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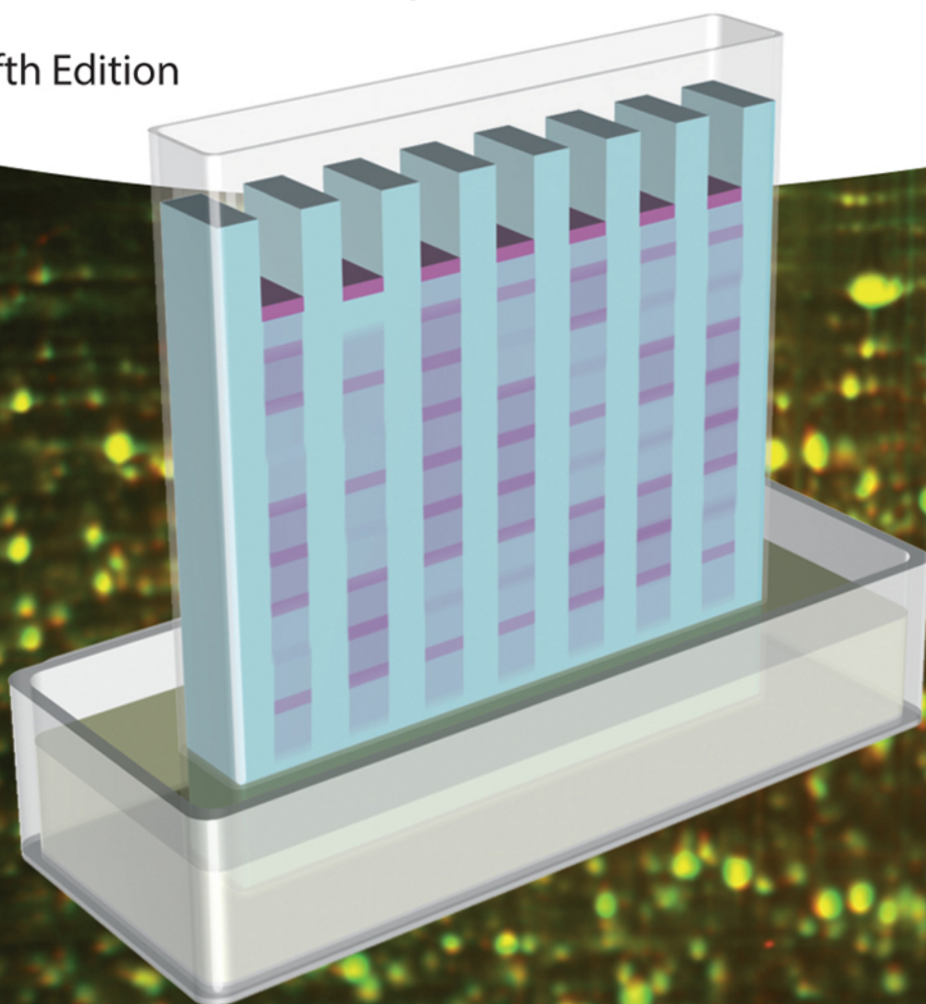
Reiner Westermeier

Electrophoresis in Practice

A Guide to Methods and Applications of DNA and Protein Separations

Fifth Edition



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Foreword

The number of electrophoretic separation methods has increased dramatically since Tiselius' pioneering work, for which he received the Nobel Prize. Development of these methods has progressed from paper, cellulose acetate membranes and starch gel electrophoresis to molecular sieve, disc, SDS and immunoelectrophoresis and, finally, to isoelectric focussing but also to high-resolution two-dimensional electrophoresis. Together with silver and gold staining, autoradiography, fluorography, and blotting, these techniques afford better resolution, sensitivity and specificity for the analysis of proteins. In addition, gel electrophoresis has proved to be a unique method for DNA sequencing, while high-resolution two-dimensional electrophoresis has smoothed the fascinating path from isolation of the protein to the gene through amino acid sequencing and, after gene cloning, to protein synthesis.

The spectrum of analytical possibilities has become so varied that an overview of electrophoretic separation methods seems desirable not only for beginners but also for experienced users. This book has been written for this purpose.

The author belongs to the circle of the bluefingers, and experienced this in Milan in 1979 when he was accused of being a money forger when buying cigarettes in a kiosk after work because his hands were stained by Coomassie. Prof Righetti and I had to extricate him from this tricky situation. According to Maurer's definition (Proceedings of the first small conference of the bluefingers, Tübingen 1972), an expert was at work on this book and he can teach the whitefingers who only know of the methods by hearsay, for example, how not to get blue fingers.

As it is, I am sure that this complete survey of the methods will help not only the whitefingers but also the community of the bluefingers, silverfingers, goldfingers, and so on, and will teach them many technical details.

February 1990

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Abbreviations, Symbols, Units

2D electrophoresis	two-dimensional electrophoresis
A	ampere
acc.	according
A,C,G,T	adenine, cytosine, guanine, thymine
ACES	*N*-2-acetamido-2-aminoethanesulfonic acid
AEBSF	aminoethyl benzylsulfonyl fluoride
AFLP	amplified restriction fragment length polymorphism
API	atmospheric pressure ionization
APS	ammonium persulfate
ARDRA	amplified ribosomal DNA restriction analysis
AU	absorbance units
16-BAC	benzyldimethyl-*n*-hexadecylammonium chloride
BAC	bisacryloylcystamine
Bis	*N*,*N′*-methylenebisacrylamide
BNE	blue native electrophoresis
bp	base pair
BSA	bovine serum albumin
C	crosslinking factor (%)
CA	carbonic anhydrase
CAF	chemically assisted fragmentation
CAM	coanalytical modification
CAPS	3-(cyclohexylamino)-propanesulfonic acid
CCD	charge-coupled device
CHAPS	3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate
CE	capillary electrophoresis
CID	collision induced dissociation
conc.	concentrated
CM	carboxylmethyl
CN-PAGE	clear native page
const.	constant
CTAB	cetyltrimethylammonium bromide
Da	dalton
DAF	DNA amplification fingerprinting

DBM	diazobenzyloxymethyl
DEA	diethanolamine
DEAE	diethylaminoethyl
DGGE	denaturing gradient gel electrophoresis
DHB	2,5-dihydroxybenzoic acid
DIGE	difference gel electrophoresis
Disc	discontinuous
DMSO	dimethylsulfoxide
DNA	desoxyribonucleic acid
DPT	diazophenylthioether
dsDNA	double stranded DNA
DSCP	double strand conformation polymorphism
DTE	dithioerythritol
DTT	dithiothreitol
E	field strength in volt per centimeter
EDTA	ethylenediaminetetraacetic acid
ESI	electro spray ionization
EST	expressed sequence tag
FT-ICR	Fourier transform – ion cyclotron resonance
GC	group specific component
GMP	good manufacturing practice
h	hour
HED	hydroxyethyldisulfide
HEPES	*N*-2-hydroxyethylpiperazine-*N*′-2-ethananesulfonic acid
HMW	high molecular weight
HPCE	high performance capillary electrophoresis
HPLC	high performance liquid chromatography
I	current in ampere, milliampere
ICPL	isotope-coded protein labeling
IEF	isoelectric focusing
IgG	immunoglobulin G
IPG	immobilized pH gradients
ITP	isotachophoresis
kB	kilobases
kDa	kilodaltons
K_R	retardation coefficient
LED	light emitting diode
LIF	laser induced fluorescence
LMW	low molecular weight
M	mass
mA	milliampere
MALDI	matrix assisted laser desorption ionization
MCE	microchip electrophoresis
MEKC	micellar electrokinetic chromatography
MES	2-(*N*-morpholino)ethanesulfonic acid

min	minute
mol/L	molecular mass
MOPS	3-(*N*-morpholino)propanesulfonic acid
m_r	relative electrophoretic mobility
mRNA	messenger RNA
MS	mass spectrometry
Ms^n	mass spectrometry with *n* mass analysis experiments
MS/MS	tandem mass spectrometry
MW	molecular weight
NAP	nucleic acid purifier
Nonidet	nonionic detergent
NEPHGE	non equilibrium pH gradient electrophoresis
NHS	*N*-hydroxy-succinimide
O.D.	optical density
P	power in watt
p.a.	per analysis
PAG	polyacrylamide gel
PAGE	polyacrylamide gel electrophoresis
PAGIEF	polyacrylamide gel isoelectric focusing
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PEG	polyethylene glycol
PFG	pulsed field gel (electrophoresis)
PGM	phosphoglucose mutase
p*I*	isoelectric point
PI	protease inhibitor
p*K*	dissociation constant
PMSF	phenylmethyl-sulfonyl fluoride
PPA	piperidino propionamide
PSD	postsource dissociation (decay)
PTM	posttranslational modification
PVC	polyvinylchloride
PVDF	polyvinylidene difluoride
r	molecular radius
RAPD	random amplified polymorphic DNA
REN	rapid efficient nonradioactive
Rf	value relative distance of migration
RFLP	restriction fragment length polymorphism
R_m	relative electrophoretic mobility
RNA	ribonucleic acid
RPA	ribonuclease protection assay
RuBP	ruthenium II tris-bathophenantroline disulfonate
s	second
SDS	sodium dodecyl sulfate
SNP	single nucleotide polymorphism

ssDNA	single stranded DNA
T	total acrylamide concentration [%]
TBE	tris borate EDTA
TBP	tributyl phosphine
TBS	tris buffered saline
TCA	trichloroacetic acid
TCEP	tris(2-carboxyethyl)phosphine
TEMED	*N*,*N*,*N′*,*N′*-tetramethylethylenediamine
TF	transferrin
TGGE	temperature gradient gel electrophoresis
ToF	time of flight
Tricine	*N*,tris(hydroxymethyl)-methyl glycine
Tris	tris(hydroxymethyl)-aminoethane
U	voltage in volt
V	volume in liter
v	speed of migation in meter per second
v/v	volume per volume
VLDL	very low density lipoproteins
W	watt
WiFi	wireless local area network (artificial abbreviation)
w/v	weight per volume (mass concentration)
ZE	zone electrophoresis

Preface

German Version

This book was written for the practitioner of electrophoresis in the laboratory. For this reason, we have avoided physico-chemical derivations and formulas concerning electrophoretic phenomena.

The type of explanation and presentation comes from several years of experience in giving user seminars and courses, writing handbooks, and solving user problems. They should be clear for technical assistants as well as for researchers in the laboratory. The commentary column offers room for personal notes.

In Part I, an introduction – as short as possible – to the actual state of the art is given. The references are not meant to be exhaustive.

Part II contains exact instructions for 11 chosen electrophoretic methods that can be carried out with one single piece of equipment. The sequence of the methods was planned so that an electrophoresis course for beginners and advanced users can be established afterwards. The major methods used in biology, biochemistry, medicine, and food science have been covered.

If – despite following the method precisely – unexplained effects should arise, their cause and remedies can be found in the troubleshooting guide in the Appendix.

The author would welcome any additional comments and solutions for the troubleshooting guide that the reader can supply.

Freiburg, March 1990 *R. Westermeier*

English Version, Fifth Edition

More than a decade has passed since the last update of this book. In the meantime, new methods have been developed in all areas of electrophoresis, workflows have been simplified, sensitivity of detection has been improved, and more experience has been added. Therefore it was high time to bring out a new, revised edition.

Many lecture tours, congresses, and hands-on workshops on proteomics and electrophoresis techniques inspired me to change the order of the chapters and update information in all sections. Since the book *Proteomics in Practice* had been published in a new edition, and new mass spectrometry methodologies have been evolved, a special chapter on proteomics was no longer needed. Furthermore, as many DNA typing methods are now performed with alternative and more automated techniques, this part could be shortened.

Freising, August 2015 *R. Westermeier*

Part I
Fundamentals

Introduction

Electrophoresis is besides chromatography the most frequently applied separation technique for the analysis of protein, glycan, and nucleic acid mixtures. With electrophoresis high separation efficiency can be achieved using a relatively simple setup of equipment. It is mainly applied for analytical rather than for preparative purposes. However, with the advent of amplification of DNA fragments with polymerase chain reaction (PCR®), and highly sensitive and powerful mass spectrometry (MS) analysis of proteins and peptides, so called "analytical amounts" of electrophoretically separated fractions can be further analyzed.

The main fields of application are biological and biochemical research, protein chemistry, pharmacology, forensic medicine, clinical investigations, veterinary science, food control as well as molecular biology. The monograph by Andrews (1986) is one of the most complete and practice-oriented books about electrophoretic methods. In the present book, electrophoretic methods and their applications will be presented in a much more condensed form.

Principle

Under the influence of an electrical field charged molecules or particles migrate into the direction of the electrode bearing the opposite charge. During this process, the substances are in aqueous solution. Because of their varying charges and masses, different molecules and particles of a mixture will migrate at different velocities and will thus be separated into single fractions.

The electrophoretic mobility, which is a measure of the migration velocity, is a significant and characteristic parameter of a charged molecule or particle. It is dependent on the pK values of the charged groups and the size of the molecule or particle. It is influenced by the type, concentration and pH of the buffer, by the

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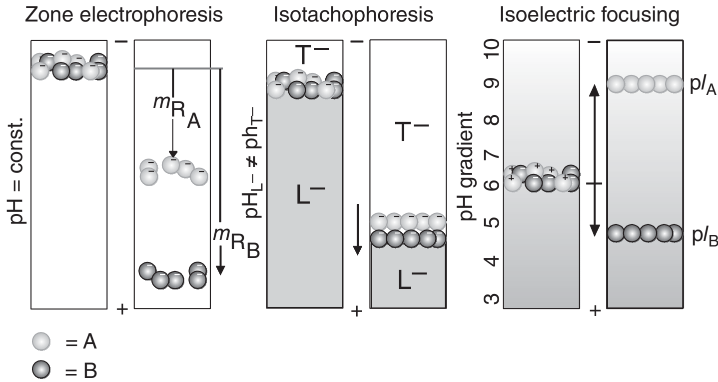

Figure p1.1 The three electrophoretic separation principles. Explanations in the text. A and B are the components of the sample.

temperature as well as by the nature of the supporting material. Electrophoretic separations are carried out in free solutions – like in capillary, microchip, and free flow systems – or in stabilizing media such as thin-layer plates, films, or gels. Detailed theoretical explanations can be found in the textbook edited by Lottspeich and Engels (2016).

Sometimes the *relative* electrophoretic mobility of substances is specified. It is calculated relative to the migration distance of a standard substance, mostly a dye like bromophenol blue, which has been applied as an internal standard. The relative mobility is abbreviated as m_r or R_m.

Three basically different electrophoretic separation methods are performed in practice nowadays:

a) Electrophoresis, sometimes called *Zone Electrophoresis* (ZE)
b) Isotachophoresis (ITP)
c) Isoelectric focusing (IEF).

The three separation principles are illustrated in Figure p1.1. There is a fourth method: “moving boundary electrophoresis,” which is described below. However this technique has no practical importance anymore.

a) In *ZE* a homogeneous buffer system is used over the whole separation time and range so as to ensure a constant pH value. The migration distances during a defined time limit are a measure of the electrophoretic mobilities of the various substances. It can be applied to nonamphoteric as well as amphoteric molecules. During the separation diffusion can lead to blurred zones, which reduces the sensitivity of detection and the resolution. Buffer reservoirs at the anodal and the cathodal side are needed to maintain the buffer conditions during the separation.
b) In *ITP*, the separation is carried out in a discontinuous buffer system. The ionized sample migrates between a leading electrolyte with a high mobility