Electrophoresis in the Separation of Biological Macromolecules

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List of abbreviations

ANS	8-anilino-1-naphthalenesulphonic acid
Bis	N,N′-methylenebisacrylamide
BSA	bovine serum albumin
C	concentration of cross-linking agent in percentage of total monomers (T)
CSF	cerebrospinal fluid
D	diffusion constant
DATD	diallyltartar diamide
DMAPN	3-dimethylaminopropionitrile
DOC	sodium deoxycholate
EDTA	ethylenediaminetetraacetic acid, disodium salt
IEF	isoelectric focusing
HDL	high density lipoproteins
LDH	lactate dehydrogenase
LDL	low density lipoproteins
POPOP	1,4-di-2-(5-phenyloxazolyl)-benzene
PPO	2,5-diphenyloxazole
SDS	sodium dodecylsulphate
σ	standard deviation
TCA	trichloroacetic acid
TEMED	N,N,N′N′-tetramethylethylenediamine
T	total concentration of monomers (acrylamide and cross-linking agent)
U	mobility
U_0	free mobility
μ	ionic strength
VLDL	very low-density lipoproteins

Preface

Electrophoresis is a method widely used in the analysis of biological macromolecules. Electrophoretic techniques are relatively simple and inexpensive, and some of them can be adapted for serial investigations, e.g. in the clinical laboratory. In this book we tried to give a review of electro-migration methodology. This attempt is justified by the fact that during the approximately forty years' history of modern electrophoresis a vast number of methodical variations have been created. To quote just one example, a selected bibliography of the papers on electrophoresis, published between 1968 and 1972, lists 8236 items (*J. Chromatography*, Supplementary Volume No. 4, 1975), while the total number of papers is undoubtedly higher. Our aim, therefore, has not been to survey the literature of electrophoresis, but to outline the principles and practice of the most important variants and to provide representative examples of their application for the study of proteins, nucleic acids and glycosaminoglycans.

In the course of the preparation of the manuscript, we soon realized that, for the user of our book, a critical assessment of the advantages and disadvantages of the individual methods would be of greater practical value than a mere collection of laboratory recipes. Nevertheless, some methods of basic importance are described in detail to enable their reproduction in experimental work. Only essential details are given of specific techniques, and for particulars, the reader is referred to the original publication. With the chapters on methods of isolating different groups of proteins, nucleic acids and glycosaminoglycans, our aim has been to demonstrate how the properties of a given group of macromolecules influence the applicability of the separation techniques. In many instances, the choice of method of electrophoretic analysis depends on the technique of isolation and purification of the sample before electrophoresis. These latter steps are important for obtaining reproducible electrophoretic results.

Manufacturers of equipment and chemicals are mentioned only in those instances when this was inevitable. In this manual we do not provide any systematic information about sources of equipment or reagents; there-

fore, we must emphasize that mentioning or omitting any industrial house does not imply any qualification.

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The Authors

I. Theoretical and Technical Bases of Electrophoretic Methods

I.1. Principles of electrophoresis. A brief survey

Biological macromolecules – proteins, nucleic acids, polysaccharides – are dispersed in solution as particles of colloidal size. These possess a certain electrical charge which is due to groups capable of dissociating electrolytically. The net charge of a given particle is determined first of all by the H^+ ion concentration of the medium and may be modified by interaction with small molecular weight ions or other macromolecules. Under the influence of an electrical field charged particles will migrate either to the cathode or to the anode, depending on the sign of their net charge. This phenomenon is called electrophoresis. The migration velocity (cm sec^{-1}) of a particle at an electrical field strength of 1 V cm^{-1} is called electrophoretic mobility. The dimension of this parameter is $cm^2\ sec^{-1}\ V^{-1}$ and its sign is the same as that of the net charge of the particle. Differences in mobilities provide the basis for separating substances from mixtures for analytical or preparative purposes. Determination of mobility is also useful for characterizing a substance.

The understanding of the behaviour of a macromolecule under the effect of an electrical field requires the establishment of a theoretical relationship between mobility and molecular parameters like electrical charge and frictional coefficient, and the elucidation of the effect of the composition of the solvent medium on these parameters. We shall give only a brief outline of these considerations. For a detailed discussion of the problem the reader should consult, for example, Overbeek and Lijklema (1959), Overbeek and Wiersema (1967), and Shaw (1969).

Consider an isolated particle suspended in a perfect insulator. If a uniform electric field (E) is applied, a force equal to QE (where Q is the net charge of the particle) will act on the particle. When the particle is set to move from rest, a frictional force $f(\mathrm{d}x/\mathrm{d}t)$ will act in the opposite direction, where f is the frictional coefficient and $\mathrm{d}x/\mathrm{d}t$ is the velocity of the particle. The resultant of these two forces according to Newton's second law equals $m(\mathrm{d}^2x/\mathrm{d}t^2)$ and thus the following equation will hold:

$$m\frac{\mathrm{d}^2x}{\mathrm{d}t^2} = QE - f\frac{\mathrm{d}x}{\mathrm{d}t} \tag{1}$$

This equation can be solved as

$$\frac{dx}{dt} = \left(\frac{QE}{f}\right)\left(1 - \exp\left[-\left(\frac{f}{m}\right)t\right]\right) \tag{2}$$

Since the value of $f/m \gg 1$ (it is 10^{12}–10^{14} for particles of molecular size) the value of $\exp(-f/m)t$ decreases to $1/e$ in 10^{-14} to 10^{-12} sec, and therefore for periods longer than about 10^{-11} sec, Eq. (2) reduces to

$$\frac{dx}{dt} = \frac{QE}{f} \tag{3}$$

This means that, when an electrical field is turned on, the particle accelerates rapidly until the electrical force is balanced by the frictional force. Then it migrates at a constant velocity according to Eq. (3).

For spherical particles the frictional coefficient is $6\pi\eta a$, where a is the radius of the particle and η is the viscosity coefficient of the solvent (Stokes' law). It may be convenient to eliminate the radius of the particle by substituting Q/a by $D\psi_0$, where ψ_0 is the potential at the surface of the sphere which is equal to Q/Da. D is the dielectric constant of the particle. The mobility (U) of the sphere is then

$$U = E^{-1}\frac{dx}{dt} = \frac{D\psi_0}{6\pi\eta a} \tag{4}$$

It is obvious that this relation, developed for charged particles moving in a perfect insulator, must be modified if it is to be applied to the electrophoresis of macromolecules, since such experiments are carried out in electrolytic solutions. One must take into account the fact that an ionic atmosphere exists around the charged colloidal particle. Owing to electrostatic forces between the charged groups of the particle and the ions in the solvent, the particle is surrounded by a diffuse cloud of ions of opposite sign to the charge of the particle. At great distances from the particle the net charge in any volume element that is large compared to atomic dimensions is zero. The presence of this ionic atmosphere around the particle results in electrophoretic mobilities slower than those predicted by Eq. (4). This difference is due to three effects. Firstly, the potential at the surface of the particle is lowered by decreasing the effective electrostatic charge. Secondly, the electrical field also acts upon the ions surrounding the macromolecular particle. As the sign of the charge of the ionic cloud is unlike that of the particle, the cloud will be moved in a direction opposite to that of the particle, thus retarding the migration of the latter (electrophoretic friction). Thirdly, a further retarding effect is related to the fact that the electric current carries ions to and from the environment of the particle. Hence the ions constitut-