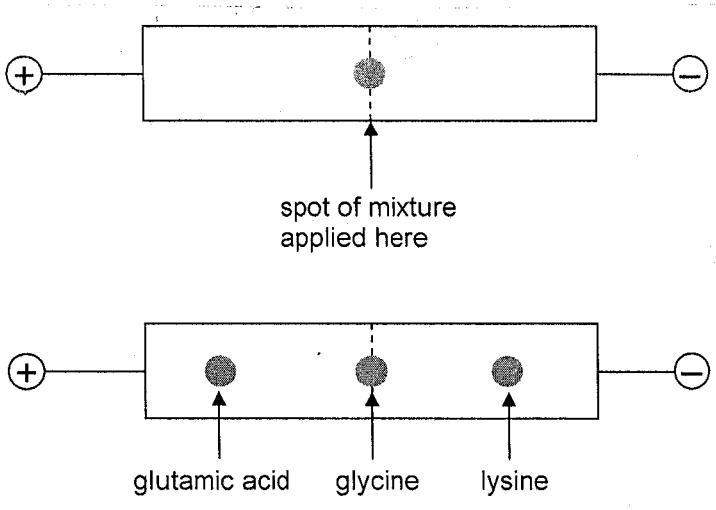
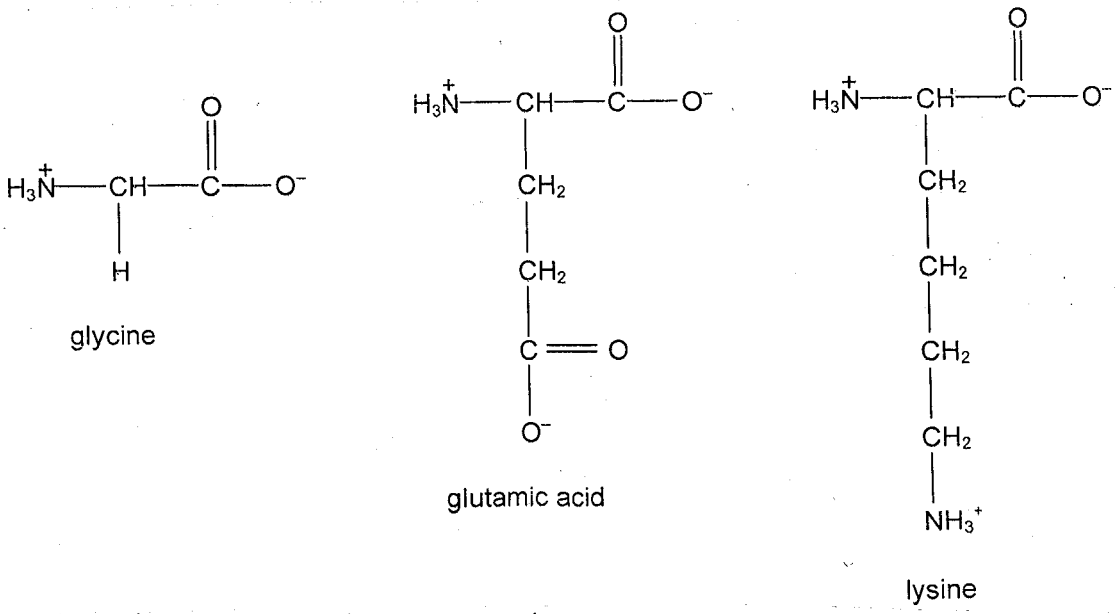
Electrophoresis (A2)

In electrophoresis there is no mobile phase, and only ions, not neutral molecules, move through the buffer solution along the plate.

The original spot will have been seperated into three spots.



results of electrophoresis of glycine, glutamic acid and lysine at pH=7

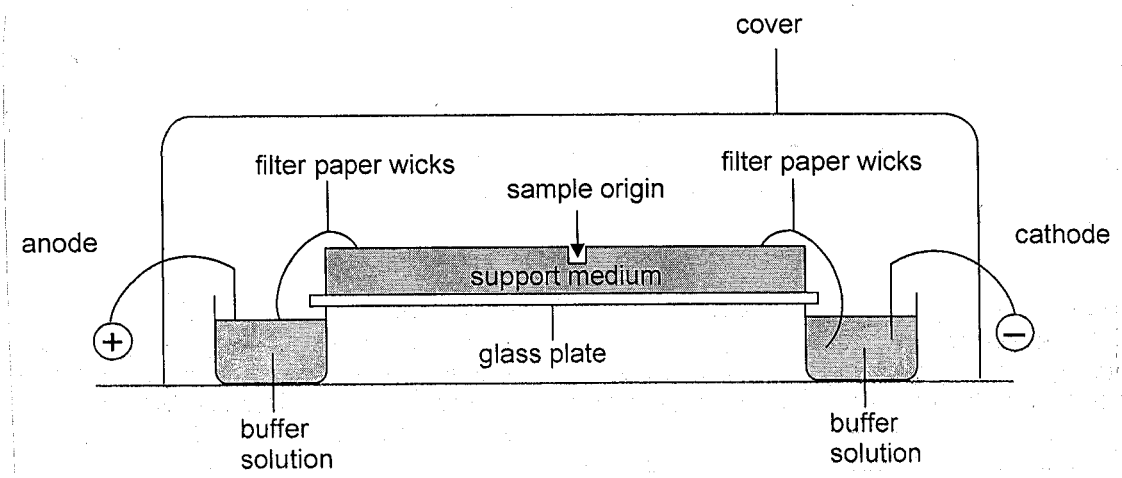


structures of glycine, glutamic acid and lysine at pH=7

Gel Electrophoresis.

Paper electrophoresis even though can be used to seperate mixture of ions but the method is imprecise.

Gel electrophoresis is commonly used in industrial, analytical or research environments.



gel electrophoresis apparatus.

The main components used in a gel electrophoresis analysis are:

- an electrophoresis chamber.
- a gel support medium soaked in conducting buffer.
- a means of generating an electric field - eg. a powerpack.
- probes for detecting and/or measuring the seperated molecules
- a means of extracting the individual products.

Gel electrophoresis covers a range of techniques that is used to seperate, analyse and purify mixtures of biological molecules such as proteins and nucleic acids.

These techniques can be adapted to:

- measure the relative masses of macromolecules.
- prepare nucleic acids and polypeptides for sequencing the component monomers, eg. purine and pyrimidine bases and amino acids.
- seperate proteins, so antibodies can be raised.

Running Gel Electrophoresis.

The sample mixture is placed in the sample well.

The mixture is seperated into its constituents by applying an electric field to the gel, which is soaked in a liquid buffer.

The gel is a sponge-like structure based on a three-dimensional polymeric network. It has the texture of a jelly.

Gel are used because their properties can be preaisely controlled during their preparation, and they are more chemically stable as a support medium.

The components in the sample mixture have an electrical charge because proteins, like amino acids, carry either an overall positive or overall negative charge, depending on the pH of their environment.

Nucleic acids such as deoxyribonucleic acid (DNA) are usually negatively charged (at the phosphate groups in the chain) at the pH used for their seperation using electrophoresis.

The molecules move in response to an electrical field applied across the mixture.

The rate of progress of the molecules depends on their size, charge and shape.

On seperation, the components are concentrated into bands or zones.

Each band or zone can be quantified using a variety of methods.

Using sensitive techniques, such as bioassays or silver staining, amounts as small as 10^{-18}g can be detected.

Applications of Gel Electrophoresis

- checking the adulteration of foods.
- chromosome sequencing.
- DNA fingerprinting.
- characterising the chemicals responsible for allergic symptoms.
- screen infants' milk for α-lactoglobulin, a protein which is lethal for small babies.

Seperations based on Rate of Migration

The rate of migration of each component in the mixture across the gel becomes constant when the force of attraction between the electrode and the oppositely charged component is equal to the friction force of the medium resisting the motion for that particular species.

The relative rates at which components move and therefore the extent to which they seperate are influenced by the strength of the field, the nature of the gel and the surrounding buffer.

Factors affecting mobility.

1. Voltage: the velocity of a molecule is directly proportional to the voltage gradient across the gel.
2. Size: smaller molecules migrate quicker than larger molecules carrying the same charge.
3. Shape: a molecule with lots of side-chains experiences more frictional resistance than a linear molecule of the same mass and charge, and will therefore move more slowly.
4. Buffer pH: proteins exist as zwitterions like amino acids, and can be either positively or negatively charged because they contain both acidic and basic groups. The extent and direction of ionisation depends on the pH of the buffer.
5. Temperature: a rise in temperature can speed up electrophoresis, but can also denature the proteins. The temperature is normally controlled by cooling the plate with a flow of water underneath.

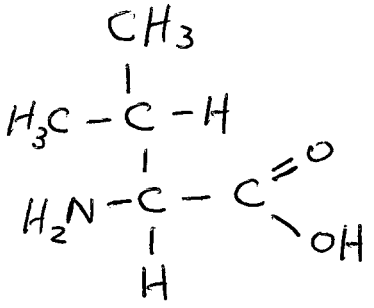
Two-way electrophoresis

If a number of components in a sample have similar electrophoretic mobilities, a complete seperation may not be achieved.

In this case, either the buffer medium or the gel support can be altered. Another option is to conduct 2-way electrophoresis in different buffers that can often separate components.

Exercise 1

a. Write down the formula for the form in which pure valine would mainly exist in aqueous solution.

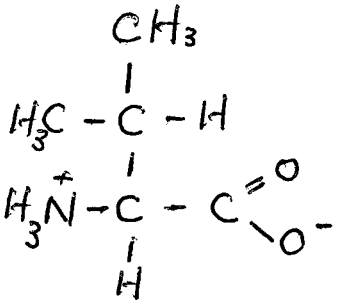


b. How would this change if a small amount of hydrogen ions were added?

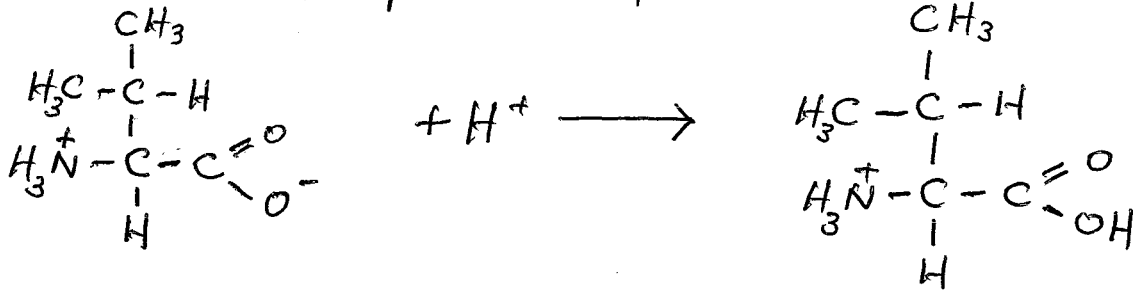
c. What factors determine the rate of movement of molecules during electrophoresis?

Workings

a.



b. The COO^- group becomes protonated.

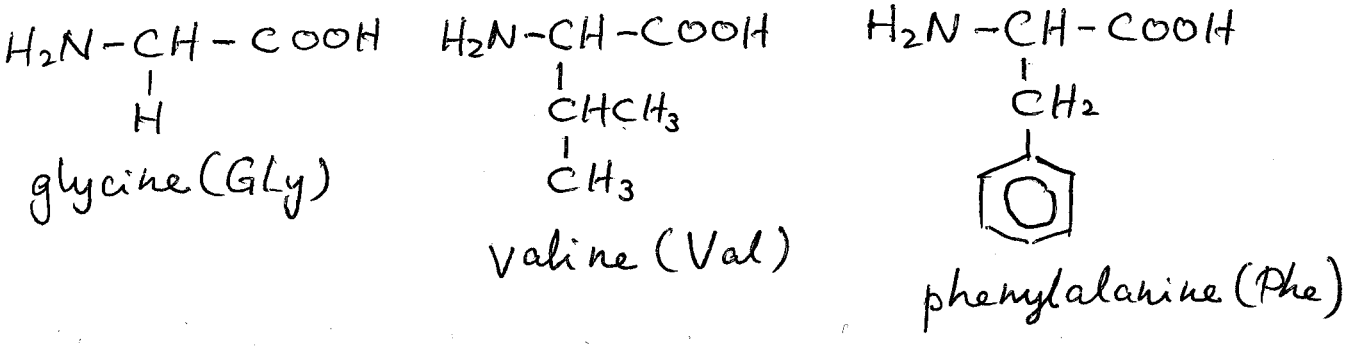


c. Electrophoresis – rate of movement of molecules determined by: charge, size, shape and temperature.

Exercise 2

A mixture of three amino acids is seperated by gel electrophoresis.

The three amino acids are glycine, valine and phenylalanine.



a. The electrophoresis is carried out in a buffer solution of pH=10. Draw the ions present in these alkaline conditions.

b. (i) Draw a sketch of the electrogram you would expect (viewed from above), labelling the amino acids as Gly, Val and Phe.

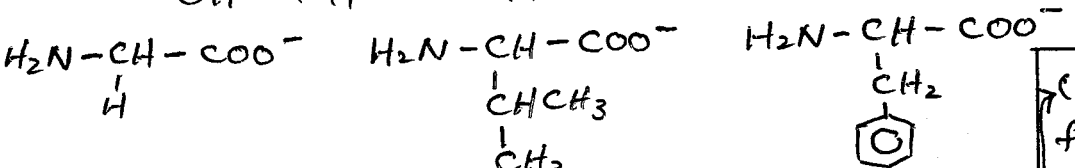
(ii) Explain your answer to part b(i).

Workings

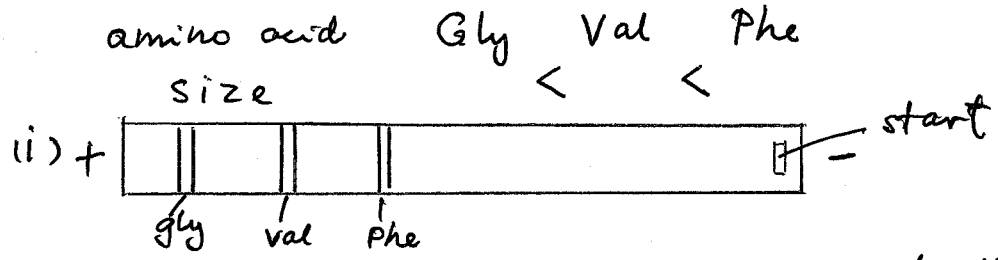
at pH = 10, present of OH^- in high concentration

$$OH^- + H^+ \longrightarrow H_2O$$

a.



b.



(gly) moving furthest, and the largest (Phy) moving the shortest distance

(ii) Each of the ion will have a −1 charge at pH=10, so the size of the ions is the only factor involved in their seperation. The molecules are seperated according to size, with the smallest

Some medical applications of gel electrophoresis

Defects in newborn babies.

Neural tube malformations can be detected from proteins leaking from the central nervous system of a foetus into the amniotic fluid (the fluid enveloping the foetus). Analysing these proteins can indicate spinal problems in new-born babies.

Sweating polypeptides.

Over 400 polypeptide spots show up in a two-dimensional electropherogram of human sweat. Many of these have been previously unidentified.

Alcohol abuse.

The extent of alcohol abuse can be investigated by analysing blood. This is because excess alcohol is associated with changes in acidic proteins and glycoproteins in blood plasma.

'Fish eye' disease.

This is an inherited condition in which lipid is laid down in the eyes making them appear opaque like fish's eyes. Its medical name is dyslipoproteinaemia. Protein samples from people with this condition have been analysed, suggesting that there is deficiency in an enzyme system associated with lipid metabolism.

Heart attacks.

Blood samples are taken at regular intervals (once a day for three days after the initial chest pain).

Studies are being done to identify more sensitive 'marker' proteins which will indicate early blockage in blood vessels.

Assessing fitness.

When a person is very much out of condition there can be a serious increase in the amount of protein in urine, which can be detected by two-dimensional electrophoresis.

As well as being a pointer to lack of physical fitness, increased protein content in the urine can also be a possible indicator of the onset of diabetes.