Analytical Science

A course (in 15 Chapters), developed as an Open Educational Resource, designed for use at 2nd year England & Wales undergraduate level and as a CPD training resource

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Chapter 8 – Capillary Electrophoresis

Contents

Introduction

Slide (16) in Chapter 7 illustrated the advantage of capillary columns over packed columns in gas-liquid chromatography. Capillary columns provide sharp, well separated peaks with short retention times. However there is a limit to how sharp the peaks can be and this is due to the fact that the mobile phase is pumped through the column.

Pumping a liquid or gas through a column results in a particular flow profile as the liquid in contact with the sides of the column is slowed down by friction and therefore the liquid in the centre of the column moves slightly faster (laminar flow). Figure (8.1) illustrates this

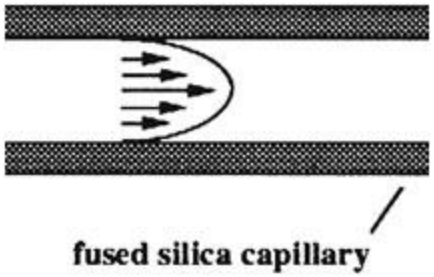


Figure (8.1) illustrates pressure driven flow. The analyte in the centre of the column reaches the detector slightly before the analyte molecules nearer the sides. This results in peak broadening.

Figure 8.1 - pressure driven flow

Continued on the next slide

If it was possible to alter the flow profile to that illustrated in figure (8.2) it would be possible to obtain sharper peaks

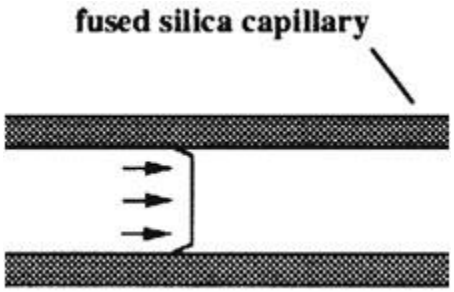


Figure 8.2 - plug flow profile

Figure (8.2) the profile of 'plug' flow. With this type of flow, friction effects are minimised so analyte molecules reach the detector at the same time.

Electrophoretic flow has the flow profile given in the above diagram and this lead to the development of the separation technique which is known as **capillary electrophoresis**.

Capillary electrophoresis (ce) is not a chromatographic method but it is a separation method and its theory parallels that of chromatography which was considered in detail in the previous two Chapters.

Definition of **electrophoresis**
The transport of electrically charged compounds in solution under the influence of an electric field

So capillary electrophoresis combines the advantages of chromatography with electrophoresis. A plot of the results of a ce separation looks very like a chromatogram but is called an **electropherogram**, the detector response is plotted against **migration time**, the ce equivalent of retention time.

As with chromatography, good separations can be achieved for small samples with short analysis times. Polar and non-polar analytes can be separated and detected at part per million concentrations.

The capillary electrophoresis techniques are:

- **Capillary zone electrophoresis (cze);** this is the simplest form of capillary electrophoresis (ce) it is also known as free solution ce.
- Isoelectric focussing;
- Capillary gel electrophoresis;
- Isotachophoresis;
- **Micellar electrokinetic capillary chromatography**.

Of these, only the two highlighted will be considered in this Chapter.

Capillary Electrophoresis - instrumentation

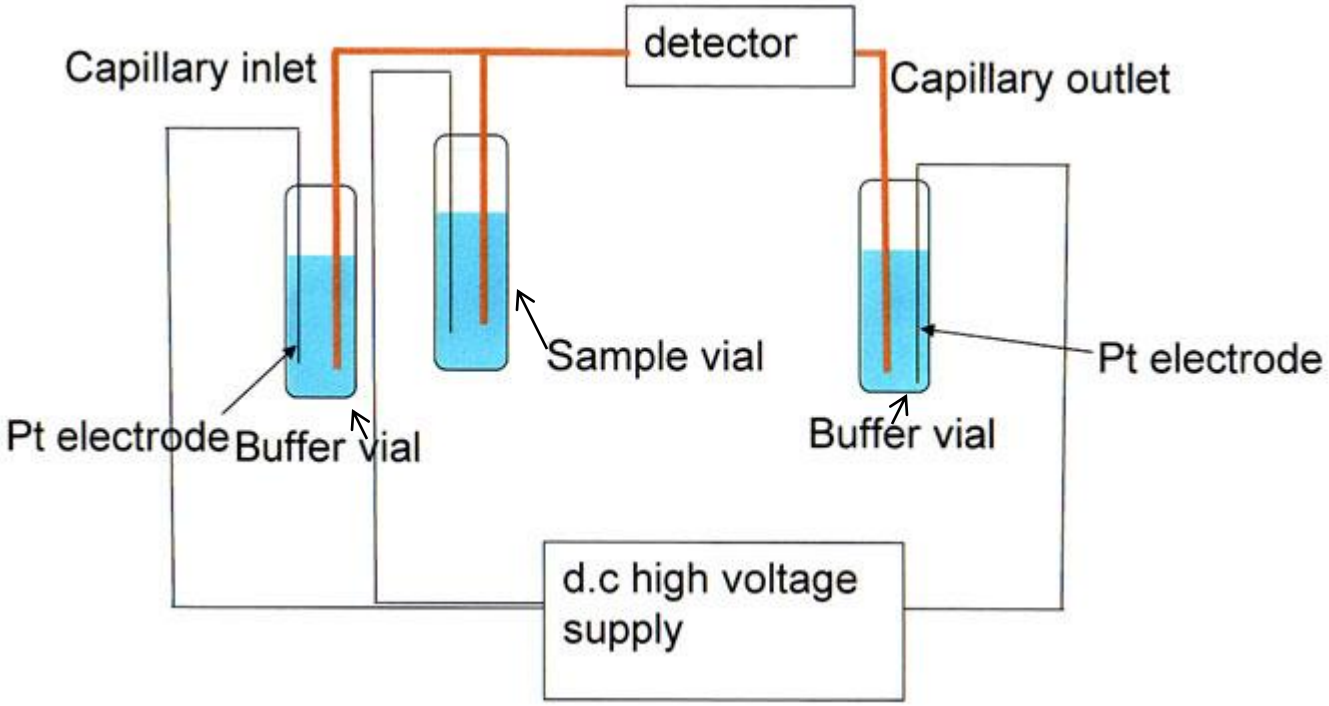


Figure 8.3 – schematic diagram of ce instrumentation

Figure (8.3) shows a schematic diagram of a capillary electrophoresis Instrumentation set up. Electrophoretic flow through a capillary column, is achieved by applying a high voltage, up to 30,000 volts, whilst the ends of the capillary are immersed in a buffer solution. To introduce the sample, the capillary and electrode are briefly immersed into the sample vial, see next slide. A small aliquot (typically nanolitre), goes into the capillary either by applying a lower voltage e.g. 2,000 volts for 10 seconds or by means of a small pressure difference across the capillary. After injection the sample vial is replaced by the buffer vial. The high voltage is applied across the capillary column, this results in the analytes migrating due to the induced **electroosmotic** flow and electrophoretic effects. The detector is situated towards one end of the capillary. A typical instrument is shown on slide 9.

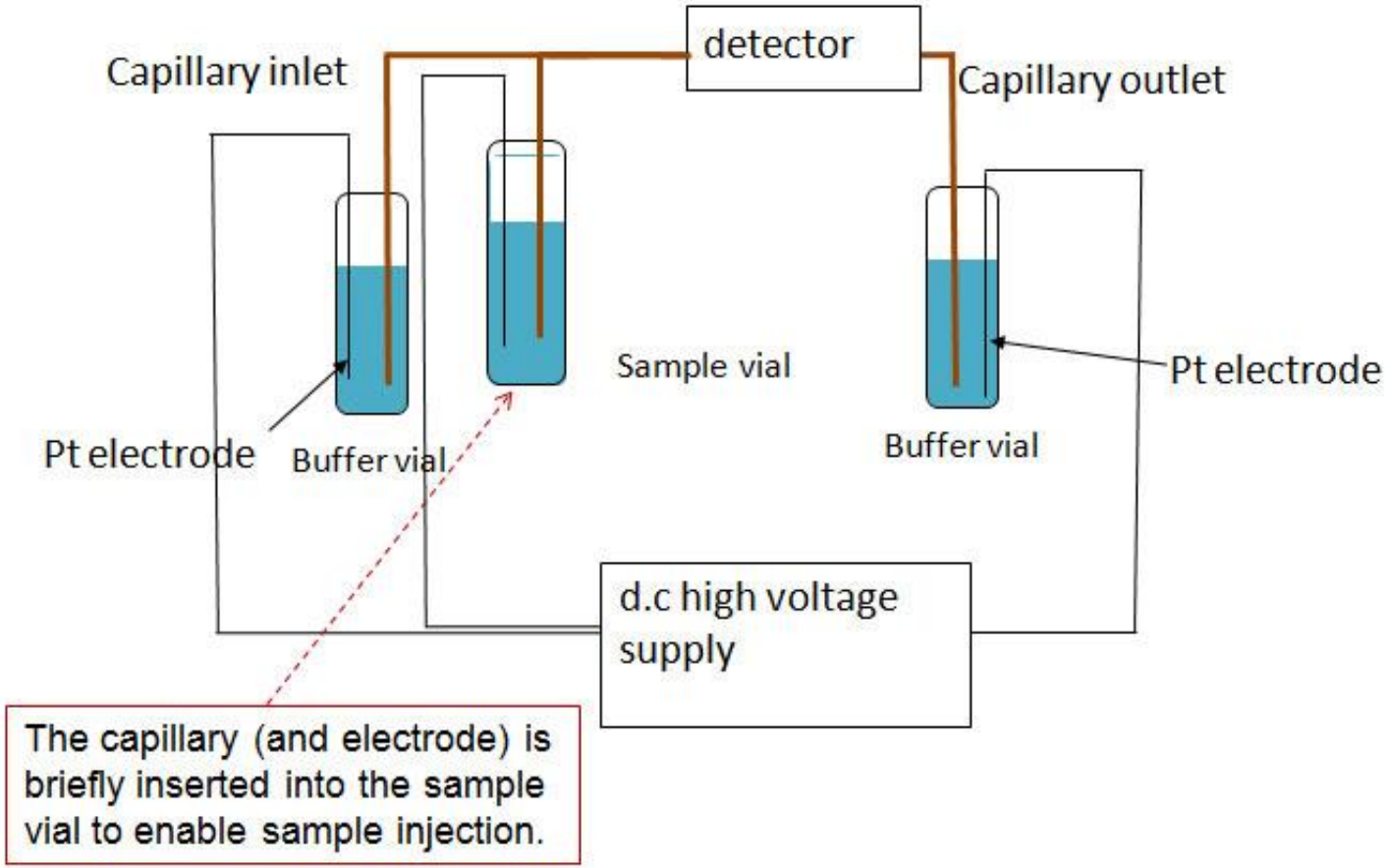
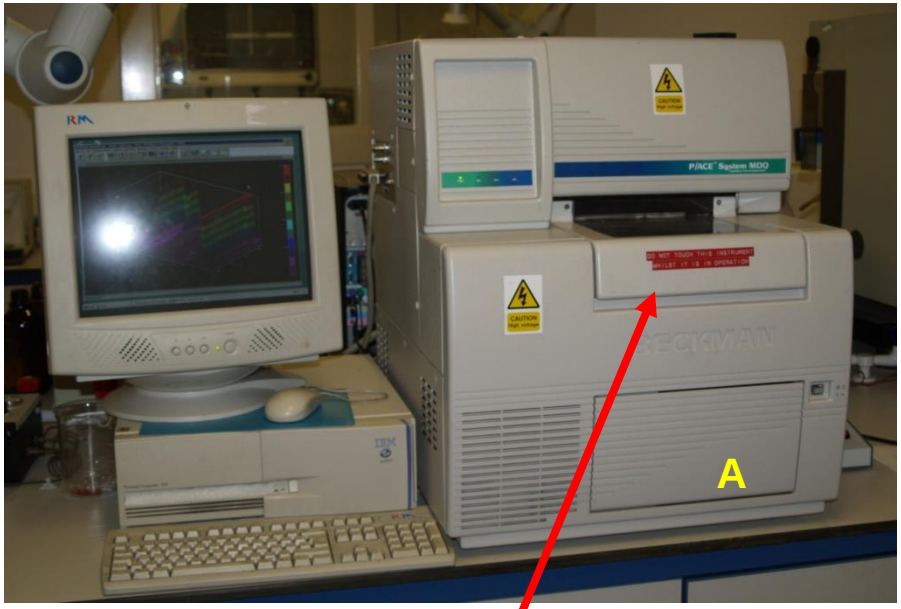
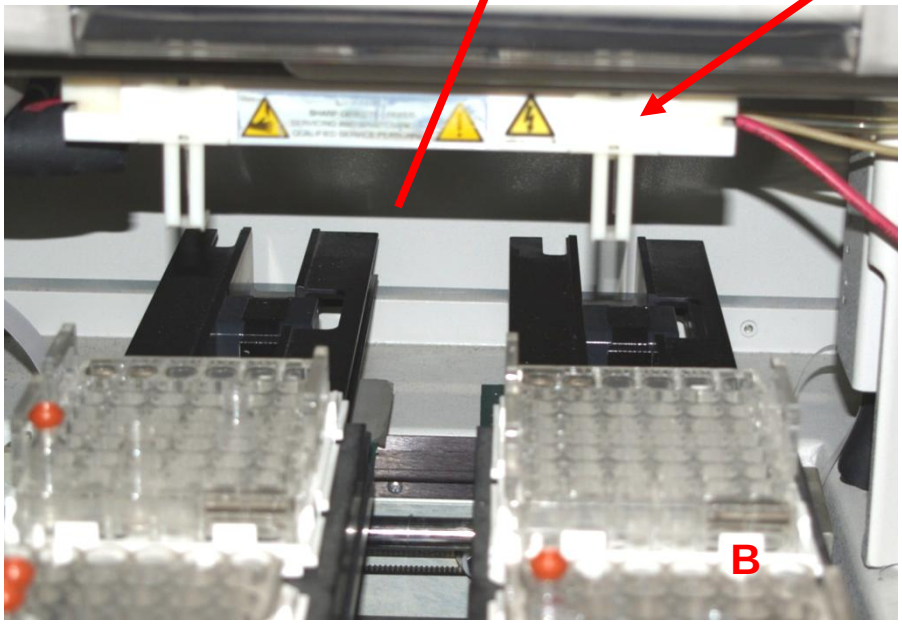


Figure 8.4 schematic diagram of capillary electrophoresis system. To inject the sample the inlet buffer vial is replaced by the sample vial for the duration of the injection and then the inlet buffer solution moved back into its original position.

Figure 8.5 - Beckman PACE ce instrument





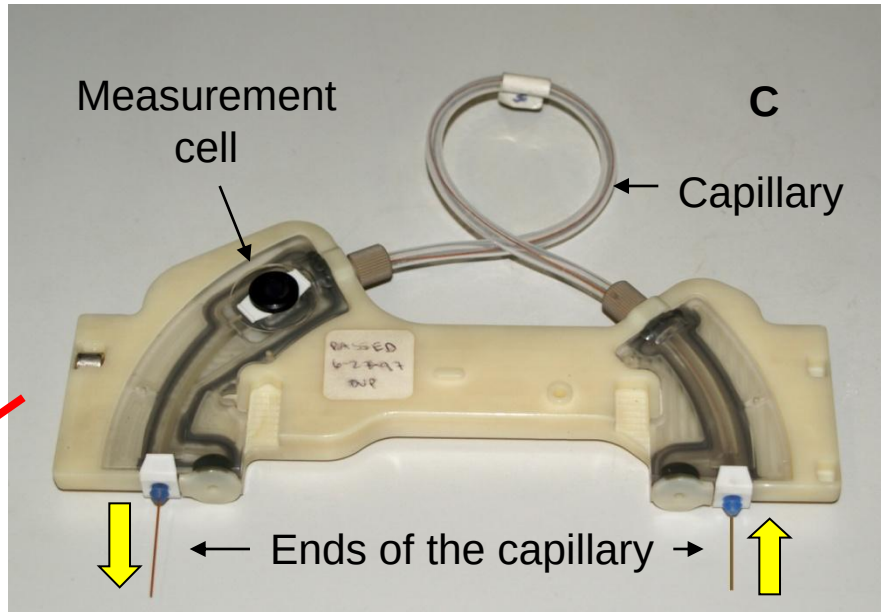


Figure (8.5) shows the instrument and data output. (B) shows the inside of the sampling compartment offering automated sampling and analysis from samples placed in a tray of sample vials. (C) shows the capillary protected by a plastic tube and the measurement cell. Figure (8.6) on the next slide shows an electropherogram when using a diode array detector

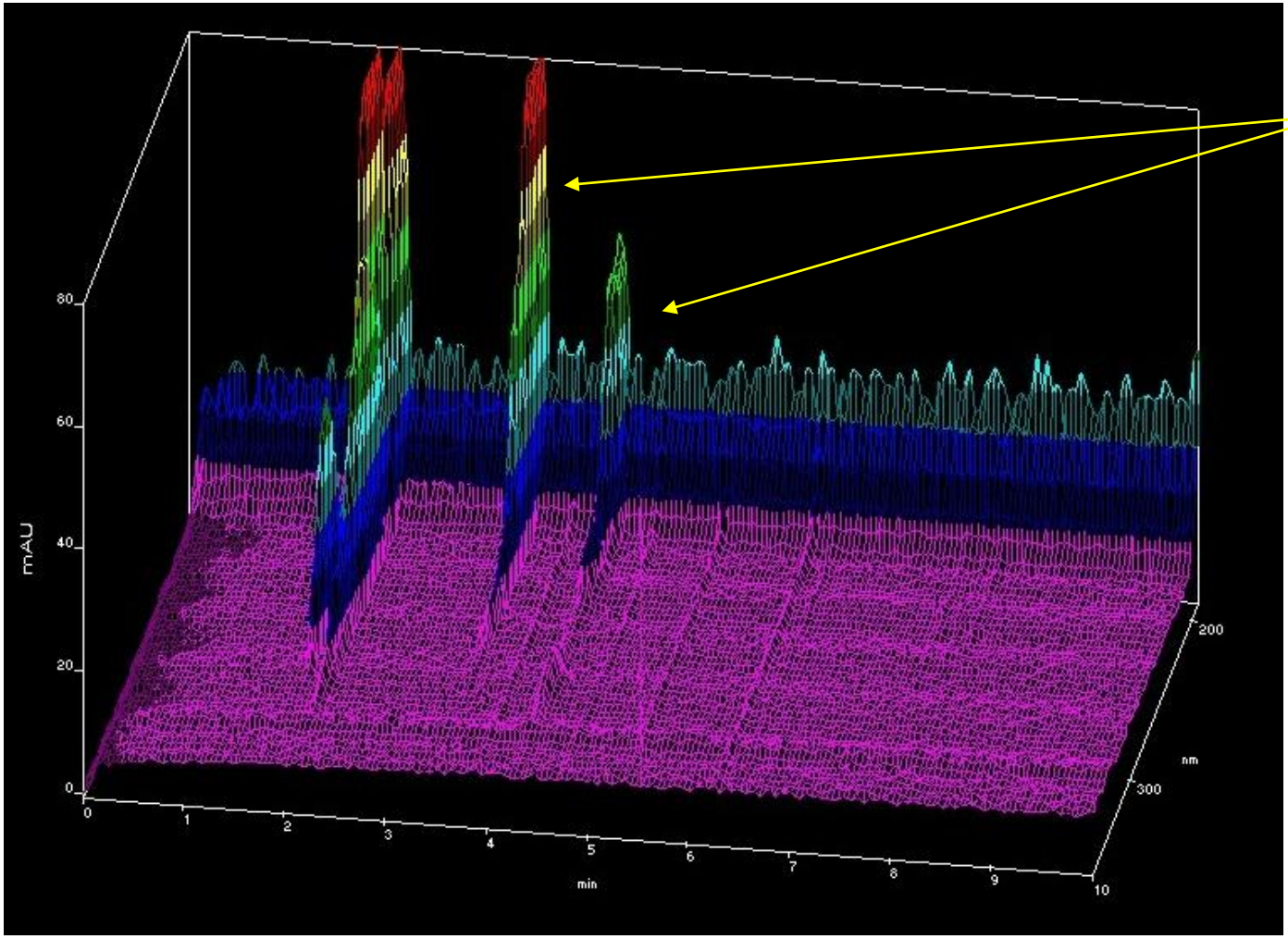


Figure 8.6 – 3 dimensional electropherogram showing the separation of 3 substances by ce

Figure (8.6) shows a typical electropherogram from a ce separation using a diode array detector. The axes are: Y (absorbance), X (time in minutes) and Z (wavelength scan between 200 – 300 nm). The UV spectrum obtained for each substance separated by the ce system can help to confirm the identity of the separated substances.

CE cells and detectors

The following analytical techniques are used to detect analytes separated using ce methodologies:

- UV - including fixed wavelength and diode-array technologies;
- Fluorescence;
- Mass spectroscopy;
- Electrochemical detection.

UV detection is probably the most common method of detection, however in order to increase the pathlength and thus analytical sensitivity, measurement cells are frequently manipulated as illustrated in figure (8.7):

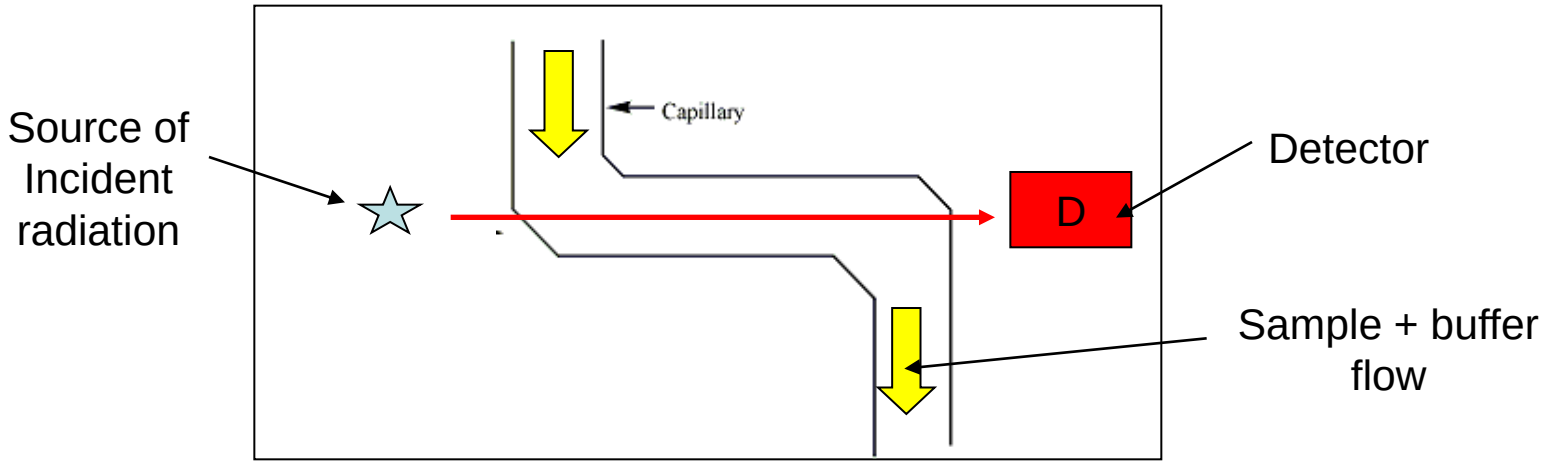


Figure 8.7 - possible cell design for UV cells

Theory of capillary zone electrophoresis (cze)

Electrophoretic mobility

Under the influence of an electric field, an electrically charged solute will migrate through a buffer with an electrophoretic velocity, ν_{ep} cm/s.

$$\nu_{ep} = \mu_{ep} E \qquad \text{Equation (8.1)}$$

where E (V/cm) is the electric field and μ_{ep} (cm^2/s) is the electrophoretic mobility which is proportional to the charge and size of the migrating species.

μ_{ep} can be expressed as:

$$\mu_{ep} = \frac{q}{6\pi\eta r} \qquad \text{Equation (8.2)}$$

where r is the radius of the migrating molecule, q is its charge, and η is the buffer viscosity.

If the charge on an analyte is zero it will have no electrophoretic velocity.

Electroosmotic flow

In addition to the electrophoretic effects, the electroosmotic flow moves the buffer and **all** analytes (not just those that are charged) through the capillary column.

When a buffer is placed inside a capillary the inner surface of the capillary acquires a charge.

In the case of silica at pH values greater than 3, the Si –OH groups are ionised to $Si\text{-}O^-$, causing this charged surface to attract buffer cations.

Two layers (a double layer of buffer ions) will be attracted to the charged surface. The layer closest to the capillary wall will be fixed due to the mutual attraction of the ions, the second layer will be able to move when the electric field is applied. This mobile layer of ions will be surrounded by solvent molecules which will be dragged along as the ions move – this is electroosmotic flow and illustrated diagrammatically as figure (8.8) on the next slide.

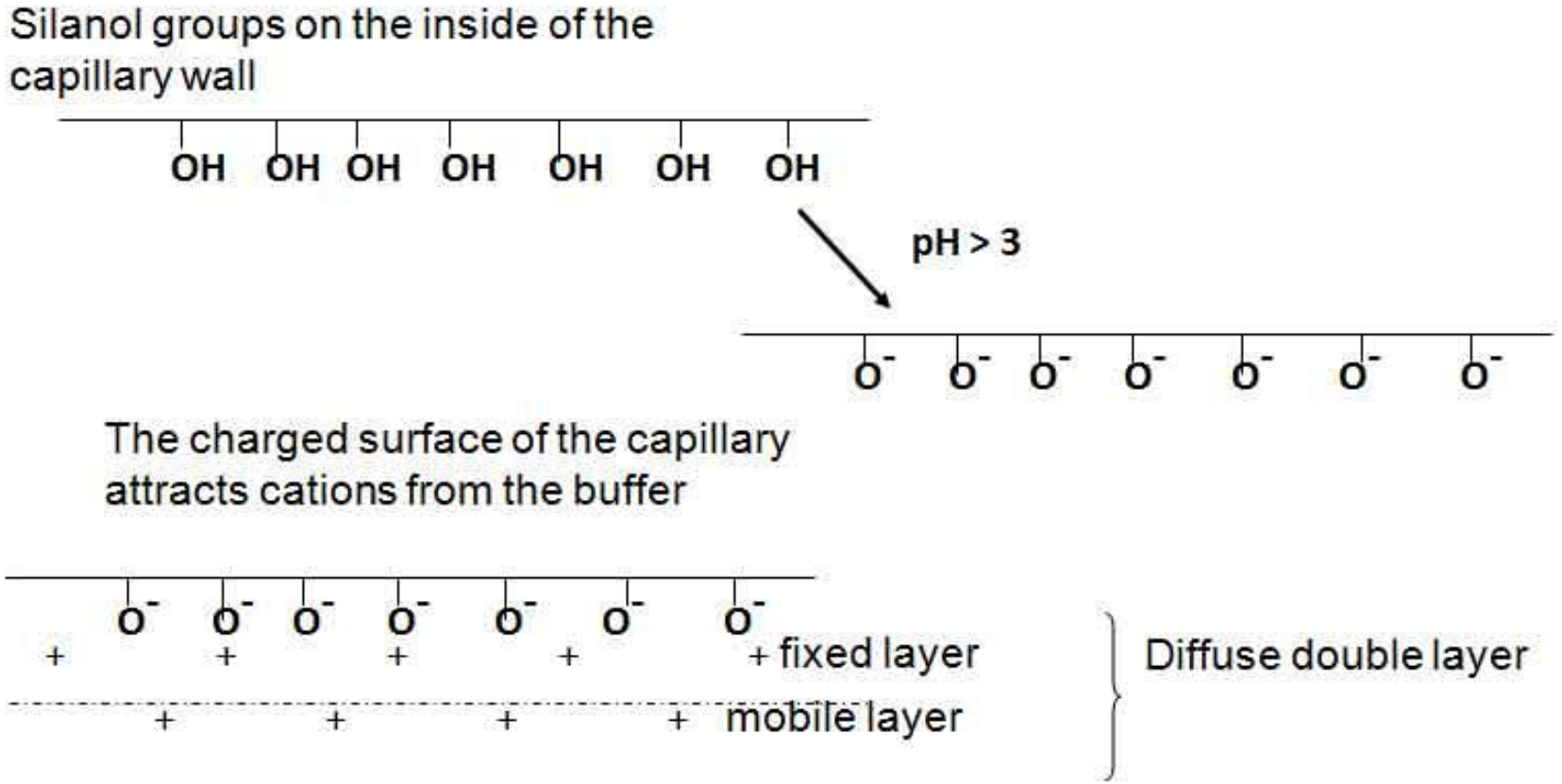


Figure 8.8 diagrammatic representation of electroosmotic flow

Effect of flows in cze

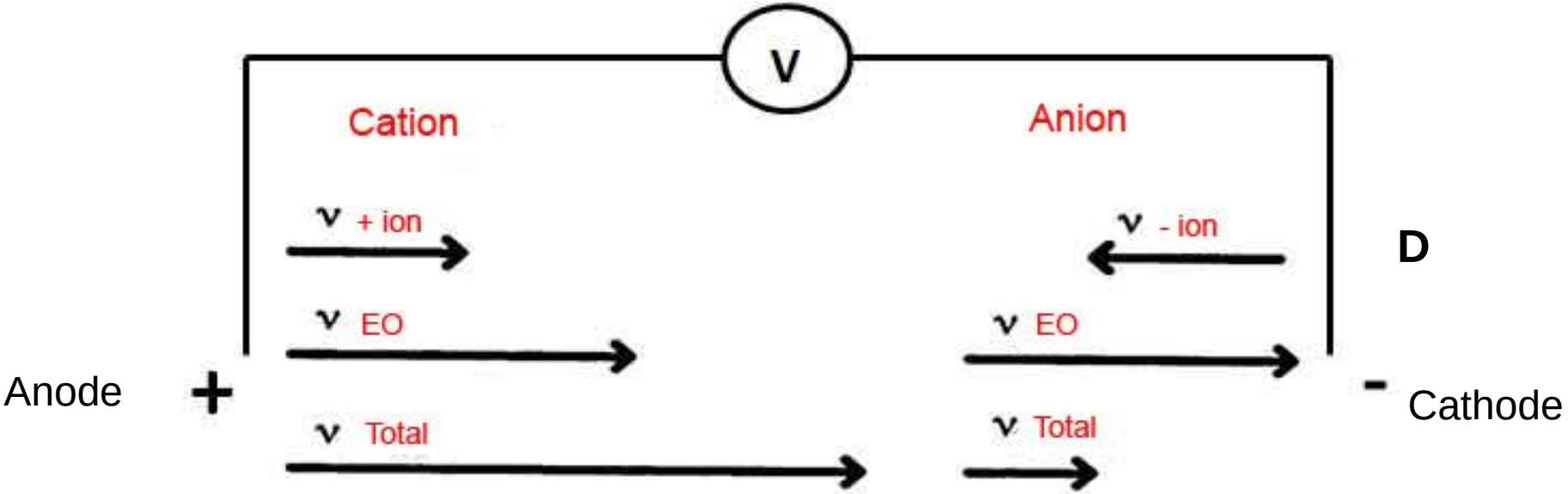


Figure 8.9 - total effect of electrophoretic and electroosmotic flows in cze

In figure (8.9) above, the length of the arrows is proportional to the velocity, therefore if the detector (D) is placed near the cathode, the cations will be detected first and the anions last, with neutral analytes in between. The electroosmotic flow is greater than the electrophoretic flow, hence despite anions being attracted to the anode (against the electrophoretic flow), they will eventually reach the cathode. **Note that anions and cations can be separated in the same sample solution but that neutral ions, which will be subject only to electroosmotic flow, will NOT be separated from each other.**

Micellar electrokinetic capillary chromatography (mecc)

Mecc combines the separation mechanism of chromatography with the electrophoretic and electroosmotic movement of solutes and solution. This is achieved by adding a surfactant, in order to create micelles, to the buffer solution which flows through the capillary. Micelles are described on the next slide.

- The advantage of mecc is that neutral analytes (solutes) can be separated in addition to charged analytes.

- As **partitioning** effects, facilitate the separation of all types of analytes, the plot of detector response versus time is known as an **electrokinetic chromatogram.**

Micelles

Micelles are aggregates of molecules known as surfactants. A surfactant is a long chain molecule which has a long hydrophobic (water-hating) tail and a hydrophilic (water-loving) head. Sodium dodecyl sulphate is a surfactant, the dodecyl hydrocarbon chain is the hydrophobic tail. This is illustrated in figure (8.10).

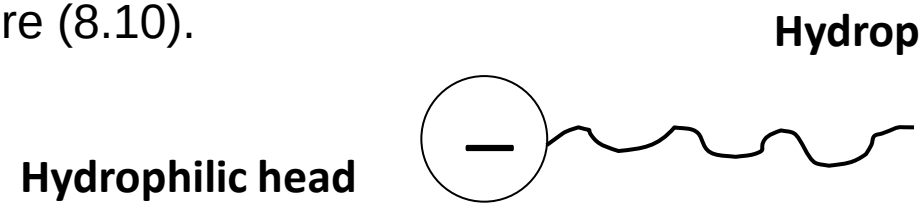


Figure 8.10 - diagram of a surfactant molecule

When the surfactant concentration in an aqueous solvent reaches the critical micelle concentration (cmc) the molecules aggregate, or clump together, with the hydrophobic tails pointing inwards, away from the water they hate, and the hydrophilic heads pointing outwards - towards the water they love – see figure (8.11)

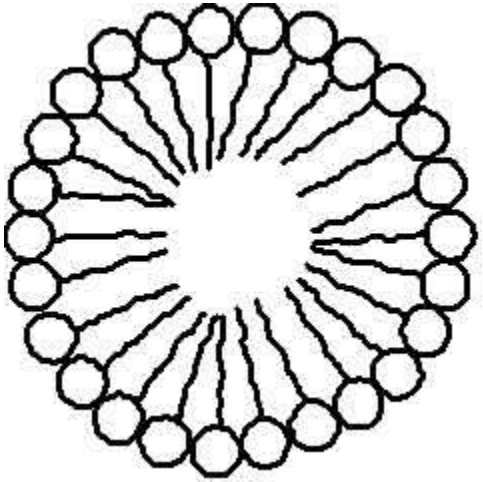


Figure 8.11 - diagram of a micelle

Continued on the next slide

In mecc, separation occurs due to:

- Electrophoretic and electroosmotic flows as in cze;
- The micelles acting as a **psuedo** stationary phase which the analytes can partition into from the buffer solution.

At a neutral to alkaline buffer pH, a strong electroosmotic flow moves in the direction of the cathode. If sodium dodecyl sulphate is used as the surfactant the electrophoretic flow will pull the negatively charged, i.e. anionic micelles, against the electroosmotic flow. As a result the overall micelle migration velocity is slow compared to the bulk of the solvent.

Analytes will separate in the order of increasing migration time - **anions, neutrals and cations**. This is because the anions will spend most of their time in the bulk of the solvent as there is mutual repulsion between the negative anion and negative micelle. The neutral analytes will be separated on the basis of how they partition between the micelles and the bulk solvent; cations will elute last as there will be a strong electrostatic attraction between the positive cation and the negative micelle. A diagram illustrating these movements is shown as figure (8.12) on the next slide.

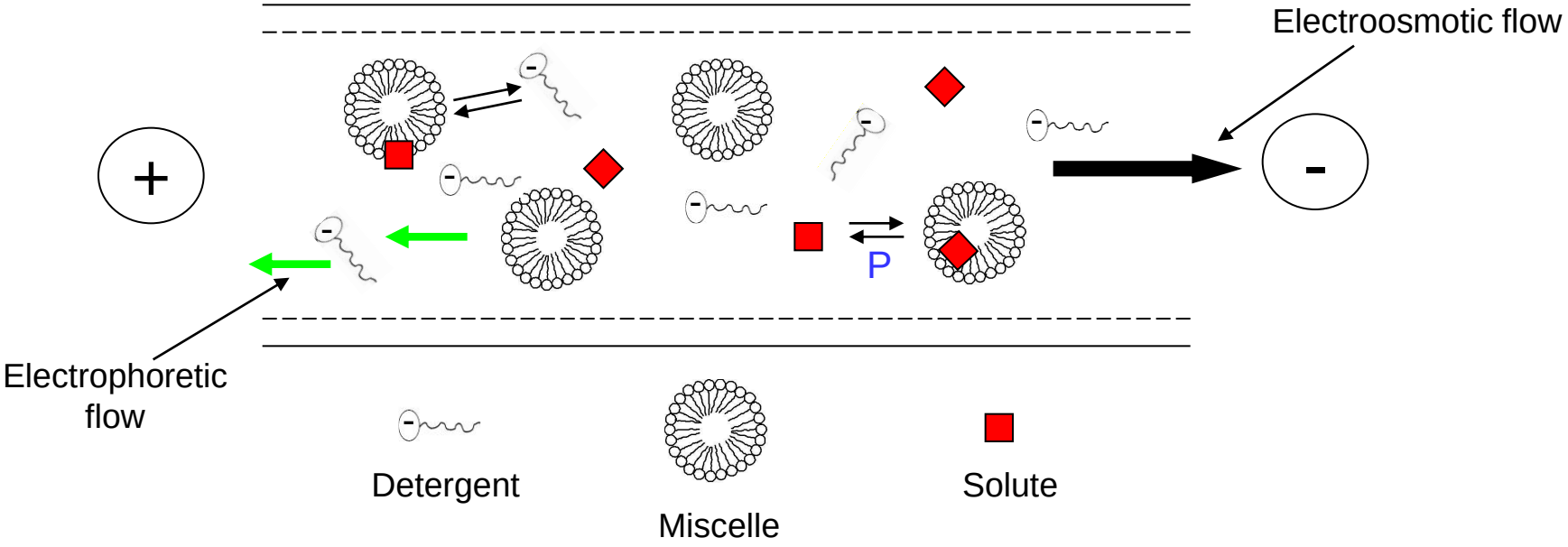


Figure 8.12 - diagram showing the processes occurring in mecc.

The equilibrium marked P is for the partition of analytes (solutes) between the bulk solvent and the micelles. Note also that the surfactant molecules also move in and out of the micelles, excess surfactant molecules remain in the bulk solvent.

Applications of capillary electrophoresis

- Capillary electrophoresis techniques have been applied to the analysis of many compounds of environmental interest such as pesticides; herbicides; and polyaromatic hydrocarbons.
- Capillary zone electrophoresis is an alternative to ion-exchange chromatography
- Many chiral enantiomers have been separated, which is of particular importance in the development of new pharmaceuticals
- Capillary electrophoresis techniques are being employed to support the development of high throughput technologies. [see figure (8.13)].

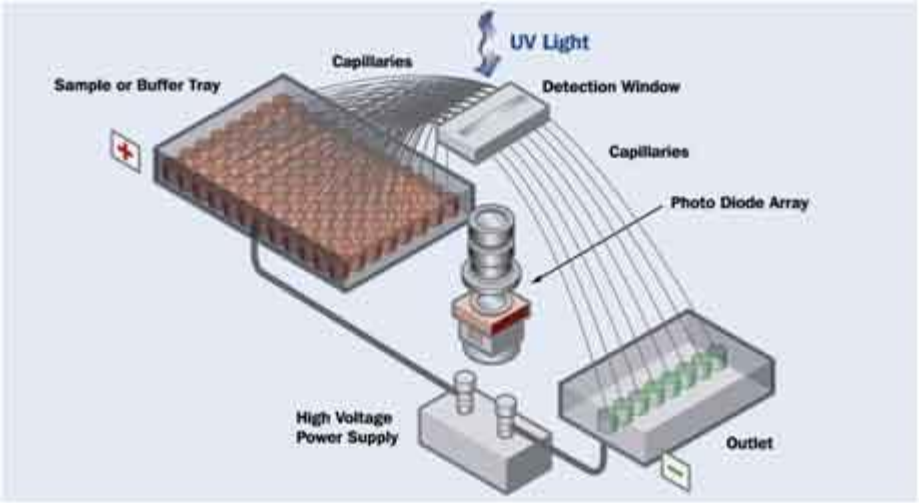


Figure 8.13

In figure (8.13), 96 samples are being analysed sequentially by ce. Each sample component reaching the detector produces a UV spectrum at the photodiode array detector. *The image is used by Permission of Advanced Analytical Technologies Inc.*

Reflection

- Capillary electrophoresis is a separation technique but not a chromatographic technique as there is no stationary phase (the micelles in mecc act as a pseudo stationary phase).
- As with chromatographic methods microlitre samples can be separated and detected
- Separation is achieved due to electroosmotic and electrophoretic effects on analytes in a buffer solution as they flow through a capillary column
- Instead of retention times in ce analytes have migration times
- Migration times are used to ‘identify’ the analyte by comparison with standards.
- The only way to characterise an analyte is to use ce in conjunction with mass spectrometry.
- It is possible to obtain qualitative and quantitative results