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DEPARTMENT OF BIOTECHNOLOGY
FACULTY OF EGINEERING & TECHNOLOGY

LT 15. Isoelectric focussing

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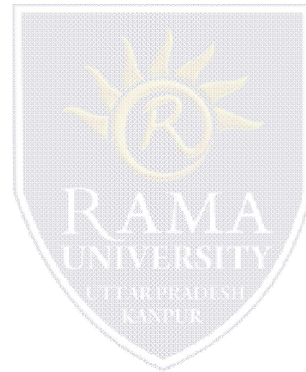

Iso -electric focussing

IEF, also known simply as electrofocusing, is a technique for separating charged molecules, usually proteins or peptides, on the basis of their **isoelectric** point (pI), i.e., the pH at which the molecule has no charge. **IEF** works because in an electric field molecules in a pH gradient will migrate towards their pI. At pI pH, proteins stop migrating. Since this method requires the proteins to move freely according to their charge under the electric field, IEF is carried out in low percentage gels to avoid any sieving effect within the gel. Polyacrylamide gels (4%) are commonly used, but agarose is also used, especially for the study of high Mr proteins that may undergo some sieving even in a low percentage acrylamide gel.

The IEF system utilizes horizontal gels in glass plate or plastic sheet. Separation is achieved by applying a potential difference across a gel that contains a pH gradient. To perform IEF, following items are needed

- Polyacrylamide gels as support media
- Ampholytes for formation of pH gradients
- Riboflavin for generation of free radical under light and initiation of photoplymerisation of gel.

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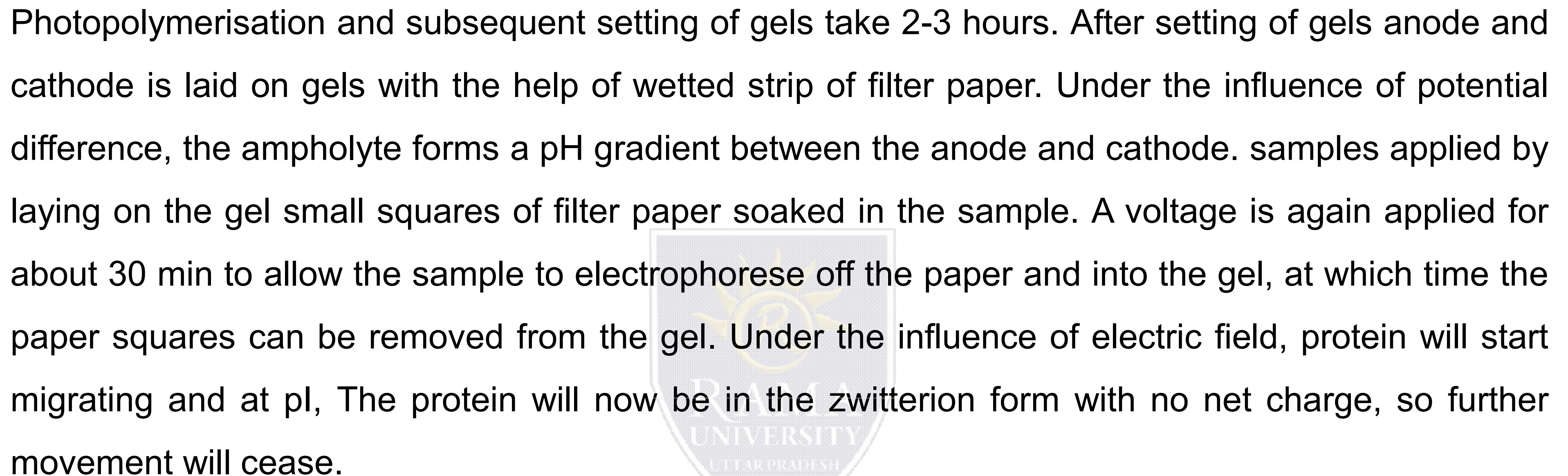


Photopolymerisation and subsequent setting of gels take 2-3 hours. After setting of gels anode and cathode is laid on gels with the help of wetted strip of filter paper. Under the influence of potential difference, the ampholyte forms a pH gradient between the anode and cathode. samples applied by laying on the gel small squares of filter paper soaked in the sample. A voltage is again applied for about 30 min to allow the sample to electrophorese off the paper and into the gel, at which time the paper squares can be removed from the gel. Under the influence of electric field, protein will start migrating and at pI, The protein will now be in the zwitterion form with no net charge, so further movement will cease.

Application of IEF

•IEF is highly sensitive analytical technique that is used for studying micr0heterogeneity in a protein.

•The method is particularly useful for separating isoenzyme

IEF in graphics

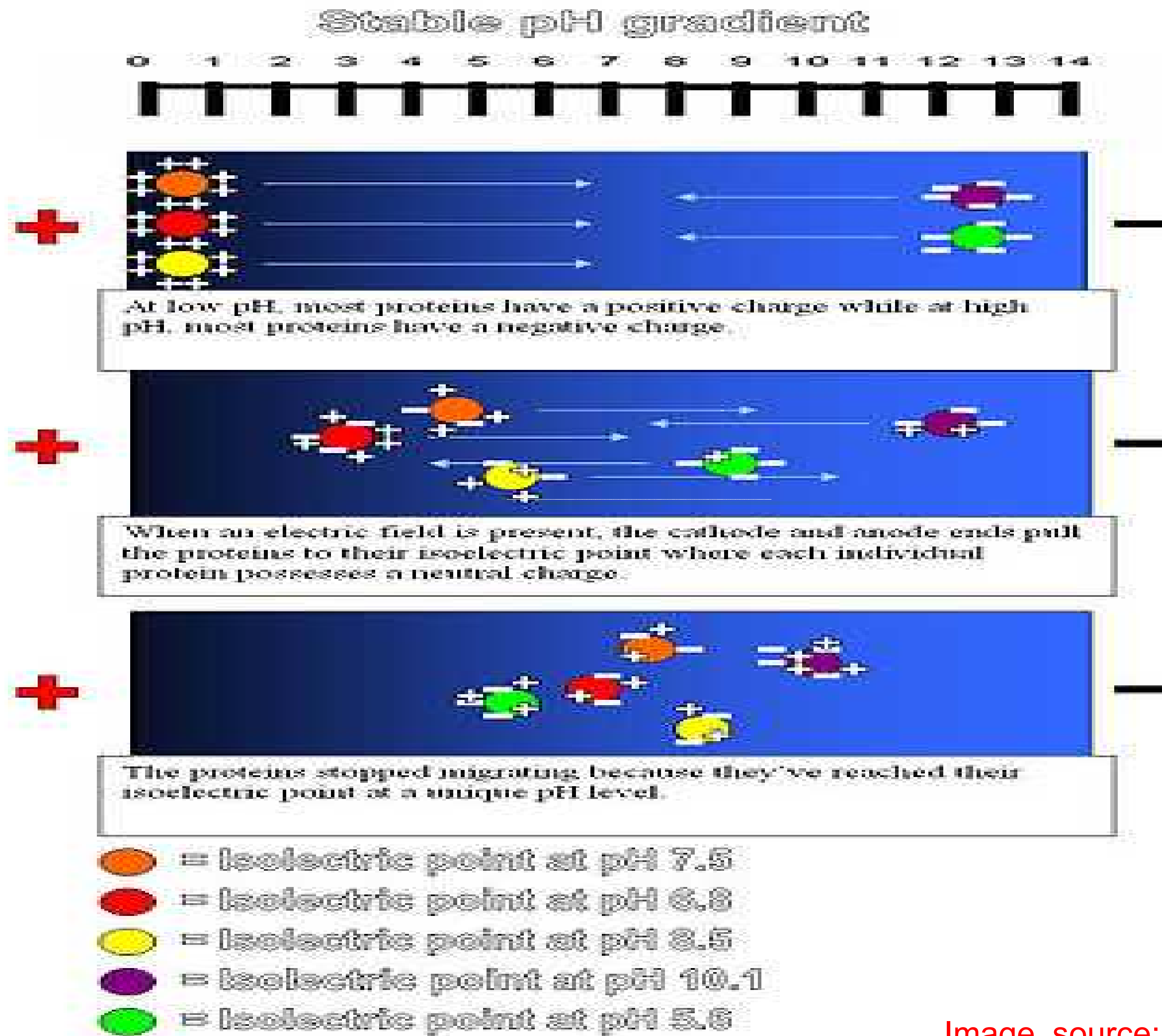
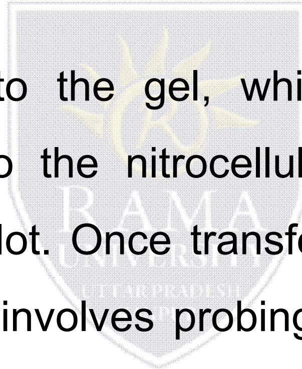


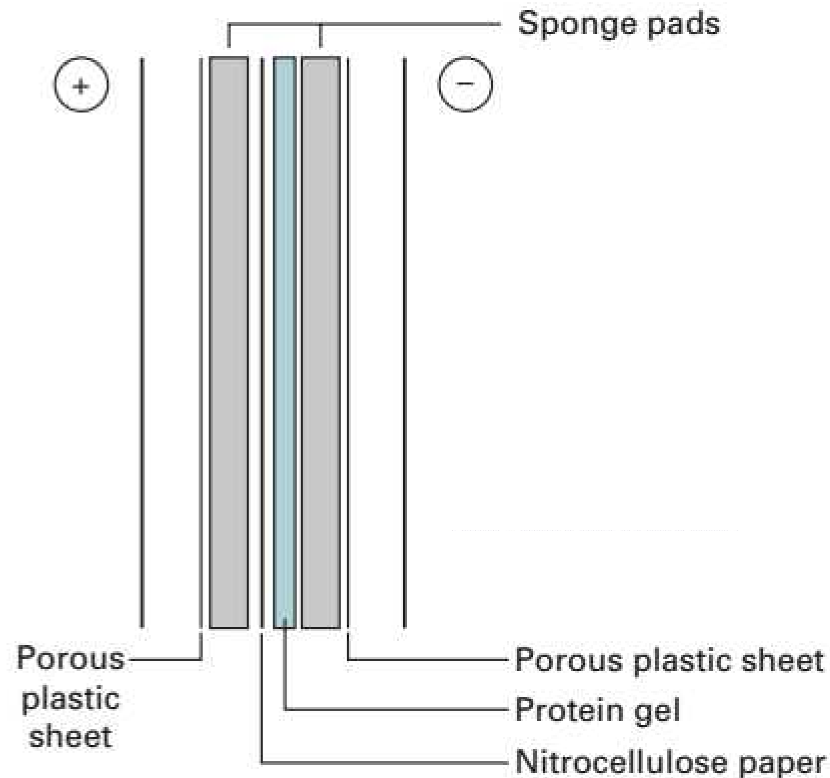
Image source: wikipedia

Protein (western) blotting

It is the process of transfer of pattern of separated proteins from the gel matrix onto a sheet of nitrocellulose paper. Transfer of the proteins from the gel to nitrocellulose is achieved by a technique known as electroblotting. In this method a sandwich of gel and nitrocellulose is compressed in a cassette and immersed, in buffer, between two parallel electrodes



A current is passed at right angles to the gel, which causes the separated proteins to electrophorese out of the gel and into the nitrocellulose sheet. The nitrocellulose with its transferred protein is referred to as a blot. Once transferred onto nitrocellulose, the separated proteins can be examined further. This involves probing the blot, usually using an antibody to detect a specific protein.



Diagrammatic representation of electroblotting. The gel to be blotted is placed on top of a sponge pad saturated in buffer. The nitrocellulose sheet is then placed on top of the gel, followed by a second sponge pad. This sandwich is supported between two rigid porous plastic sheets and held together with two elastic bands. The sandwich is then placed between parallel electrodes in a buffer reservoir and an electric current passed. The sandwich must be placed such that the immobilising medium is between the gel and the anode for SDS–polyacrylamide gels, because all the proteins carry a negative charge.

Analysis of blotted protein band using antibody

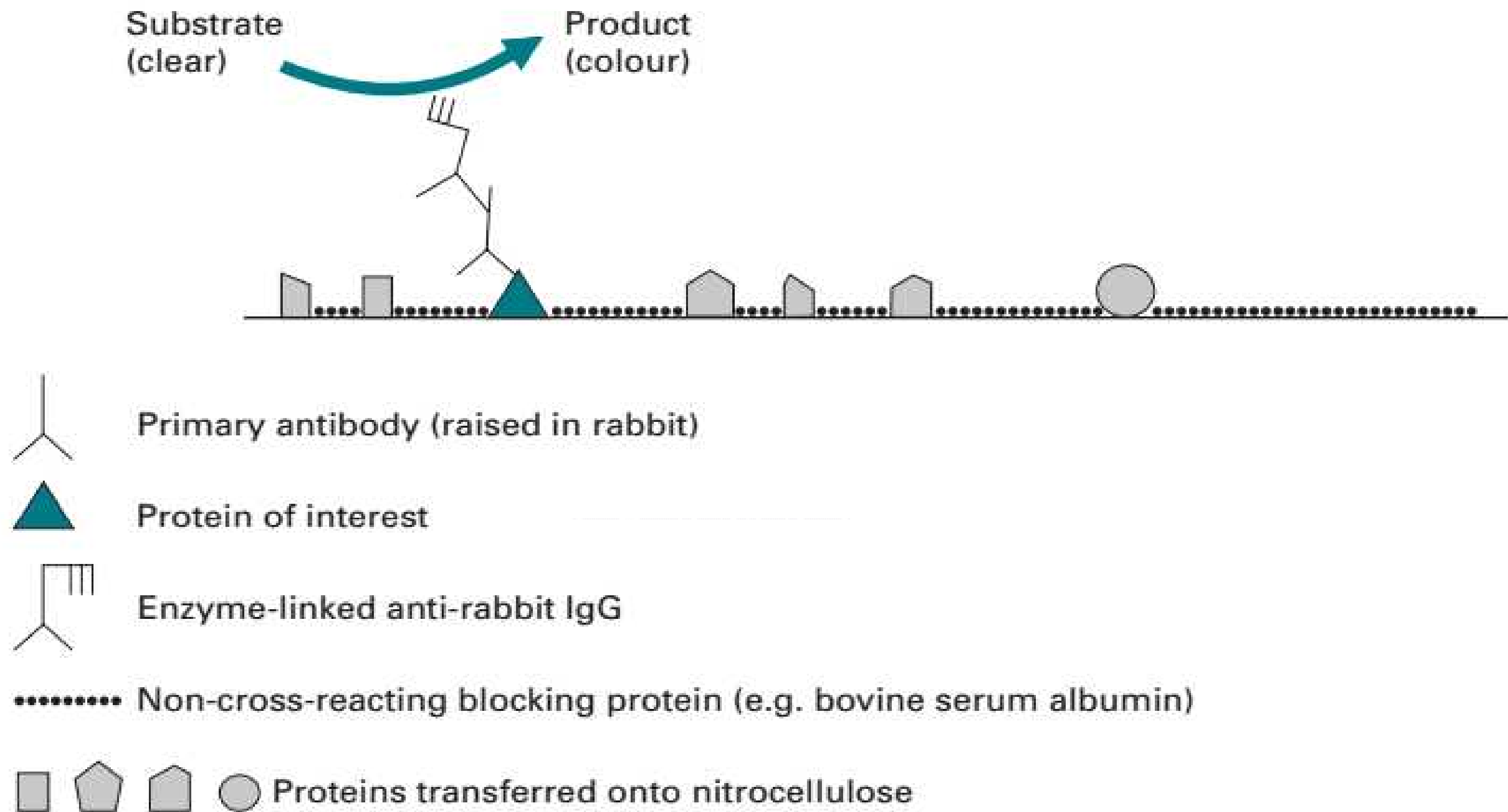


Fig. 10.12 The use of enzyme-linked second antibodies in immunodetection of protein blots. First, the primary antibody (e.g. raised in a rabbit) detects the protein of interest on the blot. Second, enzyme-linked anti-rabbit IgG detects the primary antibody. Third, addition of enzyme substrate results in coloured product deposited at the site of protein of interest on the blot.

Image source: Wilson & Walker

Test your understanding

Isoelectric focussing is also known as

a. 2D gel electrophoresis

b. Pulse Field Gel Electrophoresis

c. Western Blotting

d. Northern blotting

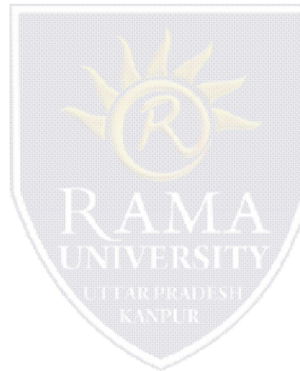
Isoelectric focussing is particularly useful for separating………………

a. Isoenzyme

b. Lipid

c. Antibodies

d. none of the above



Silver staining is used for staining protein bands during

a. SDS-PAGE

b. Chromatography

c. Centrifugation

d. Filtration

Coomasie brilliant blue is used for…………

Staining of separated protein bands

Degradation of protein band

Intercalation of protein band

None of the above

References & Further reading

1. Wilson, K, Walker, J., Principles and Techniques of Practical Biochemistry. 5th Ed. - Cambridge University Press,. Cambridge 1999.
2. Biotechniques, Theory & Practice: Second Edition by SVS Rana, Rustogi Publications.
3. Biochemical Methods of Analysis, Saroj Dua And Neera Garg : Narosa Publishing House, New Delhi.
4. Bioanalytical Techniques, M.L. Srivastava, Narosa Publishing House, New Delhi.