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# Staining

*See* **II / ELECTROPHORESIS / Detection Techniques: Staining, Autoradiography and Blotting**

# Theory of Electrophoresis

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## Principles

Electrophoresis is a very separation technique which involves the separation of charged species (molecules) on the basis of their movement under the influence of an applied electric field. It is widely used by chemists and biochemists in studies related to medical technology, environmental research, food and water analysis, pollution control and forensic investigations. The development and applications of electrophoretic separation methods are an example of the fruitfulness of using physical methods in tackling biological and biochemical problems.

The migration of charged colloidal particles in an electric field was originally given the name cataphoresis or electrophoresis. Because there has been some diversity of opinion about the definition of a colloid, and thus about the distinction between colloidal and molecular systems, there has also been some difference of opinion as to how widely the term 'electrophoresis' should be used. Some authors prefer the term ionophoresis to describe the movement of relatively small molecules or ions under such conditions.

The 1940s and 1950s witnessed very rapid developments in the applications of methods making use of the migration of particles in an electric field. These applications covered the whole range of particle sizes from the largest protein molecules to small molecules like amino acids, sugars (at high pH) and even simple inorganic ions, using the sample types of procedures and apparatus. Although it is not a form of chromatography, the differences in the rates of migration of the charged particles provide a powerful means of separating biocolloids such as proteins, polysaccharides and nucleic acids, as well as for the characterization of their components. For these reasons, and also for historical reasons, it is now general practice to use the term 'electrophoresis' to refer to all these procedures. Electrophoresis pertains to the transport of electrically charged particles – ions, colloids, macromolecular ions or particulate matter – in an electric field.

Electrophoresis experiments are usually carried out to obtain information on the electrical double layers surrounding the mobile particles, to analyse a mixture, or to separate it into components. Interpretation of experimental results requires a theory connecting the electrophoretic mobility with the fundamental quantities relating to the electric double layer – the electrical potential, charge and structure.

The electrical double layer is not restricted to the interface between electrically conducting phases. For example, if a glass rod is immersed in an aqueous electrolyte, then it will carry a double layer of ions wholly within the electrolyte phase. This double layer originates from the specific adsorption of a Helmholtz layer of anions or cations from solution onto the glass surface. The resulting excess of charge is neutralized by a diffuse or Gouy layer dispersed further out in the solution. If we consider the case of two insulating phases, namely glass and oil, the double layer at the interface may be considered to arise either from the specific adsorption of ions generated by very weak electrolytes or from the orientation of dipolar molecules. The behaviour of the diffuse or mobile component of the double layer may be correlated with a class of phenomena which includes electrokinetic effects.

Electrokinetic effects are associated with the relationship between the relative motion of two phases (generally a liquid and a solid) and the electrical properties of the interface between them. Electrokinetic phenomena arise in microheterogeneous systems, i.e. in cases when one phase is dispersed in another. Electrokinetic effects may be classified into four groups: (1) electroosmosis, (2) electrophoresis, (3) streaming potential and (4) sedimentation potential.

1. *Electroosmosis* is the movement of a liquid along a capillary, a system of capillaries or a porous plug under the influence of an externally applied electric field.
2. *Electrophoresis* is the movement of solid particles under the influence of an electric field applied to the medium in which the particles are suspended. In this case the disturbance of the double layers attached to the solid moving particles produces the effect. It may be regarded as the reverse of electroosmosis, in which the solid phase is fixed and it is the movement of the liquid phase that is induced by the applied electric field. In both electrophoresis and electroosmosis the applied potential difference sets up a mechanical force which results in the movement of one phase.
3. *Streaming potential* is the building up of potential difference between the upstream and downstream ends of liquid flow. This is caused by friction between the moving liquid layer and the wall of the capillary, the system of capillaries or the porous plug.
4. *Sedimentation potential* (Dorn effect) is the converse of electrophoresis and results in the building up of potential difference between the top and the bottom of a vessel in which dispersed solid particles are suspended in a liquid.

The theoretical treatment of the electrical double layer depends on its geometry. The double layer at a flat interface constitutes the most simple case, which we can analyse to explain many of the facts connected with double layers. The boundary between two phases is a layer of finite dimensions. The properties of the two adjacent phases change gradually over a certain distance. These changes depend both on geometrical factors and on the forces between the molecules. The density and orientation of the molecules, even in a one-component system, undergo a gradual change when going from one phase to another, e.g. from the liquid to the gas phase. In multicomponent systems the boundary layer concentrations are different from those in the bulk, leading to what is called adsorption. Though these changes near phase boundaries are limited to only a very few layers of molecules, all the properties of the phases are changed in this transition layer.

When one or both phases contain ions, the transition layer may be much more extended. In such a case, one type of ion is strongly concentrated at the phase boundary by short-range forces. When ions of one sign are adsorbed at the phase boundary, ions of the opposite sign will be attracted by the resulting electric field and will accumulate near the phase boundary. This accumulation is opposed by their Brownain movement. As a result an electrically neutral double layer is formed which may extend to a considerable thickness (a few tens of nanometres).

In order to apply simple mathematical treatment to electrokinetic phenomena it is assumed that the diffused double layer acts as a parallel plate electric capacitor whose plates are $d$ cm apart, each carrying a charge $e$ per cm$^2$. The zeta potential, i.e. $\zeta$, the potential difference between the plates, is given by eqn [1]:

$$\zeta = 4\pi ed/D \qquad [1]$$

where $D$ is the dielectric constant of the medium between the hypothetical plates. This is the fundamental equation for the quantitative treatment of all types of electrokinetic phenomena.

When a liquid is forced by electroosmosis through the fine capillaries of a porous diaphragm, two opposing factors will determine the flow, namely the force of electroosmosis and the frictional force between the moving liquid layers and the capillary wall. When the two forces become equal, the flow attains a uniform rate. If $u$ is the uniform velocity so obtained and $d$ is the effective thickness of the double layer across which the flow takes place, then the velocity gradient in the double layer may be taken as equal to $u/d$. Since the velocity at one side, i.e. the wall, is zero, and the average value on the other side, i.e. in the moving liquid, is $u$, the force due to frictional effects is equal to $\eta u/d$, where $\eta$ is the coefficient of viscosity of the liquid. If $E$ is the potential gradient across the membrane and $e$ is the charge per $cm^2$ at the boundary of movement, then the electrical force causing electroosmosis is equal to $Ee$. Hence at the steady state eqn [2] applies:

$$Ee = \eta u/d \qquad [2]$$

Substituting the value for $d$ from eqn [2] in eqn [1] we obtain eqn [3]:

$$\zeta = 4\pi\eta u/DE \qquad [3]$$

Following on from this discussion of electrokinetic phenomena, electrophoresis takes place due to the presence of an electrical double layer at the interface between the dispersed phase and the dispersion medium, and the consequent presence of a zeta potential. On applying an external electromotive force, positive and negative portions of the double layer are displaced relative to each other. Since the particles in a solution are free to move, they will migrate under the influence of the applied electric field. As has been noted previously, the double layer surrounding a particle may be treated as a capacitor. We can therefore derive a relationship for the observed velocity $u'$ of the particle from eqn [3], namely eqn [4]:

$$u' = \zeta DE/4\pi\eta \qquad [4]$$

Here the quantity $u'/E = U$ represents the particle's mobility, i.e. the velocity for a potential gradient of $1\ V\ cm^{-1}$.

Consider the case of a comparatively large spherical particle of radius $R$ carrying a charge $q$ in a medium of dielectric constant $D$. According to electrokinetic theory the potential of the particle may be given by $q/DR$. If the charge is identified with that present in the diffused double layer only, then the potential is $\zeta$ and since the thickness of the Helmholtz double layer is negligible compared with the radius of large particles, $R$ may be taken as equal to the radius of particle plus its Helmholtz layer. This can be written as shown in eqn [5]:

$$\zeta = q/DR \qquad [5]$$

From eqns [4] and [5] we get eqns [6] and [7]:

$$u' = qE/4\pi\eta R \qquad [6]$$

$$U = q/4\pi\eta R \qquad [7]$$

where $U$ is the electrophoretic mobility. If the surrounding medium is an electrolyte, the interaction between the charged and migrating particles will reduce the zeta potential, $\zeta$, of the particle. The magnitude of this effect has been evaluated by Debye and Hückel, who observed that $\zeta$ is reduced by a factor of $1/(1 + KR)$, where $1/K$ is ionic length. $K$ is of the order of $10^{-7}$–$10^{-8}$ cm and can be calculated in terms of ionic charges in the electrolyte, the concentration and dielectric constant of the electrolyte and the radius at which the ionic atmosphere would need to be concentrated to obtain the potential of the ion. The electrophoretic mobility for a comparatively large spherical particle in an electrolyte may be given by eqn [8]:

$$U = \left(\frac{q}{4\pi\eta R}\right)\left(\frac{1}{1 + KR}\right) \qquad [8]$$

Eqn [8] is not applicable to small spherical particles where the curvature of the double layer is too large for streaming to take place entirely in the direction of the applied field. In such a case the electrical force on the particle is equal to the viscous drag as given by Stokes' law. Considering the effect of the electrolyte on the zeta potential (as in eqn [8]) the result for a small spherical particle is given by eqn [9]:

$$U = \left(\frac{q}{6\pi\eta R}\right)\left(\frac{1}{1 + KR}\right) \qquad [9]$$

Debye and Hückel made an exact treatment and found that the factor 4 in eqn [8] is strictly only applicable for cylindrical particles, and it should be replaced by the factor 6 (as in eqn [9]) for spherical particles. Eqns [8] and [9] are thus the special cases of a general expression covering all sizes of particle. A slight modification can be made to eqn [9] to give the electrophoretic mobility according to:

$$U = \left(\frac{q}{4\pi\eta R}\right)\left(\frac{1}{1 + KR}\right)f(KR) \qquad [10]$$

where $f(KR)$ is a complicated function of $K$ and $R$. More elaborate equations for electrophoretic mobility have been derived by taking into account the finite sizes of ions in the double layer attached to the particle. Gorin has also given a treatment for cylindrical particles.

Looking at the consequences of the complications associated with the theoretical deviation of the relationship between the electrophoretic mobility and the shape of the particle, attempts have been made to solve the problem experimentally. However, the experimental results are equally inconclusive. Abramson established that the electrophoretic mobility is independent of the shape of the moving particles by performing experiments on the movement of spherical particles of some oils and of needles of asbestos and *m*-aminobenzoic acid coated with the same protein.

The applicability of eqns [8] and [9] (for spherical particles) can also be tested by the comparison of electroosmotic and electrophoretic mobilities using a microelectrophoresis cell made of the same material as the suspended particle, e.g. glass or quartz. If eqn [8] is correct, then the ratio of the two mobilities should be unity; on the other hand if eqn [9] is correct, the ratio should be 1.5. Experiments with spherical particles and surfaces, all coated with adsorbed protein to ensure that the surfaces were the same, indicated that the mobility ratio was approximately unity, as required by eqn [8]. Objections have been raised to this conclusion on the grounds that the independence of electrophoretic mobility with respect to the shape of particles and the value of unity for the mobility ratio were due to the use of a liquid medium containing a relatively high concentration of the electrolyte. The objectors stated that if the electrolyte concentration is less than 1 mmol $L^{-1}$ then the ratio of electrophoretic to electoosmotic mobility is unity. Other workers also questioned the conclusion, and as such the situation is somewhat uncertain. However, eqn [8] may be regarded as reasonably adequate for particles of any shape.

## Factors Affecting Electrophoretic Mobilities

Several factors have a definite influence on the electrophoretic mobilities of charged molecules or ions.

### Nature of the Charged Molecule

The nett charge, size, shape and relative mass of particles has a great influence on their electrophoretic mobilities. The charge-to-size ratio of molecules is an important parameter. The higher its charge-to-size ratio ($e/r$), the faster a molecule will migrate under given conditions.

### Nature of the Electrophoretic System

As well as the characteristics of the substances to be separated, there are several parameters relating to the electrophoretic system itself that have a pronounced effect on the electrophoretic mobilities of the molecules or ions. These parameters are as follows.

1. The ionic composition of the electrophoresis buffer.
2. The temperature.
3. The pH of the electrophoresis buffer.
4. The applied voltage.
5. In the case of zone electrophoresis, the type of support medium chosen, and if the support medium is gel, its pore size.

**Ionic composition of the electrophoresis buffer** A charged macromolecule becomes surrounded by an ionic atmosphere of opposite charges because of interactions between ionizable groups on the surface of the charged molecule and ions in the electrophoresis buffer. As a result, both its net charge and its electrophoretic mobility are decreased. This effect is quite pronounced in the electrophoretic separation of proteins, since different proteins have different amino acid side chains which interact to varying degrees with the ions in the solutions used.

In order to minimize these 'counterion' effects it is advisable to use an electrophoresis buffer with as low an ionic strength as possible. However in some cases, such as with polypeptides and polynucleotides, electrophoresis has to be carried out in solutions of high ionic strength, otherwise these macromolecules will not be soluble. It therefore becomes necessary to choose a suitable salt concentration.

**Temperature** Temperature plays a pronounced effect on electrophoresis. In an electrophoretic run, heat (Joule's heat) is generated and may affect the electrophoresis in a number of ways.

1. *Diffusion*. An increase in temperature causes an increase in the diffusion of migration zones of charged molecules. If the electrophoresis takes a long time (several hours) diffusion effects become more significant.
2. *Evaporation*. It is customary to perform electrophoresis in a closed system to avoid loss of water by evaporation, which increases with temperature. This evaporation results in the drying out of the supporting medium and also leads to an

increase in the ionic strength of the buffer during the analysis.

3. *Viscosity*. In gel electrophoresis an increase in temperature can change the viscosity of the medium. Since this takes place during the electrophoretic run, the interpretation of the results may become complex.
4. *Distortion of zones*. During an electrophoretic run, particularly in column gels if cooling is inadequate, the portions of the migration zones in the warmer parts of the gel move faster than those in the cooler parts. This difference in migration speeds produces curved bands. This may result in the overlap of neighbouring zones and consequently in poor resolution.
5. *Convection currents*. During an electrophoretic run the warmer solution in the centre of the apparatus has a lower density than the cooler solution close to the walls. This density gradient induces convection currents in the solution. Since water has its maximum density at 4°C, and the smallest variations in density of aqueous solutions are observed around this temperature, it is advisable to perform electrophoresis at a temperature as close as possible to 4°C. However, the viscosity of an aqueous solution increases as the temperature is lowered, which may result in an increase in the frictional resistance to the migration of the charged molecules. If the temperature is maintained at 4°C the electrophoretic mobilities of the charged molecules will be relatively low. It is therefore necessary to choose an optimal temperature for a particular electrophoretic run and maintain it throughout the course of analysis.

**pH of the electrophoresis buffer** pH has a marked effect on the nett charge on a protein molecule. At a definite pH value, i.e. at the isoelectric point, the nett charge on the molecular is zero. Molecules acquire a nett positive charge at pH values below their isoelectric points. At pH values above their isoelectric points, they acquire a nett negative charge. Thus different molecules (e.g. proteins) at any particular pH value will have different nett charges. To optimize the separation of a mixture of (protein) molecules the buffer pH must be chosed on the basis of the nett molecular charges. For example in an electrophoretic run two or more proteins may migrate together to give only one band. If the analysis is done at a different buffer pH value it may result in the appearance of extra protein bands, indicating the presence of other proteins in the sample.

**Applied voltage** In electrophoresis the applied voltage plays an important role. The migration velocity of a molecule is proportional to the field strength across the medium. The higher the applied voltage, the larger the field strength across the medium, and the faster a molecule will migrate. Thus, the charged molecules will migrate more quickly with increasing voltage. This saves time and reduces the diffusion of migrating molecules. However, with increasing voltage the current also increases, resulting in greater power generation (the power increases as the square of the current).

Some of this power is dissipated as heat (Joule's heat). The heat generated can have serious effects on an electrophoretic analysis, as discussed previously. It is therefore necessary to select a definite value of applied voltage. The voltage (or current) should be large enough to allow rapid migration of charged molecules, but not so large as to generate excessive heat.

**Support medium** In zone electrophoresis different types of support media are used. The selection of the most suitable support medium for a particular zone electrophoretic analysis is based on the following considerations.

1. Sample quality.
2. Size of the molecules – whether they are small or large. If a sieving gel has to be used its pore size (concentration) is chosen so as to suit the molecular size under study.
3. The time required for analysis.

## Types of Electrophoresis

Electrophoresis analysis can be divided into three main types, as listed here.

1. moving-boundary electrophoresis
2. zone electrophoresis
3. steady-state electrophoresis.

### Moving-Boundary Electrophoresis

Moving-boundary or free solution electrophoresis was first proposed by Picton and Linde in 1892 and was fully developed by Tiselius in 1930, finding wide application between 1935 and the 1950s. Its principle use was in protein research, where it provided invaluable results.

The apparatus for moving-boundary electrophoresis, in its simplest form, consists of a U-tube with a rectangular cross-section (**Figure 1**). It is partially filled with the solution to be analysed (protein solution) and a buffer solution is layered over it. Electrodes are immersed in the buffer solution.

When an electromotive force is applied across these electrodes the charged molecules move towards the appropriate electrode. If the solution under study is

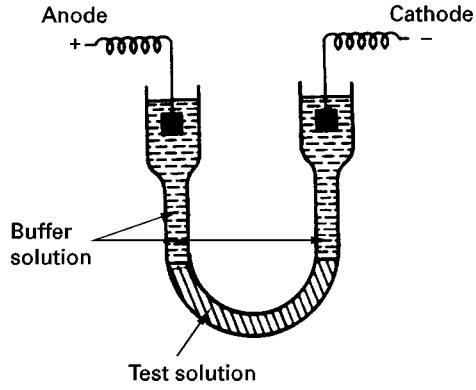


**Figure 1** Apparatus for moving-boundary electrophoresis.

coloured and contains several components with different electrophoretic mobilities, then their migration can be observed as multiple moving boundaries in the system. Where the migration boundary is not visible to the naked eye it may be made visible by causing it to fluoresce as a result of exposure to ultraviolet (UV) light. In such a case the apparatus should be made of quartz. In the case of protein solutions which have a refractive index slightly greater than that of the buffer, there will be a charge in refractive index at the protein boundary in the apparatus, which can be detected by optical methods.

After completion of the electrophoretic analysis the different fractions containing the components separated from the original sample can be analysed using appropriate methods.

Moving-boundary electrophoresis has proved to be useful in the determination of complex heterogeneous samples. However, it has a drawback in that only the slowest and fastest moving components of a sample can be obtained in pure form.

## Zone Electrophoresis

Zone electrophoresis results in the complete separation of the components of a mixture in the form of discrete zones. The separated zones may be stabilized in a number of ways which include the use of support media, density gradients and free zone techniques.

**Use of support media** An important development in electrophoresis was the use of support media for stabilizing zones of electrophoretically separated mixtures. A porous medium such as filter paper, cellulose acetate film, a gel, glass beads, granular starch or polyvinyl particles, etc., is used as the support medium. The separatory power of some of the gels exceeds that of other media or free solution.

The most common apparatus for zone electrophoresis using support media is depicted in **Figure 2**.

The sample is placed on the support medium in the form of a spot or narrow band at the sample origin. On subjecting it to electrophoresis the components of the sample separate into bands which are kept distinct by the presence of the support medium.

**Zone electrophoresis in density gradients** An important method that allows complete separation of complex mixtures involves carrying out electrophoresis in a density gradient column. The density gradients are usually solutions of substances such as ethylene glycol, glycerol, sucrose, etc., which do not ionize or interact with the materials under examination.

**Free zone electrophoresis** Although it is a complex technique, by using this method it is reasonably simple to recover the separated components at the end of analysis. The electrophoretic analysis may be performed in two ways, by rotation of the electrophoresis vessel during the run or by streaming a continuous film of buffer across the electrophoretic system, in a direction perpendicular to the applied electric field. These techniques are not in common use because they require elaborate equipment and are expensive.

## Steady-State Electrophoresis

The fundamental basis of this method is that, after electrophoresis has proceeded for a certain length of

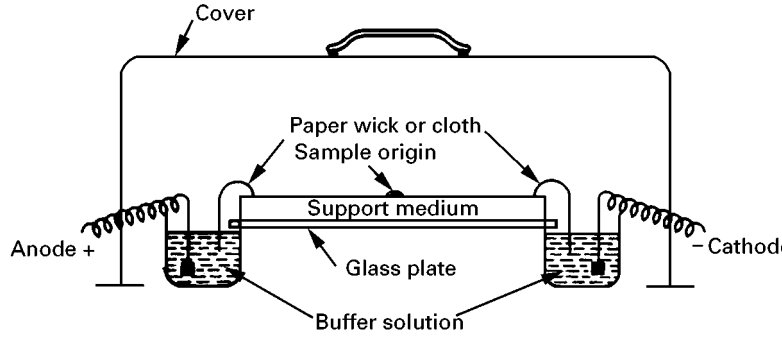


**Figure 2** Apparatus for zone electrophoresis.

time, a state known as the steady-state is reached in which no further changes to either the position or the width of the zone of the separated components occur with time. Steady-state electrophoresis involves two types of techniques, isoelectric focusing and isotachophoresis.

**Isoelectric focusing** In this technique the charged components of the sample move through the medium under the influence of an applied electric field until they ultimately reach a position in the pH gradient where their nett charge is zero and hence they do not migrate any more. This pH is their isoelectric point. The individual bands may be collected from the apparatus, and examined chemically or biologically.

This technique has very high resolving power, and may be used preparatively. It is not applicable to some compounds (proteins) that precipitate at their isoelectric point, which makes their recovery difficult.

**Isotachophoresis** This technique is particularly applicable to the separation of charged components with small relative molecular masses, such as some drugs and polypeptides of medical interest. In this technique all the charged components become stacked one behind the other, depending upon their electrophoretic mobilities.

To achieve a good separation of two charged components from one another by isotachophoresis, their electrophoretic mobilities must differ at least by 10%.

## Comparison of Moving-Boundary and Zone Electrophoresis

In moving-boundary electrophoresis a differential movement of the charged particles towards one or the other of the electrodes is observed. Separation takes place as a result of differences in mobilities. The mobility of a particle is approximately proportional to its charge-to-mass ratio. However, this technique suffers from several disadvantages, one of the most serious being the tendency of the separated components to mix by convection as a consequence of thermal and density gradients and mechanical vibrations. Thus, careful thermal regulation and isolation from mechanical vibrations is essential. Also, elaborate optical systems are often required for locating and measuring the fraction, making this method quite expensive.

Many of these experimental difficulties associated with moving-boundary or free solution electrophoresis may be avoided if separations are carried out in a supporting medium (zone electrophoresis), such as on paper. This prevents convection currents from distorting the electrophoretic pattern. This technique closely resembles the various chromatographic methods, with the additional parameter of a superimposed electric field. The separations mainly depend upon the properties of the medium and may result primarily from the electrophoretic effect or from a combination of electrophoresis and adsorption, ion exchange, or other distribution equilibria.

## Practical Problems

The Joule's heat generated in an electrophoretic run causes various problems, including changes in the pH of the buffer medium, diffusion and distortion of the zones, and evaporation and convection currents. It should be remembered that these effects may occur simultaneously, which may complicate the results. It is therefore necessary to optimize the temperature for a particular analysis and keep it constant.

In paper electrophoresis there is liable to be variability in the quality of paper from batch to batch, and also perhaps within one sheet. Some papers have adsorptive properties that may affect the electrophoretic mobility of the molecules under study. Ionizable groups may produce an electroosmotic effect on the paper, which in turn may distort the migration characteristics of the separating components. The physical and chemical inhomogeneity of the support medium also has a pronounced effect on the migration of substances which in turn affects the results.

The method used for the detection of sample components separated by electrophoresis depends on the type of electrophoresis, the nature of the molecules to be detected, and the purpose of analysis. Some detection methods are listed in **Table 1**.

Separations using electrophoretic methods may not be adequate for very complex mixtures. Resolutions may be improved substantially by combining electrophoresis with some other separation technique. The sample subjected to electrophoresis in one direction while the other separation analysis is carried out in a perpendicular direction (so-called two-dimensional techniques).

## Applications

Electrophoresis involves the separation of charged species on the basis of their movement under the influence of an applied electric field. It has found wide applications in the characterization of biological molecules (proteins and nucleic acids). The main applications of electrophoresis have been in the separation of biological molecules, which includes molecules with

**Table 1** Methods of detection for quantitative analysis of sample components separated by electrophoresis

| | |
|---|---|
| Optical methods | UV absorption<br>Staining<br>Fluorescence |
| Raidiochemical methods | Liquid scintillation counting<br>Autoradiography |
| Biological assay and immuno-methods | Immunoelectrophoresis<br>Rocket electrophoresis |

relatively lower relative molecular masses such as amino acids, and also molecules of higher relative molecular masses such as proteins and polynucleotides (including RNA and DNA molecules). An example of the use of paper electrophoresis follows.

Paper electrophoresis has been extensively used in almost all laboratories where proteins and other similar macromelecular electrolytes are investigated. The apparatus (Figure 2) consists of two electrode chambers placed 15 cm apart. There is also a device which can support up to six (30 cm) filter paper strips between the electrodes. A d.c. supply source (0–250 V) is used to apply the desired voltage across the electrodes. The two electrode chambers are filled to equal heights with the buffer solution. The buffers commonly used for this purpose are (1) Aronsson and Gronwall buffer, i.e. dimethyl barbiturate buffer, which is a mixture of 20.60 g sodium dimethyl barbiturate and 2.80 g barbituric acid with a pH of 8.6, and (2) Consden and Powell buffer, i.e. borate buffer, which is a mixture of 1.77 g sodium hydroxide and 9.60 g orthoboric acid with a pH of 8.6.

Whatman paper (M540) strips (about 30 cm long) are cut and dipped in a container of buffer until they are thoroughly wet. The excess buffer is then removed by laying them out on a large sheet of filter paper. The strips are then immersed into the electrode chambers so that the ends of the strips dip in the buffer solutions. The sample is applied at the centre of the paper. The paper strips are allowed to stand for about 1 h, to equilibrate the bed with the liquid evenly throughout the paper. The power supply is then switched on and the voltage adjusted to about 75 V. Excellent sharply defined separations of serum proteins into five fractions within a span of 2 cm can be obtained within 1 h. If the run is extended to 16 h, a pattern approximately 12 cm long with five fractions may be obtained. On completing the run the fractions are measured by staining. The dye most commonly employed for this purpose is amido black 10B. The paper strip is dried and developed in a dye bath containing a saturated solution of the dye in a mixture of methanol and glacial acetic acid (9 : 1 v/v). Staining is allowed for 10 min with constant shaking.

After the electrophoretogram has been stained, the excess of dye is removed (destaining) by dipping the stained paper in baths of methanol–glacial acetic acid (9 : 1 v/v) several times. This destaining procedure is a slow process and can be made more efficient and faster by opting for electrophoretic destaining. After destaining, the paper strip is dried and scanned in a densitometer, i.e. the strip is illuminated and moved along the light source, the transmittance showing the distribution of the separated compounds. On plotting the reciprocal of transmittance against the wavelength one or more maxima are observed, depending upon the number of components. The amount of each component can be estimated by measuring the area under each peak. The estimation of serum proteins can also be done by an elution method. The destained paper strip is cut transversely into small pieces 5 mm wide, one unstained small strip at the end of the paper providing the blank value. The elution is done in 1.0 mol $L^{-1}$ NaOH in 50% ethanol + 0.25% (0.25 g per 100 mL) ethylenediaminetetraacetic acid (EDTA). After elution is over, the optical density is measured using a colorimeter.

A more rapid separation of serum proteins can be achieved using polyacrylamide gel electrophoresis. However, paper electrophoresis is still of particular interest where small amounts of protein need to be isolated for further analysis or testing.

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