

# Electrophoresis

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## 2 Electrophoresis of amino acids

Electrophoresis is a way of analysing amino acids and proteins.

This technique separates charged molecules based on their ability to migrate when an electric field is applied to the system.

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## 3 Electrophoresis of amino acids

If the amino acid composition of a protein is to be investigated, the protein is first treated with acid to hydrolyse the peptide bonds between the amino acids.

The sample mixture is then applied to a support, such as paper or a polymer gel, which is saturated with a buffer of a certain pH, used as the conducting liquid.

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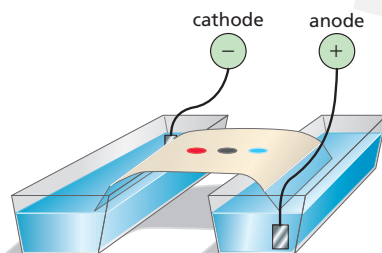
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## 4 Electrophoresis of amino acids



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## 5 Electrophoresis of amino acids

An electric field is applied across the support, and those amino acids bearing negative charges at the buffer pH migrate to the positive electrode (anode),

whereas those bearing a positive charge migrate to the negative electrode (cathode).

Those amino acids with no net charge remain stationary.

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## 6 Electrophoresis of amino acids

| Amino acid    | R group in R—CH(NH <sub>2</sub> )CO <sub>2</sub> H |
|---------------|--|
| glycine       | —H   |
| lysine        | —(CH <sub>2</sub> ) <sub>4</sub> NH <sub>2</sub>   |
| aspartic acid | —CH <sub>2</sub> CO <sub>2</sub> H                 |

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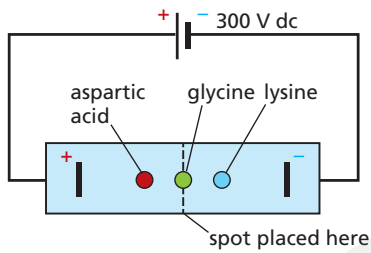
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## 7 Electrophoresis of amino acids



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## 8 pH & electrophoresis

If the pH of the buffer solution in the electrophoresis apparatus is neutral, amino acids with an extra acid group will be overall negatively charged ions and move towards the positive plate.

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## 9 pH & electrophoresis

and amino acids with one extra amine group will be overall positively charged and migrate towards the negative plate.

And those that have only one acid and one amine group will be zwitterionic and neutral and stay in the middle.

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## 10 pH & electrophoresis

Earlier we saw how amino acids change their charges in acidic and alkaline conditions. Their charge on the ions depends on the pH.

Therefore pH will affect the movement of ions during electrophoresis.

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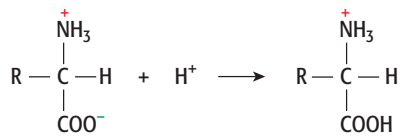
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## 11 pH & electrophoresis

In low pH (acidic), glycine will be positively charged and move towards the negative plate.



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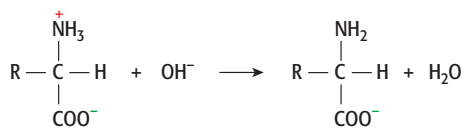
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## 12 pH & electrophoresis

In high pH (alkaline), glycine will be negatively charged and move towards the positive plate.



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## 13 factors affecting mobility

The rate/speed at which the ions move towards the oppositely charged electrode depends on the size and charge on the ions.

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## 14 factors affecting mobility

Size: smaller molecules migrate quicker than larger molecules carrying the same charge.

Magnitude of charge: highly charged ions will move more quickly.

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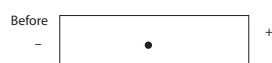
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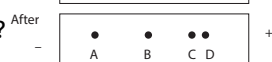
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## 15 skillcheck

i Which spot shows lysine?



ii Which spot shows alanine?



iii The other two spots show tyrosine and serine.

State which is which, and explain your answer.

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