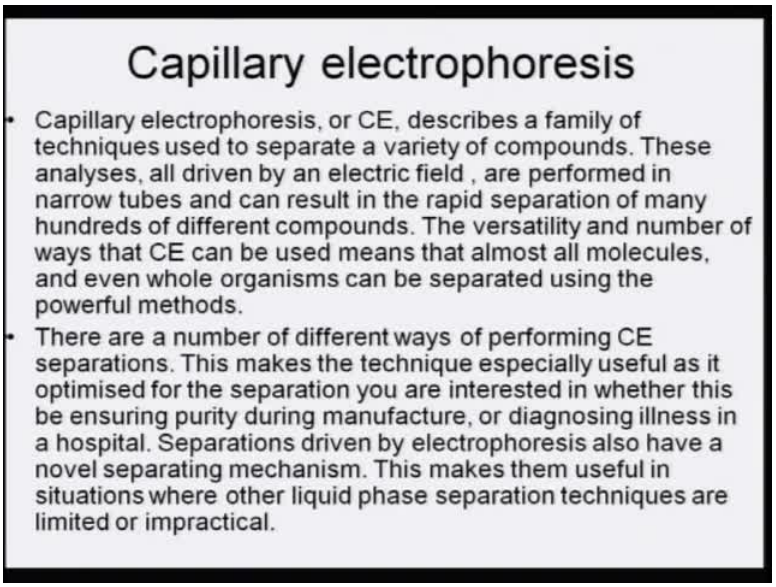

Capillary electrophoresis - this is another very specialized technique, which I wanted to draw your attention at. Capillary electrophoresis or CE, as which we would refer all along, describes a family of techniques used to separate a variety of compounds. These analyses, all driven by an electrical field, are performed in narrow tubes and can result in the rapid separation of many hundreds of different compounds. The versatility and the number of ways that CE can be used means that almost all molecules and even the whole organisms can be separated using the powerful methods.

There are a number of different ways of performing CE separations. This makes the technique especially useful as it optimizes further separation you are interested in,

whether this is to be ensuring purity during manufacture or diagnosing illness in a hospital and many such intricate analyses.

Separations driven by electrophoresis also have a novel separating mechanism. This makes them useful in situations where other liquid phase separation techniques are limited or not are practical to be applied.

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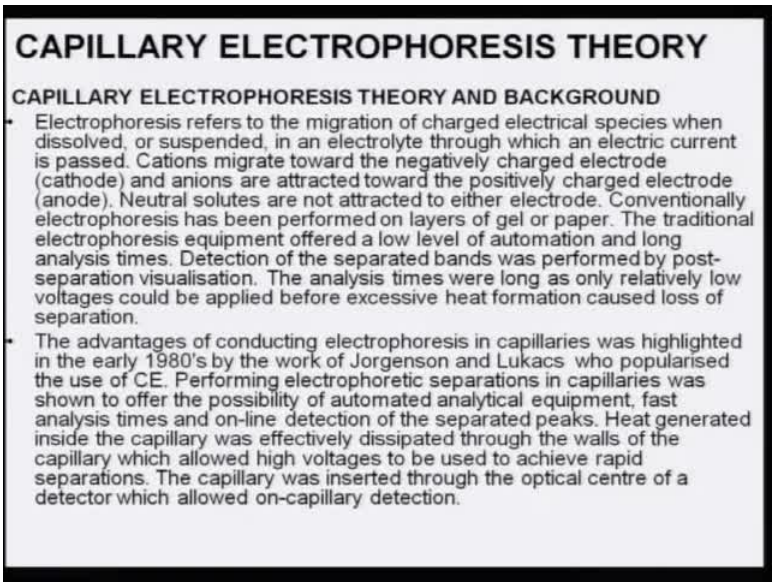

Capillary electrophoresis theory - how does it work? Capillary electrophoresis theory and its background shows that the migration of charged electrical species, when dissolved or suspended in an electrolyte through which an electrical current is passed, the cation migrates towards the negatively charged electrode, as you would know and the anions are attracted towards the positively charged electrode; neutral solutes are not attracted to either electrodes.

Conventionally, electrophoresis has been performed on layers of gel or paper. The traditional electrophoresis equipment offered a low level of automation and long analysis times. However, the detection of the separated bands was performed by post separation visualization. The analysis times were long as only relatively low voltages could be applied before excessive heat formation caused loss of separation.

So, this was the earliest, you know, the infant stage of capillary electrophoresis. However, the advantages of conducting electrophoresis in capillaries was highlighted in

the year 1980's by the work by Jorgenson and Lukacs, who popularized the use of capillary electrophoresis. Performing the electrophoretic separation in capillaries was shown to offer the possibility of automated analytical equipment, fast analysis times and online detection of the separated peaks.

Heat generated inside the capillary was effectively dissipated through the walls of the capillary, which allowed high voltages to be used to achieve rapid separations. The capillary was inserted through the optical center of a detector, which allowed on-capillary detection. So, you see that one can see the detection while the separation is taking place.

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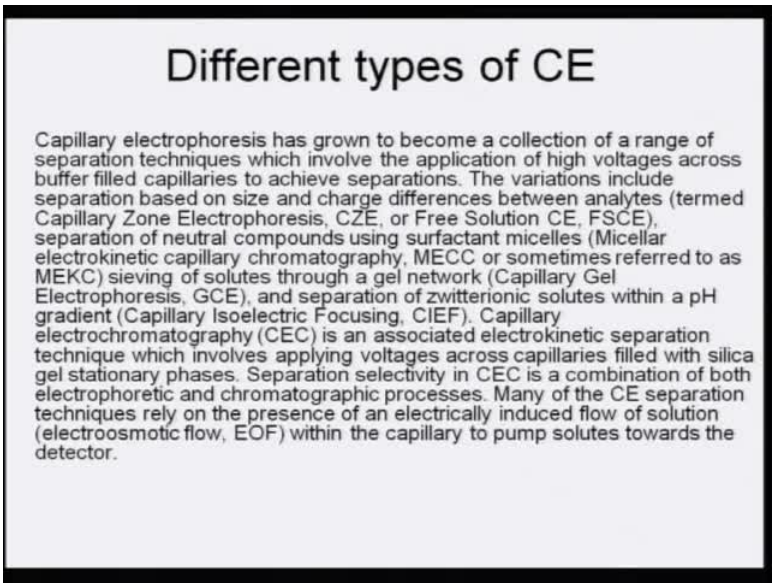

Different types of capillary electrophoresis - capillary electrophoresis has grown to become a collection of a range of separation techniques which involve the application of high voltages across buffer filled capillaries to achieve separations. The variations include: separation based on size and charge differences between analytes - capillary zone electrophoresis, that is, CZE or free solution, that is, CE, separation of neutral compounds using surfactant micelles - Micellar Electrokinetic Capillary Chromatography or sometimes referred to as MEKC, sieving of solutes through the gel network, that is, capillary gel electrophoresis and separation of zwitterionic solutes within a pH gradient, which is called capillary isoelectric focusing. Capillary electro

chromatography is an associated electrokinetic separation technique, which involves applying voltages across the capillary filled with silica gel stationary phases.

Separation selectivity in CEC is a combination of both electrophoretic and chromatographic processes. Many of the capillary electrophoresis separation techniques rely on the presence of the electrically induced flow of solution, that is, electroosmotic flow within the capillary to pump the solutes towards the detector. So, how the process takes place, how there are different, you know, different types of capillary electrophoresis activity taking place gives an idea that what a huge range it is all by itself. Almost like equivalent to a GC or an HPLC and in the modern times, capillary electrophoresis is really being used largely by analysts.

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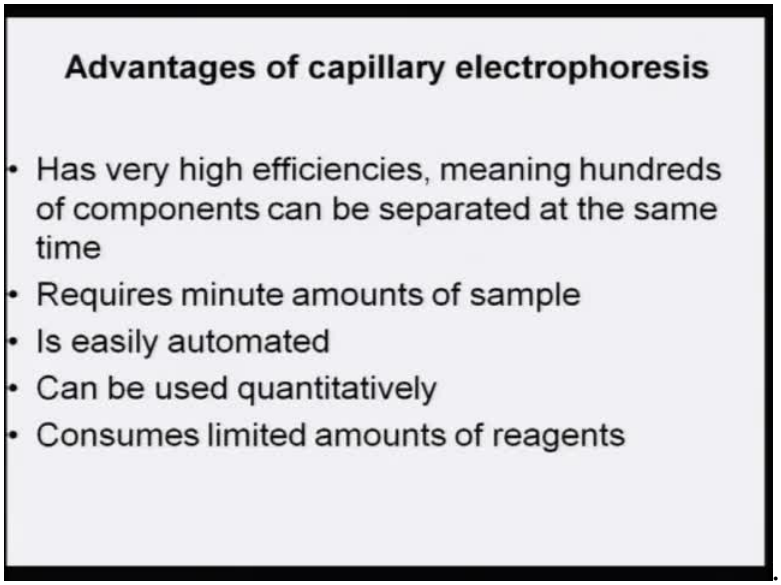

Advantages of capillary electrophoresis - has very high efficiency, meaning hundreds of components can be separated at the same time, requires minute amounts of samples, is easily automated, can be used quantitatively and consumes limited amounts of reagents. So, you see in all aspects, it is one of the most efficient and economical method.

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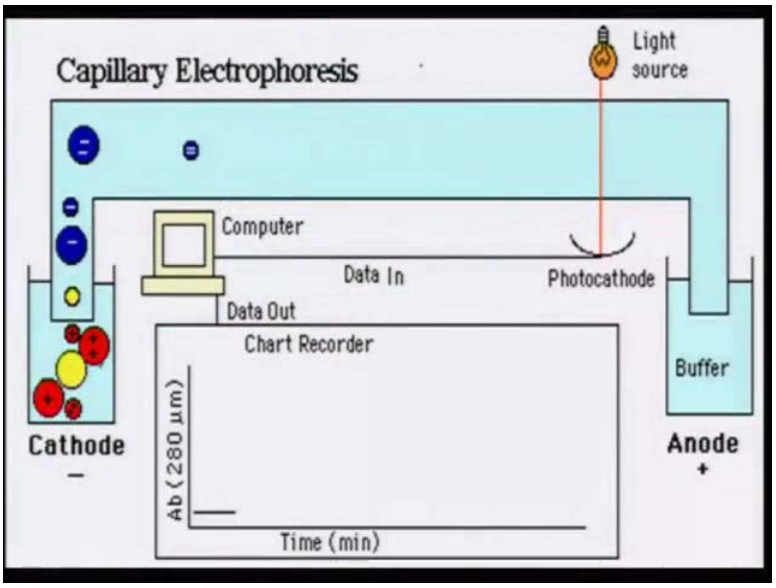

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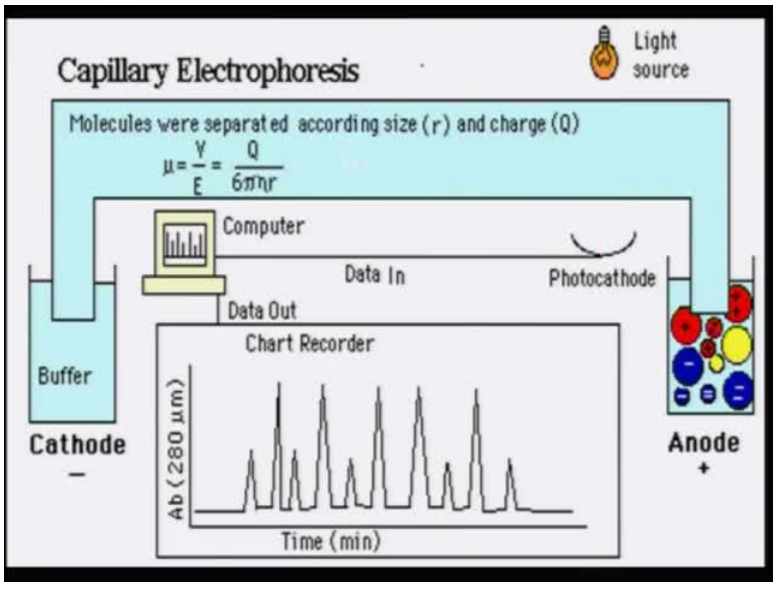

Capillary electrophoresis - if one has to see it in pictorial way, I have taken this example and would retain it for some time so that one can see how these charged ions are first separated and how they are, you know, transferred one by one to the different electrodes and they are trapped there and there is a recorder, which gives a recording to show how they are analyzed simultaneously.

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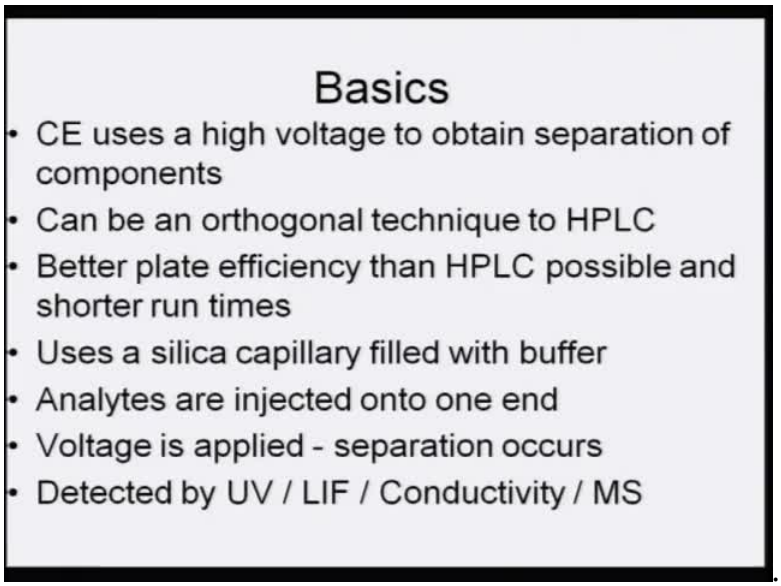

Basics - the capillary electrophoresis uses a high voltage to obtain separation of components, can be an orthogonal technique to HPLC that means it can be in competition with the HPLC machine, better plate efficiency than HPLC possible and shorter run times. So, you see it has an edge over the HPLC because it requires only very small time to run and do the analysis. Uses a silica capillary filled with buffer, analytes are injected onto one end, voltage is applied - separation occurs and it is detected either by a UV or a conducting device or a mass detector.

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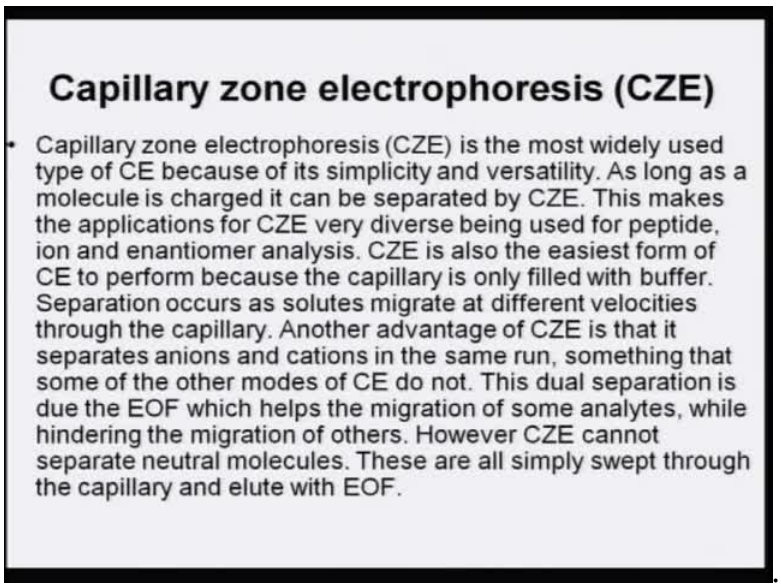

Capillary zone electrophoresis - capillary zone electrophoresis, that is, CZE is one of the widely used type of capillary electrophoresis because of its simplicity and versatility. As long as a molecule is charged, it can be separated by CZE. That means for capillary zone electrophoresis, the molecule must have some kind of charge; otherwise, neutral molecule cannot be separated. This makes the application of CZE very diverse, being used for peptide ion and enantiomer analysis. CZE is also the easiest form of capillary electrophoresis to perform because the capillary is only filled with buffer.

Separation occurs as solutes migrate at different velocities through the capillary. Another advantage of CZE is that it separates anions and cations in the same run as what we saw in the picture, which was shown earlier, Sometimes and something that some of the other modes of capillary electrophoresis do not.

This dual separation is due to the electric charges that are present, which help the migration of some analytes, while hindering the migration of the others. However, CZE cannot separate neutral molecules; this have has I have told time and again. There are only simple swept through the capillary and elute with the help of this electroosmotic pressure.

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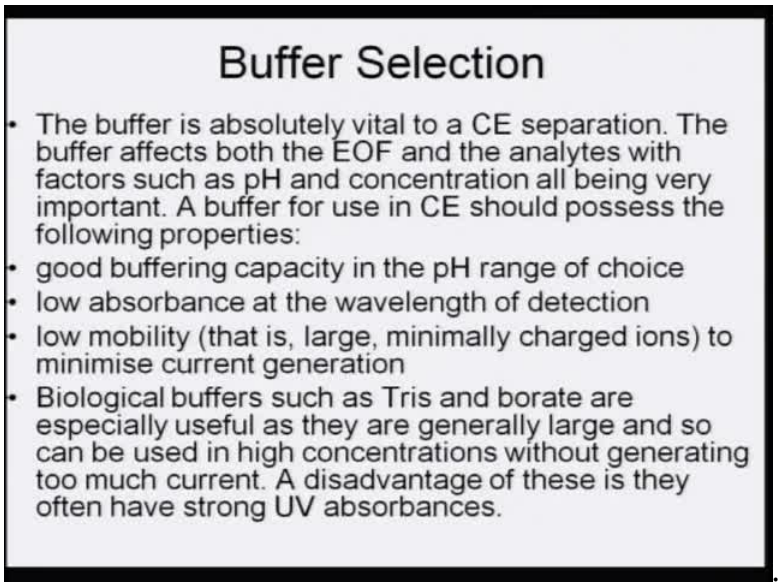

Buffer selection - that because everything is happening in this buffer solution, and so, the selection of the buffer solution is very crucial. The buffer is absolutely vital to a capillary

electrophoresis separation. The buffer affects both the electroosmotic pressure and the analytes with factors such as pH and concentration, all being very important.

A buffer for use in CE should possess the following properties: it should be a good buffering capacity in the pH range of choice; low absorbance at the wavelength of detection; low mobility, that is, large minimum charged ions to minimise current generation.

Biological buffer such as tris and borate are especially useful, as they are generally large, and so, can be used in high concentrations without generating too much current. A disadvantage of these is they have often strong UV absorbances.

So, now, I said it should not have an absorbance in the region where the analyte is present, but this some of these buffers are very strong UV absorbers. So, that is a little tricky. One needs to choose the UV the buffer solution, which does not interfere with the UV activity of the analyte.

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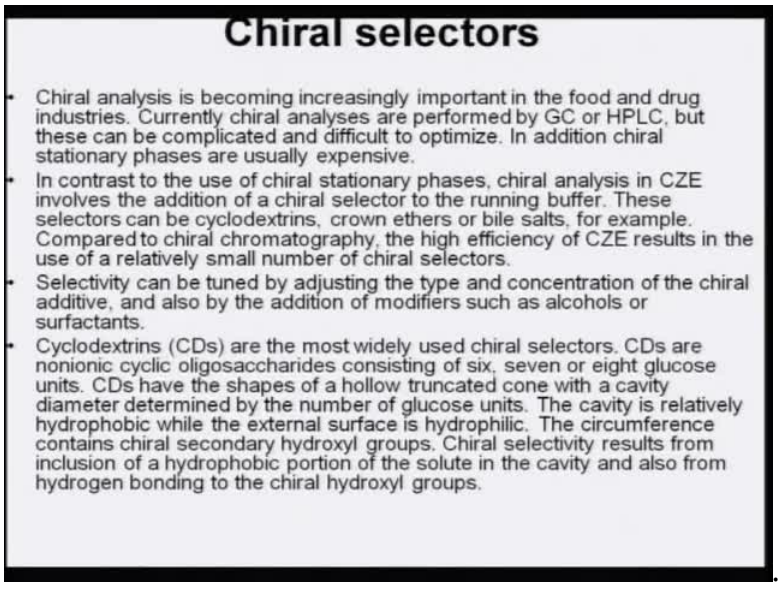

There are chiral selectors and chiral analysis is becoming increasingly important in the food and drug industries. Currently, chiral analyses are performed by GC or HPLC, but now the capillary zone electrophoresis is picking up. Cyclodextrins are most widely used chiral selectors. CDs are nonionic cyclic oligosaccharides consisting of 6, 7 or 8 glucose units.

A little while ago, I have shown you what cyclodextrins looks like. CDs have the shape of a hollow truncated cone and a cavity diameter determined by the number of the glucose units. The cavity is relatively hydrophobic, while the external surface is hydrophilic. So, cyclodextrin molecule has an exterior, which is hydrophobic has an interior, which is hydrophobic and has an exterior, which is hydrophilic. The circumference contains chiral secondary hydroxyl groups.

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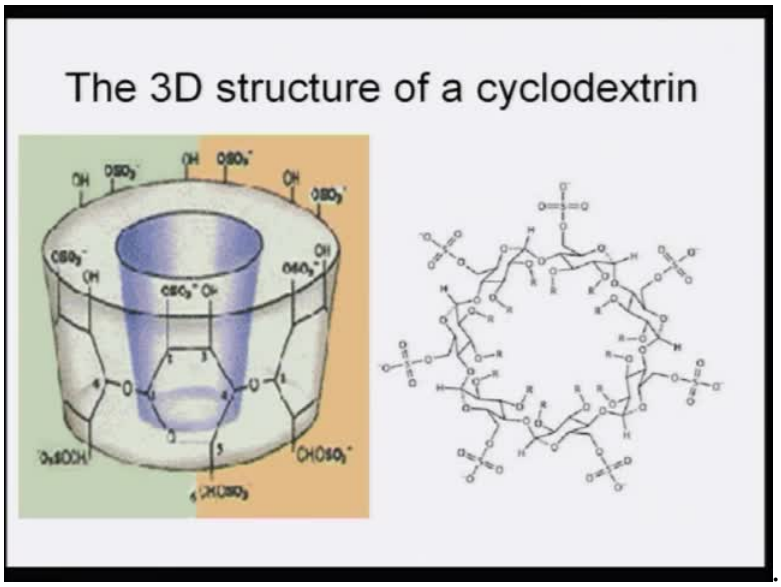

Chiral selectivity results from inclusion of a hydrophobic portion of the solute in the cavity and also from hydrogen bonding in the chiral hydroxyl groups. So, that is how the chirally it is selected. Now, if you see the 3D picture of cyclodextrin, it will be much more clear to you that how it forms the circumference and the inner diameter is hydrophobic and the outer diameter is hydrophilic.

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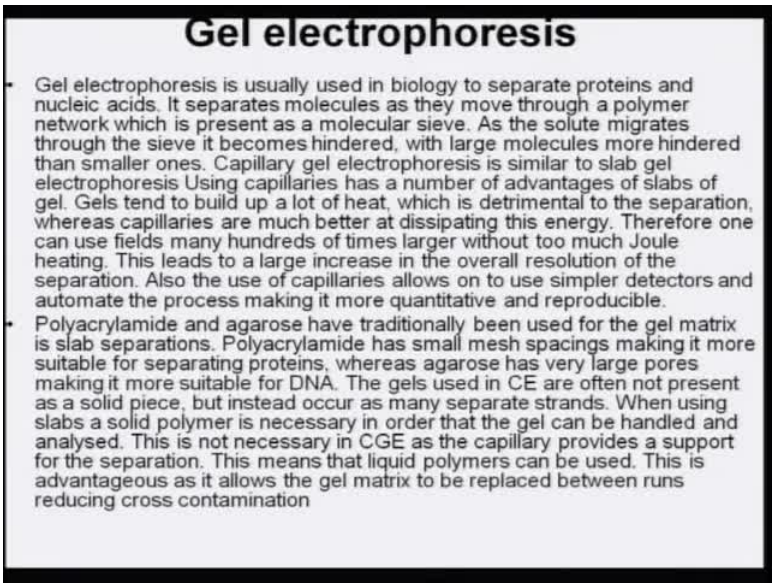

Gel electrophoresis - gel electrophoresis is usually used in biological system to separate proteins and nucleic acids. It separates molecules as they move through a polymeric network, which is present as a molecular sieve. As a solute migrates through the sieve, it becomes hindered, with large molecules more hindered than the small ones.

Capillary gel electrophoresis is similar to slab gel electrophoresis. Using capillaries has a number of advantages of slabs of the gel. Gels tend to build up lot of heat, which is detrimental to the separation, whereas capillaries are much better at dissipating this energy.

Therefore, one can use fields many hundreds of times larger without too much joule heating. This leads to a large increase in the overall resolution of the separation and that is how also the use of capillaries allows the use of detectors and automates the process, making it a quantitative and reproducible method.

Polyacrylamide and agarose have traditionally been used as gel matrix in slab separations. Polyacrylamide has small mesh spacing making it more suitable for separating proteins, whereas agarose has very large pores making it more suitable for DNA. The gel used in capillary electrophoresis often are present as a solid piece, but instead occur as many separate strands. While using a slab, a solid polymer is necessary in order that the gel can be handled and analysed.

This is not necessary in the CGE method as the capillary provides a support for the separation. This means that liquid polymers can also be used. This is advantageous as it allows the matrix to be replaced between runs reducing cross contamination. So, one can keep on changing the gel as and when required, when it is felt that the gel is contaminated and the gel electrophoresis is nothing, but a kind of a mesh through which some can pass and others will be restricted.

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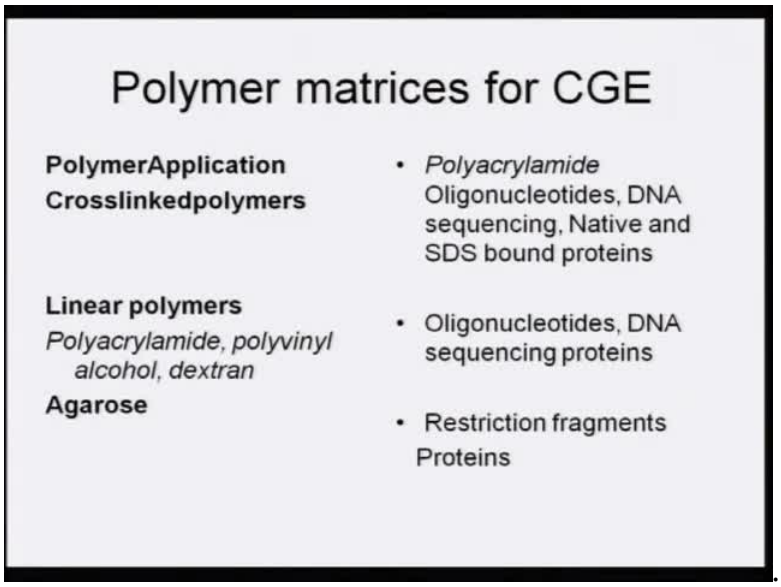

So, for polymer matrix for CGE, the applications are that polymer application, cross linked polymers are used and for polyacrylamide oligonucleotides, DNA and so on can be used.

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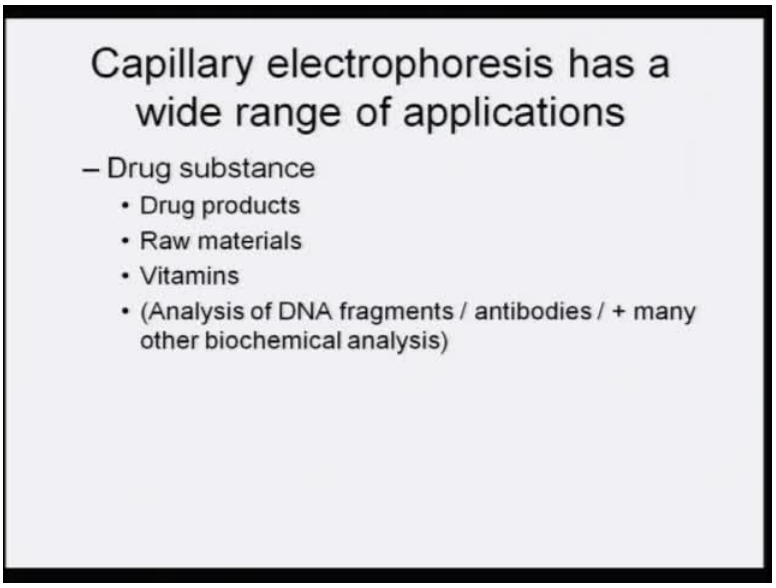

So, here is a kind of compability. What can be used for what kind of molecules? Capillary electrophoresis has a wide range of applications in drug substance, drug products, raw material identification, vitamin and identification, analysis of DNA fragments, antibodies, many other biological analysis and the list is exhaustive.

So, with this we have come to an end of the capillary electrophoresis, which is such an important area for the nucleic acid and the protein analysis.