Theory of Zone Electrophoresis of Reversibly Interacting Systems

TWO ZONES FROM A SINGLE MACROMOLECULE*

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Recently (1), a moving boundary theory of electrophoretic transport was formulated for interacting systems of the type

\[ P + nHA = P(HA)_n \]  

where \( P \) represents a macromolecular ion in solution and \( P(HA)_n \) its complex with \( n \) moles of a small uncharged constituent, \( HA \), of the solvent medium. It was assumed that the complex migrates with a greater electrophoretic mobility than the uncomplexed macromolecule and that equilibrium is established instantaneously. Computer solution of the conservation equations showed that under appropriate conditions the electrophoretic patterns will resolve into two moving peaks as a result of production of gradients of \( HA \) concentration in the electrophoresis column. The computations predicted the essential features of the electrophoretic behavior of proteins in acidic media containing varying concentrations of carboxylic acid buffer and provided a theoretical basis for our previous interpretation in terms of the electrophoretic behavior of proteins in acidic media containing varying concentrations of carboxylic acid buffer and provided a theoretical basis for our previous interpretation in terms of the electrophoretic behavior of proteins in acidic media containing varying concentrations of carboxylic acid buffer and provided a theoretical basis for our previous interpretation in terms of the electrophoretic behavior of proteins in acidic media containing varying concentrations of carboxylic acid buffer.

**FORMULATION OF THEORY**

As previously (1), the molar concentrations of \( P, P(HA)_n \), and \( HA \) are designated as \( C_1 \), \( C_2 \), and \( C_3 \), and their diffusion coefficients as \( D_1 \), \( D_2 \), and \( D_3 \). The electrophoretic mobilities of \( P \) and \( P(HA)_n \) are designated as \( \mu_1 \) and \( \mu_2 \); the mobility of the uncharged molecules, \( HA \), is zero. \( K \) is the equilibrium constant of Reaction 1, \( E \), the time of electrophoresis, and \( x \), the position in the electrophoresis column. The electric field strength, \( E \), is assumed to be the same throughout the electrophoresis column; i.e. electrophoresis is assumed to be ideal in the sense that the contribution of the protein to conductivity is negligible. A further simplification is afforded by ignoring the dependence of \( \mu_1 \) and \( \mu_2 \) on concentration of \( HA \).

The conservation equations and the equation expressing the assumption of local chemical equilibrium at every instant are identical with Equations 2 of Reference 1. The transformation of the conservation equations to a moving frame of reference, conversion of the differential equations to finite difference equations, and the details of their numerical solution on the Los Alamos IBM 7094 electronic computer are the same as described previously (1) except for the choice of initial and boundary values.

The initial values are set with protein occupying a single zone at the center of the space mesh.

\[ C_1(0, x_i) = C_2(0, x_i) = 0 \quad i \neq I/2 \]
\[ C_1(0, x_{I/2}) = C_{10} \]
\[ C_2(0, x_i) = K C_1(C_{30}) = C_{10} \]
\[ C_3(0, x_i) = C_3 \quad i = 0, 1, 2 \ldots I \]

As boundary values we take for all \( t_t \)

\[ C_1(l_t, 0) = C_2(l_t, x_j) = 0 \]
\[ C_2(l_t, 0) = C_2(l_t, x_j) = 0 \]
\[ C_3(l_t, 0) = C_3(l_t, x_j) = 0 \]

and use a sufficiently large mesh that during the time of interest no appreciable disturbance of the initial values reaches its extremities.

The above initial conditions correspond to experimental ones for zone electrophoresis, i.e. a sharp zone of solution containing equilibrium concentrations of \( P, P(HA)_n \), and \( HA \) imbedded in a column of solvent containing the same concentration of \( HA \), stabilization against convective mixing being achieved by using a solid supporting medium or a density gradient produced by an inert solute. The concentrations of \( P \) and \( P(HA)_n \) in the initial equilibrium solution are designated as \( C_{10} \) and \( C_{30} \), respectively. The concentration of \( HA \) is generally expressed as \( pH = -\log C_3 \), although the symbol \( C_{30} \) is used to indicate initial concentration. The following values for the several molecular parameters were used in all the computations: \( \mu_1 = 3 \times 10^{-4} \text{ cm}^2 \text{sec}^{-1} \text{volt}^{-1}, \mu_2 = 5 \times 10^{-4}, D_t = D_3 = 3.14 \times \)

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$10^{-7}$ cm$^2$ sec$^{-1}$, and $D_2 = 5.5 \times 10^{-4}$. The latter value is typical of small molecules having molecular weights of the order of 100.

$E$ was assigned a value of $\pm 10$ volts cm$^{-1}$, $C_{10} + C_{20} = 1.4 \times 10^{-4}$ M, and $C_{20}/C_{10} = 1$. The results of the computations are presented graphically as plots of $(C_1 + C_2)$ against position in the electrophoresis column, i.e. theoretical zone electrophoretic patterns, accompanied by plots of pH $A$ against position. In several cases, $C_1$ and $C_2$ are also plotted against position. Migration velocities per unit field, $10^5 \times v$ cm$^2$ set$^{-1}$ volt$^{-1}$, are shown above or beside corresponding zones in the theoretical electrophoretic patterns.

**RESULTS**

Computations have been made for two values of $C_{30}$ with the use of about a 10-fold range of values for $n$, i.e. the order of the reaction with respect to HA, in each case. The results summarized in Figs. 1 through 4 show that, as with moving boundary electrophoresis (1), the nature of the theoretical zone electrophoretic patterns is determined by the interplay of three factors:

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![Image](https://via.placeholder.com/150)
(a) the value of $n$, and (b) the concentration gradients of $IIA$ produced by (c) the continual reequilibration of Reaction 1 during differential transport of $P$ and $P(HA)_n$. For relatively small values of $n$ (Fig. 1), the patterns show a single, slightly skewed zone migrating at a velocity which is approximately the weighted average of the mobilities of $P$ and $P(HA)_n$. For intermediate values (Fig. 2), the patterns resolve into two zones for short times of electrophoresis, although the relative amounts of macromolecule contained in the zones do not correspond to the initial equilibrium composition of the macromolecule solution. As electrophoresis proceeds, the slower moving, minor zone spreads excessively as the two zones blend into a single trailing one which eventually becomes very broad indeed. Thus, for the longest time of electrophoresis (Fig. 2F), the breadth of the zone is about 7 cm, the maximum having migrated 9.3 cm. (The role of reequilibration in producing this phenomenon is illustrated in Fig. 3.) Such broad trailing zones are often observed in practice and are usually attributed either to adsorption of the protein on the solid supporting medium or to a known electrophoretic heterogeneity as in the case of $\gamma$-globulin. Clearly, in the absence of demonstrated heterogeneity, interaction of a single macromolecule with an uncharged constituent of the solvent must also be entertained as a possible explanation of trailing zones.

Finally, for large values of $n$ (Fig. 4), the patterns show two well resolved and stable zones which migrate at velocities approaching the mobilities of $P$ and $P(HA)_n$. The relative amounts of macromolecule in the two zones also approach the initial equilibrium composition, but, as is typical of reaction systems of the type $A + B \leftrightarrow C$, the concentration of protein never reaches zero between the zones. These results show that a single macromolecule, which interacts cooperatively and reversibly with an uncharged constituent of the solvent with concomitant change in electrophoretic mobility, can give two well resolved and intense zones despite instantaneous establishment of equilibrium. The small uncharged constituent may be undissociated buffer acid, buffer base, or a contaminant.

**DISCUSSION**

Zone electrophoresis is a powerful method for separating macromolecules and has found extensive application to the analysis of biological tissues and fluids, to characterization of different molecular forms of enzymes and other biologically important macromolecules and their subunits, and to genetic analysis. Accordingly, it is imperative that cognizance be taken of the fact that multiple zones need not necessarily indicate heterogeneity. The results presented above show that a single macromolecule interacting with the solvent can give two well resolved and intense zones despite instantaneous establishment of equilibrium. The situation can be even more complex than that. Thus, a single macromolecule, which isomerizes reversibly at rates comparable to the rates of electrophoretic separation of the isomers, can give three peaks on moving boundary electrophoresis (2, 3) and three spots on chromatography (4, 5). Fortunately, there is an unambiguous method for distinguishing between interactions and true heterogeneity. The macromolecule in the zones is isolated and the resulting fractions analyzed electrophoretically under conditions identical with those used in the original separation. For interactions the fractions will behave like the unfractiected material and show two or more zones, while for heterogeneity a single zone will be obtained. This test is also applicable to heterogeneous mixtures in which each component interacts with the solvent or undergoes isomerization. The requirements which must be fulfilled to assure validity of the fractionation test have recently been described (6).

The above considerations also apply to zone ultracentrifugation, in which case the reactive constituent of the solvent can be an ion.

Finally, the results described herein indicate that zone electrophoresis should prove useful for investigating reversible interactions of macromolecules with uncharged reactants and zone ultracentrifugation for interactions with either small uncharged reactants or ions.

**SUMMARY**

A theory of zone electrophoresis has been formulated for systems of the type

$$P + nHA \leftrightarrow P(HA)_n$$

where $P$ represents a macromolecular ion in solution and $P(HA)_n$ its complex with $n$ moles of an uncharged constituent, $HA$, of the solvent medium. It is assumed that the complex migrates with a different electrophoretic mobility than the uncomplexed macromolecule and that equilibrium is established instantaneously. Numerical solution of the conservation equations with the use of a digital computer shows that under appropriate conditions the electrophoretic patterns will resolve into two stable and intense zones. Other conditions may yield a single but very broad, trailing zone. These results also apply to zone ultracentrifugation.

**REFERENCES**

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