TECHNIQUES

This column deals with old and new TECHNIQUES for laboratory and field investigations and their applications. In this issue we look at electrophoresis.

Electrophoresis

Imagine this: half asleep on a bus you overhear part of a conversation between two medical scientists. You hear words such as pathology, blood, disease and electricity. Perhaps the first image to spring to mind is of a dark castle in a thunderstorm, with Baron Frankenstein and his experiments. You listen closer and hear another word mentioned several times — **electrophoresis**. This is not a Gothic horror fantasy — the scientists are discussing a technique commonly used today in routine medical laboratory analysis.

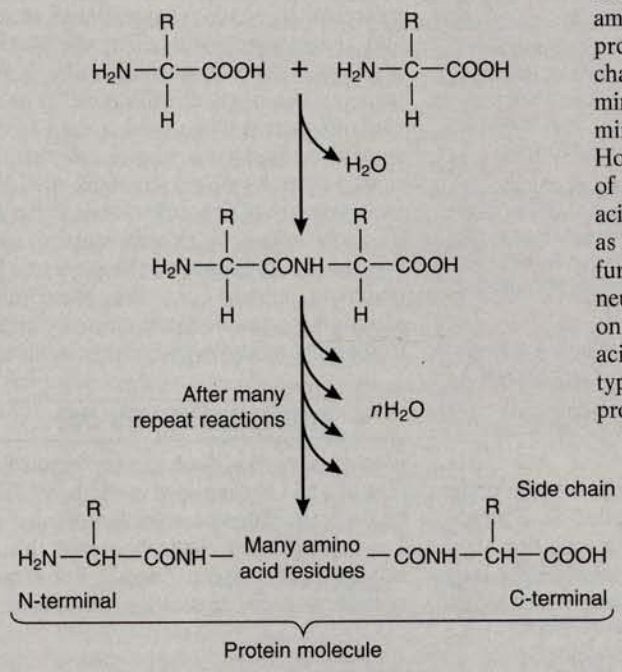
TWO PATIENTS

Elsewhere on the bus are two people on their way to hospital for an appointment at the outpatients clinic. The first is a young man who was referred to the chest clinic by his GP, because he has been having difficulty in breathing. The second is a middle-aged woman who has been suffering from back-ache, tiredness, and a series of infections. They too have a strong link with the conversation you have just overheard.

In each case the doctor who saw them requested a range of investigations, including blood tests. The medical laboratory scientist had carried out electrophoresis on each patient's serum — the fluid obtained from blood after it has clotted. Electrophoresis was used to separate the various proteins in the serum so that they could be identified. Any abnormalities were revealed by comparison with the pattern of proteins in the serum from a normal, healthy person. Interpretation of the results, together with other tests, will help the doctor at the outpatients clinic to diagnose the disease causing each patient's symptoms.

BOX 1 AMINO ACIDS AND THEIR IONS

Each individual amino acid is a zwitterion because it contains an acidic carboxyl group (–COOH) and a basic amino group ($-NH_2$). Proteins are chains of amino acids produced by a condensation reaction between the amino group of one amino acid and the carboxyl group of the next:



Although there are many amino acid residues in the protein molecule, each chain has only one terminal $-NH_2$ and one terminal –COOH group. However, the side chain of each individual amino acid residue (represented as –R) may possess a further acidic or basic (or neutral) group, depending on the particular amino acid. Thus, each protein type has its own ionic properties.

WHAT IS ELECTROPHORESIS?

If an electric field is applied to a solution containing charged particles they move towards one or other electrode depending on their charge. This effect is called electrophoresis, and is true not only of small ions such as Na^+ and Cl^-, but also of large, charged molecules such as proteins and nucleic acids. Electrophoresis was first developed in 1930 by the Swedish chemist Arne Tiselius to separate different types of proteins from one another. The importance of the technique to biochemical research and medical diagnosis was recognised in 1948 with the award of a Nobel prize to Tiselius.

ELECTROPHORESIS OF PROTEINS

Since the chief use of electrophoresis in a routine clinical biochemistry laboratory is in the analysis of proteins, I will use proteins as a model to illustrate the principles involved. Each protein molecule is a polymer of many amino acids, and contains many acidic and basic chemical groups (see Box 1). Such a molecule is said to be **amphoteric**. In solution at a given pH, each of these individual groups within the molecule will either carry a charge or else be electrically neutral, depending on (i) whether it is an acidic or basic group, and (ii) the pH at which that particular group ionises.

At high pH the acidic groups ionise:

$$-COOH \longrightarrow -COO^- + H^+$$

The basic groups stay uncharged (as $-NH_2$) and the resultant overall net charge is negative. At low pH the basic groups ionise:

$$-NH_2 + H^+ \longrightarrow -NH_3^+$$

The acidic groups stay uncharged (as –COOH) and the net charge is positive. At intermediate pH values the net charge on the protein molecule is a reflection of the balance of these groups in the molecule and the pH at which they ionise. Ionisable molecules that contain a mixture of acidic and basic groups are called **dipolar ions**, or **zwitterions**.

There is a particular pH for each protein, called its isoelectric point, where there are equal numbers of positive and negative groups so that its net charge is zero. This depends on the number and types of amino acids present in the protein.

PUTTING THEORY INTO PRACTICE

In the application of electrophoresis to the study of biological molecules it is not enough just to apply an electric current to a solution containing the substances. We also need a solid material to support the

Terms Explained

Amphoteric Having both acidic and basic properties.

Anode/cathode A conducting wire by which an electric current is led into or out of a sample. The general name for such a wire is electrode. Anode is the electrode by which current is led in. Cathode is the electrode by which current is led out. Because electric current flows from positive to negative electrode, the anode is positive and the cathode is negative.

Ion An electrically charged particle, so called because it moves in an applied electric field. Anions move towards the anode and are therefore negative (unlike charges attract), cations move towards the cathode and are therefore positive.

Zwitterion An ion that is both positively and negatively charged. Also called a dipolar ion.

solution, which allows the sample under investigation to be applied at a single, clearly defined starting position (the **origin**), while reducing interference from other factors (e.g. diffusion) which would cause blurring of the bands as they move. For routine electrophoresis of serum proteins, a sheet of cellulose acetate, soaked in buffer to maintain the required pH, is used — it is convenient to handle and gives useful separation of proteins quickly.

In addition to their ionic charge, macromolecules also have properties such as size and shape which will affect the way in which they move in the electric field. For instance, the size of the molecules is especially important when a gel is used as the support medium, as large molecules can be slowed down by the gel structure. Also, the ionic strength of the buffer solution has an influence in that a high ionic strength suppresses the ionisation of the proteins. By understanding and controlling these interactions it is possible to use electrophoresis to separate mixtures of such molecules.

THIS IS WHAT YOU DO!

Electrophoresis is done in a buffered solution of a pH chosen to give the best separation of the different molecules of interest. For most protein electrophoresis, this is usually a pH between 7 and 9. For serum proteins a special application device is used

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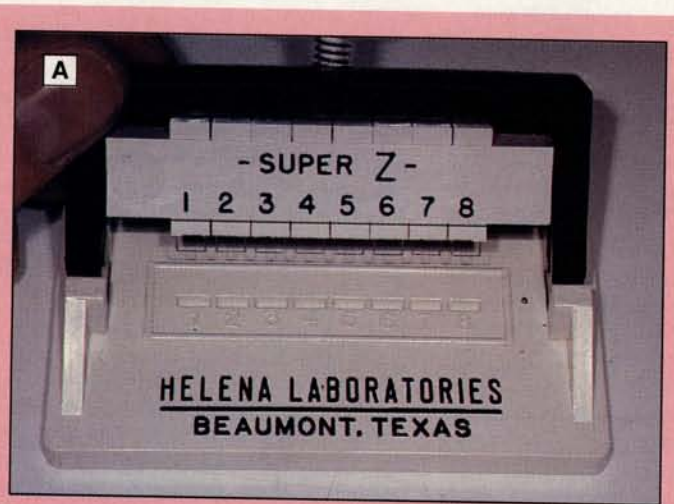
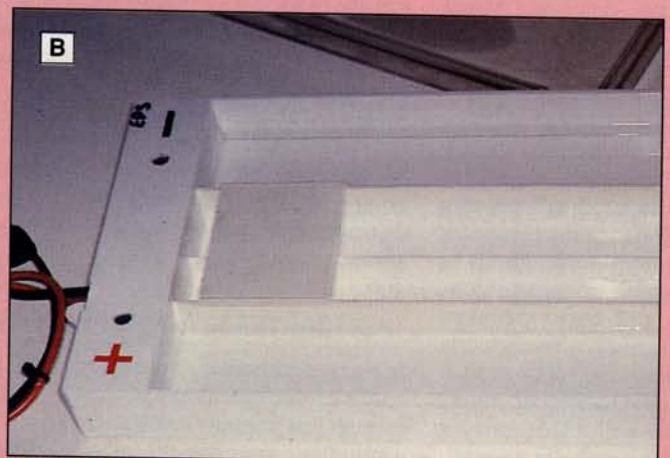
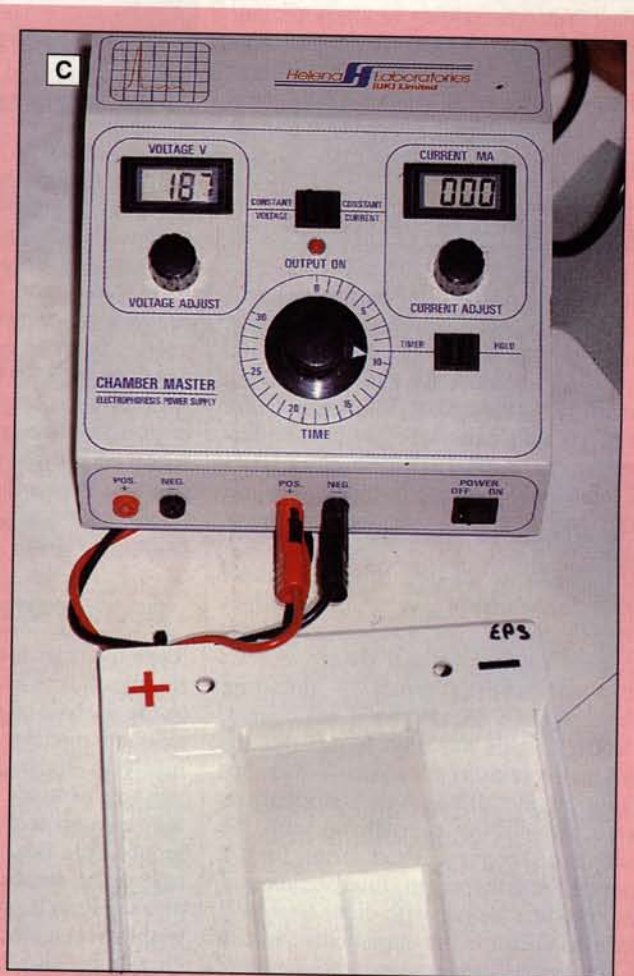
(Above) Samples 1 and 2 are from the same patient whose serum contains a paraprotein. Sample 1 is a more dilute version of sample 2. Sample 5 shows a deficiency in the α_1-band (see Figure 1).

(Below) Examples of equipment used in electrophoresis. (A) Serum application device. (B) Cellulose acetate strip in electrophoresis chamber. (C) Cellulose acetate strip in electrophoresis chamber with power supply.







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to place a small amount on the strip of cellulose acetate, which has been already soaked in buffer — several different samples can be run side by side on the same strip. The strip is then supported in a tank containing an anode and a cathode, each in its own isolated compartment which also contains the buffer. Each end of the strip must be in good contact with the buffer in each compartment — paper wicks may be used for this purpose. The lid is placed on the tank, and the power is switched on. It is left on for the time the proteins take to move in the electric field and become separated. The current is then switched off, and the strip is removed to be stained in order to make the proteins visible. The choice of stain depends on the substance(s) of interest. Also, the cellulose acetate membrane can be treated (after staining) to make it transparent. This allows it to be studied using a scanning densitometer (see Box 2).

NICE PATTERN — BUT WHAT DOES IT MEAN?

Returning to the two patients on the bus, routine electrophoresis of their serum has indeed revealed abnormalities. To understand these, first consider the pattern of normal serum (see Figure 1). This consists of five main bands or zones of protein: nearest to the positive electrode there is a large band of the serum protein, albumin, further along the strip we encounter four bands that each contain many different proteins known as globulins (classified as α_1, α_2, β and γ).

Patient 1 lacks the α_1 band. This is due to an inherited deficiency of a protein known as α_1-antitrypsin, which normally protects other proteins against digestion by the body's own proteolytic enzymes, chiefly an elastase released by white blood cells. Deficiency of this serum protein causes a lung condition called emphysema, and can also cause liver damage in newborn babies.

Patient 2 has a single abnormal band — called a paraprotein — in the γ-globulin zone, and the remainder of the γ-globulins are virtually non-existent. Further work will confirm that this patient is suffering from multiple myeloma, a condition in which bone marrow cells (Greek *myelos* = marrow) proliferate and produce abnormally large amounts of a single immunoglobulin. It is this protein which is seen as the paraprotein band in the electrophoresis pattern. Production of other immunoglobulins is suppressed, hence the reduction in the rest of the γ-globulin zone. The symptoms include bone damage (clearly visible on X-ray), anaemia, and low resistance to infection (due to the suppression of the other normal immunoglobulins, the proteins that defend the body against invasion).

For both these patients electrophoresis has been an important step in the diagnosis of their conditions. Further investigations and treatment will improve their quality of life — thanks to the movement of charged molecules in an electric field! ■

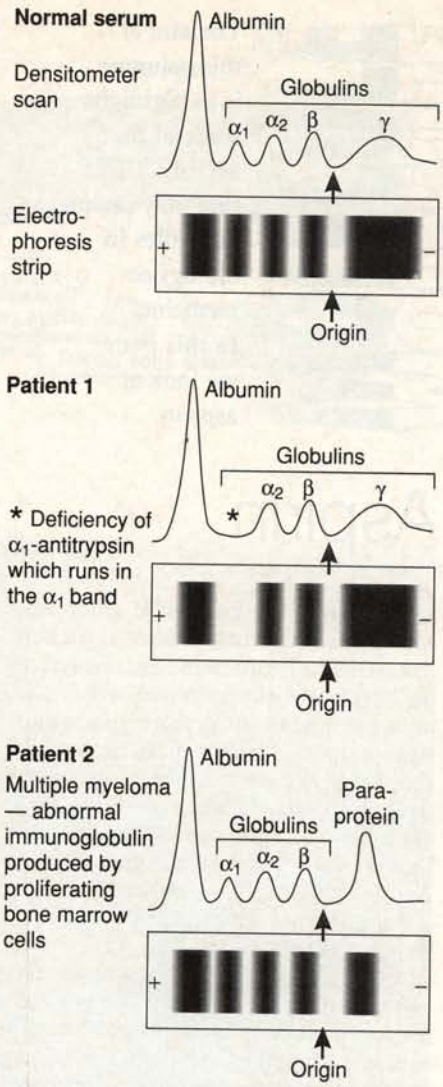
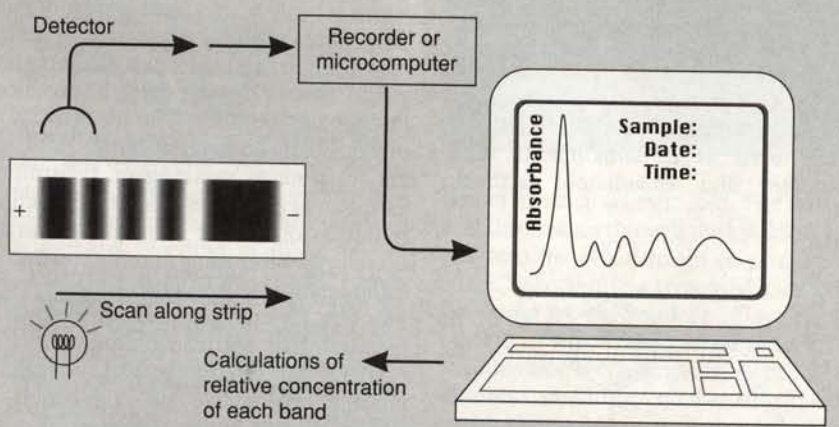


Figure 1 Serum electrophoresis patterns.

BOX 2 THE SCANNING DENSITOMETER

The electrophoretic strip, after staining and clearing, is scanned by a beam of light. The wavelength is chosen to suit the stain used. A photoelectric detector measures the amount of light passing through the strip, and the output is taken to a recording device (a chart recorder or a microcomputer system).



The resultant record of the absorbance by the different bands can be studied and the relative concentrations calculated. As the total protein and albumin levels for each patient's serum can be measured by other routine techniques, these values can be used to estimate the protein content of individual bands for each patient. This is useful, for instance, in monitoring the progress of patients suffering from myeloma.

THINGS TO DO

(1) In the routine clinical application described above, serum was used for the protein electrophoresis. What would be seen if plasma was used instead of serum? Why is it therefore usually avoided?

(2) Find out what you can about thalassaemia (a disease due to a defect in the genes coding for the protein molecules of haemoglobin). How is electrophoresis useful in diagnosing this disease?

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