ELECTROPHORESIS IN PRACTICE

Reiner Westermeier

Electrophoresis in Practice

A Guide to Methods and Applications of DNA and Protein Separations

Second Edition

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Westermeier

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Preface

The number of electrophoretic separation methods has increased dramatically since Tiselius' pioneer 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 focusing 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 not only help the whitefingers but also the community of the bluefingers, silverfingers, goldfingers etc. and will teach them many technical details.

Weihenstephan, February 1990
Prof. Dr. Angelika Görg

Foreword

German version

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

The type of explanation and presentation stems 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 will be given. The references are not meant to be exhaustive.

Part II contains exact instructions for 11 chosen electrophoretic methods, which 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 methods have been covered.

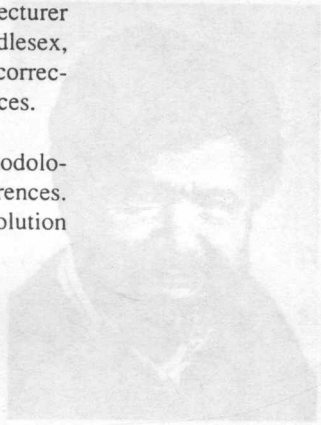
If - despite following the method precisely - unexplained effects should arise, their cause and the remedies can be found in the trouble-shooting guide in the appendix.

The author would be thankful for any additional comments and solutions for the trouble-shooting guide which the reader could supply.

Freiburg, March 1990 R. Westermeier

English version, First Edition

The author is grateful to Dr. Michael J. Dunn, Senior Lecturer at the National Heart and Lung Institute, Harefield, Middlesex, UK, for his kind engagement of reading the manuscript, correcting the english and for his excellent and informed advices.



In this version, some updates have been made to methodological aspects, new experiences, applications, and the references. A new drawing program is used, which allows higher resolution in the explanatory figures.

Leonberg, February 1993 R. Westermeier

English version, Second Edition

The author thanks Professor Görg for her tips for the state of the art of high resolution two-dimensional electrophoresis, and Dr. Gabriel Peltre, Institute Pasteur, Paris, for valuable hints on the practice of immunoelectrophoresis, agarose isoelectric focusing, blotting, and titration curves.

This version has been updated in the wording, the way of quoting the references, and in the methodology. A few figures, hints for problem solving, and a few very important references have been added. The main differences to the previous issue, however, are constituted by the addition of the lately developed methods for DNA typing and the methodology for vertical gels. Thus, section II contains now 15 chosen electrophoretic methods.

Freiburg, November 1996 R. Westermeier

Biography

Reiner Westermeier (born in 1951)

1976	Diplom Engineer
1981	Dr.-Ing., Technical University Munich, Weihenstephan, Germany
1976–1983	enganged in the development of new electrophoresis systems and applications at the Technical University Munich
1984–1987	employed as an electrophoresis products and applications specialist at LKB Instrument GmbH and
1987–1990	at Pharmacia Biotech
1991	founded a new company called ETC Elektrophorese-Technik for the development of new electrophoresis methods, media, and equipment
since January 1996	manager scientific support at Pharmacia Biotech in Freiburg, Germany
	several international cooperations and lecturing tours author of several publications and a book *(Electrophoresis in Practice,* First Edition, VCH)

Abbreviations, symbols, units

A	Ampere
acc.	according
A,C,G,T	Adenine, cytosine, guanine, thymine
ACES	N-2-acetamido-2-aminoethanesulfonic acid
A/D-transformer	Analog-digital transformer
AFLP	Amplified restriction fragment length polymorphism
APS	Ammonium persulphate
ARDRA	Amplified ribosomal DNA restriction analysis
AU	Absorbance units
BAC	Bisacryloylcystamine
Bis	N, N'-methylenebisacrylamide
bp	Base pair
BSA	Bovine serum albumin
C	Crosslinking factor [%]
CAPS	3-(cyclohexylamino)-propanesulfonic acid
CDGE	Constant denaturing gel electrophoresis
CE	Capillary electrophoresis
CHAPS	3-(3-cholamidopropyl)dimethylammonio-1-propane sulfonate
CM	Carboxylmethyl
CMW	Collagen molecular weight
concd	Concentrated
const.	Constant
CTAB	Cetyltrimethylammonium bromide
Da	Dalton
DAF	DNA amplification fingerprinting
DBM	Diazobenzyloxymethyl
DDRT	Differential display reverse transcription
DEAE	Diethylaminoethyl
DGGE	Denaturing gradient gel electrophoresis
Disc	Discontinuous
DMSO	Dimethylsulfoxide
DNA	Desoxyribonucleic acid
DPT	Diazophenylthioether
DSCP	Double strand conformation polymorphism
dsDNA	Double stranded DNA
DTE	Dithioerythreitol
DTT	Dithiothreitol
E	Field strength in V/cm
EDTA	Ethylenediaminetetraacetic acid
ESI	Electro spray ionization

GC	Group specific component
h	Hour
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 A, mA
IEF	Isoelectric focusing
IgG	Immunoglobulin G
IPG	Immobilized pH gradients
IPG-Dalt	2D electrophoresis: IPG/SDS electrophoresis
Iso-Dalt	2D electrophoresis: IEF/SDS electrophoresis
ITP	Isotachophoresis
kB	Kilobases
kDa	Kilodaltons
K_R	Retardation coefficient
LDAO	Lauryldimethylamine-N-oxide
LMW	Low Molecular Weight
mA	Milliampere
MALDI	Matrix assisted laser desorption ionization
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
MW	Molecular weight
NAP	Nucleic Acid Purifier
Nonidet	Non-ionic detergent
O.D.	Optical density
P	Power in W
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
pI	Isoelectric point
PI	Protease inhibitor
pK value	Dissociation constant
PMSF	Phenylmethyl-sulfonyl fluoride
PVC	Polyvinylchloride
PVDF	Polyvinylidene difluoride
r	Molecular radius
RAPD	Random amplified polymorphic DNA
REN	Rapid efficient nonradioactive
RFLP	Restriction fragment length polymorphism
R_f value	Relative distance of migration
R_m	Relative electrophoretic mobility
RNA	Ribonucleic acid
RPA	Ribonuclease protection assay
s	Second
SDS	Sodium dodecyl sulfate
ssDNA	single stranded DNA
T	Total acrylamide concentration [%]
t	Time, in h, min ,s
TBE	Tris borate EDTA
TCA	Trichloro acetic acid
TEMED	N,N,N',N'-tetramethylethylenediamine
TF	Transferrin
TGGE	Temperature gradient gel electrophoresis
TMPTMA	Trimethylolpropane-trimethacrylate[2-ethyl-2(hydrohymethyl)R 1,3-propandiol-trimethacrylate]
Tricine	N,tris(hydroxymethyl)-methyl glycine
Tris	Tris(hydroxymethyl)-aminoethane
U	Volt
V	Volume in L
v	Speed of migation in m/s
VLDL	Very low density lipoproteins
v/v	Volume per volume
W	Watt
w/v	Weight per volume (mass concentration)
ZE	Zone electrophoresis
2D electrophoresis	Two-dimensional electrophoresis

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Fundamentals

Section II

Methods

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Introduction

For no other biochemical separation method nowadays does one find so many new developments and methods as for electrophoretic separation techniques. With this method a high separation efficiency can be achieved using a relatively limited amount of equipment. 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. It will become increasingly important to be able to choose and carry out the appropriate electrophoresis technique for specific separation problems.
The monograph by Andrews (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.

Andrews AT. Electrophoresis, theory techniques and biochemical and clinical applications. Clarendon Press, Oxford (1986).

Principle: Under the influence of an electrical field, charged molecules and particles migrate in 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 speeds and will thus be separated into single fractions.

The electrophoretic mobility which influences the speed of migration, is a significant and characteristic parameter of a charged molecule or particle and is dependent on the pK value of the charged group and the size of the molecule or particle. It is influenced by the type, concentration and pH of the buffer, by the temperature and the field strength as well as by the nature of the support material. Electrophoretic separations can be carried out in free solutions as in capillary electrophoresis or systems without support phases but also in stabilizing media such as thin-layer plates, films or gels.

Chrambach A. The practice of quantitative gel electrophoresis. VCH Weinheim (1985).

Mosher RA, Saville DA, Thormann W. The Dynamics of Electrophoresis. VCH Weinheim (1992).

Detailed theoretical explanations can be found in the books by Chrambach (1985) and Mosher *et al.* (1992).

The *relative* electrophoretic mobility of substances is usually specified. It is calculated relative to the migration distance of a standard substance applied in the same run so as to compensate for different field strengths and separation time.

The relative mobility is abbreviated as mr or Rm.