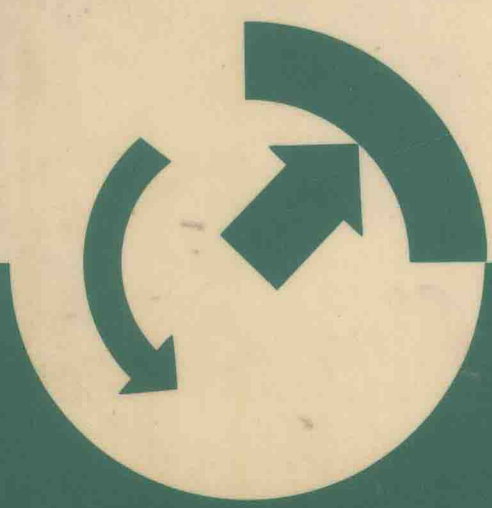
Advanced Methods in the Biological Sciences

Edited by V. Neuhoff and A. Maelicke



Andreas Chrambach

The Practice of Quantitative Gel Electrophoresis

VCH

Andreas Chrambach

The Practice of Quantitative Gel Electrophoresis

Dr. A. Chrambach
Department of Health
and Human Services
National Institutes of Health
Bethesda MD 20205, USA

Prof. Dr. V. Neuhoff
Max-Planck-Institut für
experimentelle Medizin
Hermann-Rein-Str. 3
D-3400 Göttingen

Prof. Dr. A. Maelicke
Max-Planck-Institut für
Ernährungsphysiologie
Rheinlanddamm 201
D-4600 Dortmund

1st edition 1985

Editorial Director: Dr. Hans F. Ebel

Deutsche Bibliothek Cataloguing-in-Publication-Data

Chrambach, Andreas:
The practice of quantitative gel electrophoresis / Andreas Chrambach. – Weinheim; Deerfield Beach, FL: VCH, 1985.
(Advanced methods in the biological sciences)
ISBN 3-527-26039-0 (Weinheim)
ISBN 0-89573-064-2 (Deerfield Beach, FL)
ISSN 0178-9244
NE: GT

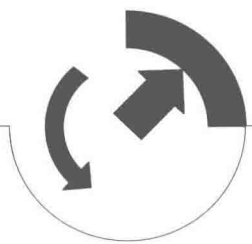
Composition: kühn & weyh software GmbH, D-7800 Freiburg
Printing: Zechnersche Buchdruckerei, D-6720 Speyer
Bookbinding: Klambt-Druck GmbH, D-6720 Speyer
Cover Design: Klaus Grögerchen, D-6901 Lampenhain
Printed in the Federal Republic of Germany

Chrambach

The Practice of Quantitative Gel Electrophoresis

Advanced Methods in the Biological Sciences

Edited by V. Neuhoff and A. Maelicke



Distribution:
VCH Verlagsgesellschaft, P.O. Box 1260/1280, D-6940 Weinheim (Federal Republic of Germany)
USA and Canada: VCH Publishers, 303 N.W. 12th Avenue, Deerfield Beach, FL 33442-1705 (USA)

ISBN 3-527-26039-0 (VCH Verlagsgesellschaft)
ISBN 0-89573-064-2 (VCH Publishers)

ISSN 0178-9244

Preface

The topic of the book is gel electrophoresis as a *unity*. This topic comprises polyacrylamide and agarose gel electrophoresis, isotachophoresis and electrofocusing. Our attempt will be to show that all of these are not competing methods of separation, but rather that each of them has its well defined area of applicability and provides the greatest efficiency of separation only within this unique area.

The electrophoretic separations for which the methods described in this book are preponderantly designed are those of *native* macromolecules, in contradistinction to the dissociated and denatured gene products. This bias derives from the axiom that biochemistry aims at an account of biological function in terms of chemical structures and reactions. Necessarily, these structures comprise the sum of post-translational molecular forms which participate in a given enzymatic, receptor, hormonal or other function.

A presentation of the quantitative, *theory-based* approaches to the separation of charged molecular species by gel electrophoresis is useful because these rational approaches provide a greater efficiency of separation than the arbitrarily selected methods. However, the sure way to prevent the average biochemist from using these (as any other) theory-based approaches in practice is to present the theoretical treatments. Therefore, this book contains no separation theory. It is exclusively a *practical* guide to separation of proteins or other charged molecular species. The results of theoretical considerations, leading to optimal efficiency of separation, are being provided only in form of computer output and the simple manipulations needed to obtain it. It is, in fact, this unique feature of computer programs of being able to imbed complex theory while providing results simply, that has made it possible for the average biochemist – most of us – to reap the fruit of separation theory in providing a more efficient separation practice.

A philosophical point needs to be made concerning this practical, non-theoretical presentation. It has to be realized that in areas such as the regulation of the concentrations, pH and other physical parameters of the trailing phase by the leading phase in moving boundary electrophoresis, a real understanding can only be conveyed by theoretical and mathematical language, i.e. in a commonly un-understandable form. To the degree that a qualitative, approximative and descriptive approach is used, the full truth is not being presented. But – the presentation becomes also commonly understandable. Since communication is needed to serve the average biochemist in his separation problems, this book has in those instances deliberately sacrificed scientific rigor and depth of presentation. It risks, therefore, to be considered inadequate by the theoretician. I hope, nonetheless, that it will provide approximative useful insights to the practitioner in search of more effective separation methods.

The separation methods of interest to this book are exclusively *general* ones, which are equally applicable to the physical identification and isolation of any charged macromolecule. This restriction is due to the fact that in most applications, there is no information prior to separation concerning a specific property of the macromolecule by which it would distinguish itself from the majority of other species. To find such a property would require a trial-and-error approach, or playing for luck, which is precisely the experimental approach which the rational, systematic and quantitative methods outlined in this book attempt to overcome. Thus, specific affinity methods will not be treated, although clearly these, and any other separation method exploiting a specific peculiarity or composition of the macromolecule of interest, are preferable to general experimental approaches if and when such a specific handle for fractionation is known.

Although this book is exclusively a practical guide to separation, it should not be read linearly from beginning to end as one would read a recipe. The reason for this is that our particular recipe deals with terminology, with polymerization chemistry, with particular forms of apparatus, the detailed description and discussion of which necessarily interrupts the narrative. The reader is therefore advised to use this book as a *handbook*, by selecting from the Table of Contents those sections dealing with the separation steps which he wants to apply to his problem. Then, facing terminology, polymer chemistry or apparatus he is unfamiliar with, he should trace back by means of the Table of Contents to the particular pages dealing with those points to obtain the necessary information.

I should explain why the *apparatus* needed to do quantitative gel electrophoresis is described and critically discussed in exhaustive detail. This would not be necessary if at this time such apparatus were commercially available. However, only some items of the needed apparatus are available, and nearly always in imperfect form. Other items need to be constructed or assembled by the user. The detailed critical review of the equipment thus serves as a guide for those intent on building or improving their own apparatus. At the same time, it serves, hopefully, as a reminder to the manufacturers of instrumentation that the equipment needs of efficient gel electrophoresis are still largely unfilled, or remain filled in non-optimal fashion.

This book does not attempt an *exhaustive* presentation of separation by gel electrophoretic methods. Rather, an attempt is being made to provide the reader with the same *sufficient set* of separation tools (rationales, apparatus, procedures, computer programs) that have made it possible to efficiently characterize and isolate diverse proteins in the author's laboratory. Necessarily, this approach is limited by the knowledge and insight of the author, but it is *practical* in the sense that the tools described here have been tried out and work.

The credits given to authors and instrument manufacturers necessarily omit those whose work I have been unfamiliar with, and whose ideas and findings should have found their way into this book but didn't. To remedy the situation in future editions of this or similar works, let me ask you, the reader, to communicate any such omissions and rectifications to me.

Bethesda,
in June 1984

A. Chrambach

Glossary of Terms

ALPHA	upper buffer phase
BETA	stacking-phase buffer, as prepared
Bis	*N,N'*-methylenebisacrylamide
BV	buffer value
C1	concentration of constituent 1 (M)
C2	concentration of constituent 2 (M)
C3	concentration of constituent 3 (M)
C6	concentration of constituent 6 (M)
%C	crosslinking agent (g/100 mL) × 100/%T
CMC	critical micelle concentration
CZE	continuous zone electrophoresis
DATD	*N,N'*-diallyltartardiamide
EDA	ethylenediacrylate
EF	electrofocusing
Ferguson plot	plot of log (R_f) vs. %T
GAMMA	resolving-phase buffer, as prepared
i	current (amps)
I	ionic strength
IEF	isoelectric focusing
ITP	isotachophoresis
K_R	retardation coefficient [– d log (R_f)/d%T]
κ	specific conductance $(1/\text{ohm}\cdot\text{cm})\cdot 10^{-6}$
KP	potassium persulfate
LAMBDA	buffer phase containing constituent 2 displacing constituent 3
M_o	free electrophoretic mobility ($cm^2/s/volt$)
MBE	moving boundary electrophoresis
MW	molecular weight
NU, ν	boundary displacement
PAGE	polyacrylamide gel electrophoresis
PI	operative resolving phase containing constituent 1 (set with constituent 3)
pI	isoelectric point
PCA	perchloric acid
r	resistivity (ohm cm)
R	ionic mobility relative to Na^+ (in computer output)
$\overline{R}$	geometric mean radius (nm)
R_f	electrophoretic mobility relative to front moving boundary
RM	net mobility relative to Na^+

RM(1,ZETA)	RM of constituent 1 in phase ZETA, trailing ion net mobility, stacking phase
RM(2,BETA)	RM of constituent 2 in phase BETA, leading ion net mobility, trailing phase
RM(1,PI)	RM of constituent 1 in phase PI, trailing ion net mobility, resolving phase
RM(1,4)	computer output designation for RM(1,ZETA)
RM(2,2)	computer output designation for RM(2,BETA)
RM(1,9)	computer output designation for RM(1,PI)
RN	riboflavin
SCAM	synthetic carrier ampholyte mixture
SDS	sodium dodecylsulfate
%T	total gel concentration (acrylamide plus Bis) (g/100 mL)
TCA	trichloroacetic acid
TEMED	*N,N,N′,N′*-tetramethylethylenediamine
Y_o	extrapolated R_f at %T = 0 on the Ferguson plot

Contents