Module : 9
Electrophoretic Separation Methods

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Electrophoretic Separation Methods

Different rates of migration of charged particles/molecules in an electric field are useful for separation. This principle can be utilised quite effectively for the separation of biomolecules as most of them are having charges. Each such molecule has a characteristic pH, known as isoelectric pH, at which, the biomolecule is neutral. One can control the operating pH by addition of few drops of acid or alkali. Thus, if the operating pH is set above the isoelectric pH (pI), the molecule becomes negatively charged and if the operating pH is set below the isoelectric pH, it is positively charged.

Compound	M_w	pI(25^0C)
Aspartic acid		2.77
Lysine		9.74
Ovalbumi	43000-45000	4.7
Borine serum albumin	68000	4.95
Misoglobin	~17000	7.33
Cytochrome C	~12000	9.28

Forces in Electrophorosis:

As discussed in Chapter 4, the electrophoretic mobility of a charged spherical ion in an electrolytic solution becomes,

$$u = \frac{v}{E} = \frac{ze}{6\pi\eta R_p} \qquad (9.1)$$

For charged colloids, the system contains small amount of electrolytes to carry appreciable current. This causes redistribution of counter and co-ions around charged colloids i.e., within electrical double layer.

If $\kappa R_p < 0.1$, then $u = \frac{2 \in_0 \in \zeta}{3\eta}$ (9.2)

If $\kappa R_p > 0.1$, then $u = \frac{\in_0 \in \zeta}{\eta}$ (9.3)

Typical values for electrophoretic mobility in free solution are in the orders of 10^{-8} $m^2/s.V$ for ions and 10^{-9} $m^2/s.V$ for proteins. The mobility increases as size of ion increases.

Complicating factors in Electrophoresis

During the flow of electric current in an electrolytic solution, there may be significant increase in temperature due to Joule heating. Thermal power generated is $P = VI = I^2R$, where, P is the energy generated per unit time, V is the electric potential, I is the current, R is the electrical resistance. Joule heating has the following is unwanted due to following reasons:

Firstly, I^2R heating is undesirable since the local differences in temperature causes local difference in density and hence creates convection. Secondly, large increase in temperature may destroy fragile biochemicals.

Since, increase in temperature, is associated with the change in density, there exists a probability of significant buoyancy effects. This results into a circulation, like natural convection.

$$\rho = \overline{\rho} - \overline{\rho}\beta\left(T - \overline{T}\right) \quad (9.4)$$

Where, ρ and $\overline{\rho}$ are densities at temperature T and $\overline{T}$ and β is the coefficient of volumetric expansion. The extent of convection is dictated by the numerical value of a non-dimensional number, known as Grashoff number.

$$Gr = \frac{\overline{\rho}\beta g h^3 \Delta T}{\eta^2} = \frac{\overline{\rho} g h^3 \Delta \rho}{\eta^2} \quad (9.5)$$

$\overline{\rho}$ = average fluid density; h= characteristic length. As *Gr* increases, convection becomes stronger. There exist several methods to avoid convection:

i. Efficient cooling reduces ΔT and prevents thermal degradation.

ii. Electrophoresis is conducted at 4^0C, where water density is maximum.

iii. Difference between plates '*h*' is kept small or electrophoresis is done in small tube, pores of a gel, membrane or paper capillary.

Introduction of gel/membrane to stop convection leads to sieving of molecules/particles which is undesirable as it involves hindered migration.

Electroosmosis is another complicating factor in electrophoresis. Fixed charges on chamber wall or gel are subjected to a force due to presence of the electric field. In this case, charges are fixed and fluid moves. This fluid movement is defined as electroosmosis.

$$v_{osm} = u_{osm} E = \frac{\in_0 \in \zeta_{wall} E}{\eta} \quad (9.6)$$

Effect of electroosmosis is reduction in separation. The extent of reduction is a function of geometry of the flow channel. The deleterious effects of electroosmosis can be prevented, if wall is coated by a material like methyl cellulose which haas a low zeta potential, so that the mobility due to electroosmosis is marginalized.

Gel membrane and paper electrophoresis

To prevent the natural convection, electrophoresis is conducted in a stabilizing porous media, *e.g.,* gel or membrane. This method is known as zone electrophoresis. This system

behaves exactly like, chromatography. Some stabilizing media should be used. These should have the following characteristics:

i. Inert, should not react with species.

ii. No residual charges on the media so that no ion exchange or electroosmosis takes place.

iii. Pore size should be greater than the molecular size so that no hindered transport occurs.

A typical schematic of laboratory scale gel electrophoresis set up is presented in Fig. 9.1.

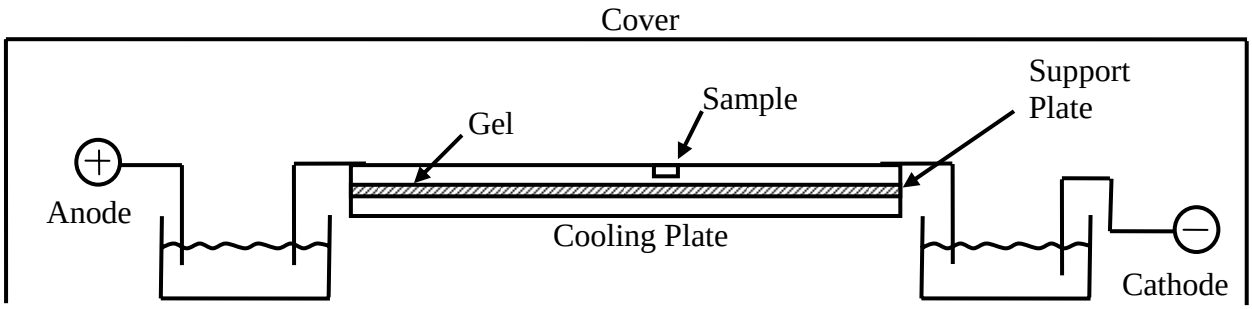
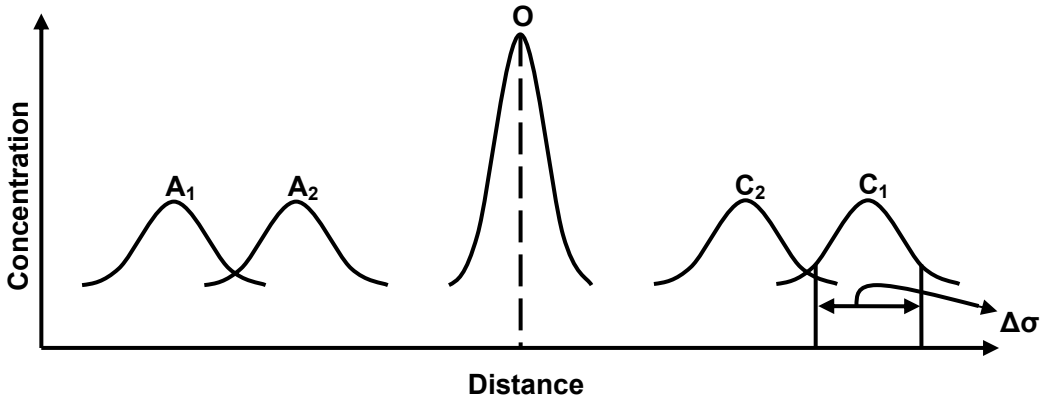


Fig. 9.1: Laboratory gel electrophoresis cell



A_1, A_2: anions

O: zero charge

C_1, C_2: cations

1) A thin layer of gel is polymerized onto support plate (glass).
2) Support plate is placed on coolant plate so that joule heating is prevented.
3) Gel layer should be 5mm for better heat dissipation.
4) Cover is to control evaporation and safety reason as high voltage may be used.

a) A small sample is placed in the sample well in the center of gel slab. When current is on, solute particle migrate towards cathode/anode.
b) Since the width of feed sample is small so that each solute migrates as a zone, with velocity u_i. the zones eventually separates out from each other.
c) Products are often detected by UV absorbance, RI, etc..

The most common electrophoresis is polyacrylamide gel electrophoresis (PAGE).

The advantages of this method are listed below:

i) It is an excellent anti-convective gel.
ii) Easy to polymerize in place
iii) Can be polymerized in the variety of pore sizes.
iv) Little residual charge on the polymer.

SDS-Page

When a solution of protein at pH 7 is treated with 1% sodium dodecyl sulphate (SDS) (w/v) and 0.1 (M) mercaptoethanol to destroy disulfide bonds, the polyreptide chain is unfolded and protein is converted to rod like structure. About 1.4 g SDS is bound to 1 g protein to make a complex. For each protein, the attachment occurs in the same ratio and

it is remarkable that each protein-SDS complex has the same charge to mass ratio. Therefore, all complexes have similar electrophoretic mobility in free solution. But SDS-protein complexes have different sizes and can be separated based on protein size by using sieving property of polyacrylamide gel. This method is helpful in estimating relative masses.

Zonal electrophoresis

Like chromatography, a pulse of feed results a Gaussian distribution response at the exit of the set up.

$$c_i = c_{\max,i} \exp\left[-\frac{\left(z - z_i\right)^2}{2\sigma_{1,i}^2} \right] \qquad (9.7)$$

Where, $\sigma_{1,i}$ = standard deviation of electrophoresis system; z_i = the location of maximum peak. The total electrophoretic velocity of the species becomes,

$$v_{net} = uE + u_{osm}E \qquad (9.8),$$

where the electric field strength is,

$$E = \frac{V}{L} \qquad (9.9)$$

L is the maximum length of migration. If t_R is the average retention time to migrate a distance z_i, then the expression of retention time is,

$$t_{Ri} = \frac{z_i}{u_i} = \frac{z_i L}{\left(u_i + u_{osm}\right)V} \qquad (9.10)$$

The expression of z_i is thus obtained.

$$z_i = v_i t_{Ri} = \left(u_i \frac{V}{L} + u_{osm} \frac{V}{L} \right) t_{Ri} \qquad (9.11)$$

Since, the chromatographic run is for a fixed time, we have, $t_{Ri} = t_{\exp t}$. Thus, different particles will traverse different distance. The width of the concentration curve is given by Einstein's equation.

$$\sigma_{1,i}^2 = 2D_{eff,i}t_{\exp t} \tag{9.12}$$

$D_{eff,i}$ is effective axial dispersion coefficient in electrophoresis system. Thus inserting the expression of experimental time, the following equation is obtained.

$$\sigma_{1,i}^2 = \frac{2D_{eff,i}z_i L}{(u_i + u_{osm})V} \tag{9.13}$$

The resolution (R) between two zones can be obtained as,

$$R = \frac{1}{4}\frac{V}{L}\left(\frac{t_{\exp t}}{2\overline{D_{eff}}}\right)^{\frac{1}{2}}(u_1 - u_2)$$

$$= \frac{1}{4}\left(\frac{v}{2\overline{D_{eff}}(u_{\max} + u_{osm})}\right)^{\frac{1}{2}}(u_1 - u_2) \tag{9.14}$$

Where, $t_{\exp t} = \frac{L}{v_{\max}} = \frac{L^2}{(u_{\max} + u_{osm})V}$ and $\overline{D_{eff}} = \frac{D_{eff1} + D_{eff2}}{2}$.

This is generally set to the fastest migrating species travels a distance '*L*'. This is done by adding a dye as marker. It may be noted that *R* increases if *v* increases.

Solved Problems

1. A batch electrophoresis is done in PAGE where μ_{osm} =0; μ_A =1.05*10^{-5} cm^2/v.s. ;

D_A=D_B= 1.5*10^{-7} cm^2/s.

E= 125 v/cm. for 2.5 hrs

Find location of peaks (distance from feed well), peak widths and resolution of two proteins?

Solution:

$$z_i = u_i t_{expt} = \mu_i E t_{expt}$$

$$z_A = \left(1.05\times10^{-5}\right)(125)(2.5\text{ hr})\times 3600 = 11.81\text{ cm}$$

$$z_B = \left(1.033\times10^{-5}\right)(125)(2.5\text{ hr})\times 3600 = 11.62\text{ cm}$$

$$\sigma_{1,i}^2 = 2D_{eff} t_{expt} = 0.0027\text{ cm}^2$$

$$\sigma_{1,i} = 0.052\text{ cm} \qquad \text{for both proteins}$$

Width is $4\sigma_1 = 4\times 0.052 = 0.208$ cm

$$R = \frac{1}{4}\frac{v}{L}\left(\frac{t_{expt}}{2D_{eff}}\right)^{\frac{1}{2}}(\mu_1 - \mu_2)$$

$$= 0.9202$$

R increases by increasing E and increasing t_{expt}

2. A batch electrophoresis is done in PAGE where $\mu_{osm} = 0$. The mobilities of two proteins are $\mu_A = 1.0\times10^{-5} cm^2/V.s$ and $\mu_B = 1.02\times10^{-5} cm^2/V.s$. The diffusivities of them are, $D_A = 1.5\times10^{-7} cm^2/s$ and $D_B = 1.7\times10^{-7} cm^2/s$. The experiment is conducted at $E = 100\ V/m$ for 3 *hours*. Determine the location of the peaks, peak widths and resolution of two proteins?

Solution:

Migrate distance:

$$Z_A = \mu_A E t_{exp} = 10^5\times100\times(3\times3600) = 10.8\ cm$$

$$Z_B = \mu_B E t_{exp} = \left(1.02\times10^{-5}\right)\times100\times(3\times3600) = 11.02\ cm$$

Therefore, location of peak of A is 10.8 *cm* and that of B is 11.02 *cm*.

$$D_{eff} = \frac{D_A + D_B}{2} = 1.6 \times 10^{-7}\ cm^2/s$$

$$\sigma_{1,i}^2 = 2D_{eff} t_{R,i} = 2 \times 1.6 \times 10^{-7} \times (3 \times 3600)$$

$$= 3.46 \times 10^{-3}\ cm^2$$

$$\sigma = 0.0587\ cm$$

$$\text{Width} = 4\sigma = 4 \times 0.0587 = 0.235\ cm$$

$$\text{Resolution} = R = \frac{1}{4} E \sqrt{\frac{t_{\exp}}{2D_{eff}}} (\mu_1 - \mu_2)$$

$$= \frac{1}{4} \times 100 \times \sqrt{\frac{3 \times 3600}{2 \times 1.6 \times 10^{-7}}} (1.02 - 1) \times 10^{-5}$$

$$= 0.918$$

R can be increased by decreasing E or by increasing t_{exp}.

References:

1. P. C. Wankat, Rate Controlled Separations, Springer, New Delhi, 2005.