Gel Electrophoresis of Proteins:

a practical approach

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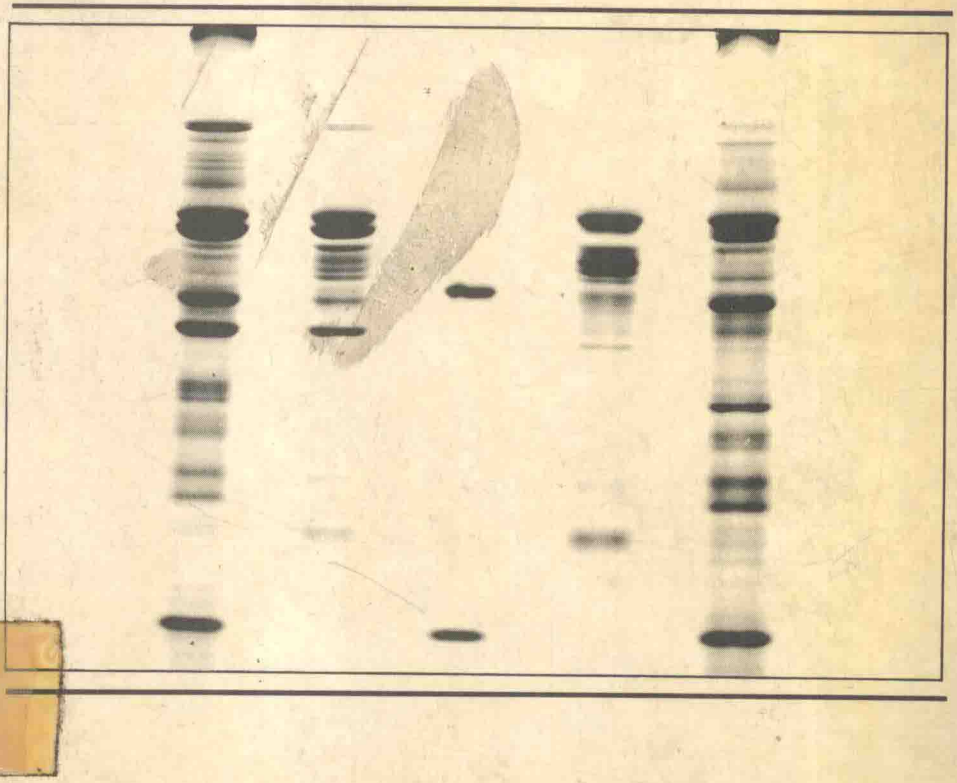

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Preface

The development of separation methods has played a significant role in the elucidation of biological systems. Of the various techniques in common use, one of the most important is gel electrophoresis. This book and its companion volume (see back cover for contents) are designed to provide details of gel electrophoretic procedures for the separation of macromolecules. The main emphasis of each book is on the practical aspects of the electrophoretic techniques in current use. Several revisions of some chapters were necessary in order to prevent undue repetition whilst including important practical topics and we thank the authors concerned and particularly the publishers for their patience and understanding during this exercise. Thanks are also due to Irene Hames for her skillful proofreading of the text at all stages.

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IMPORTANT NOTICE

HEALTH HAZARDS OF GEL ELECTROPHORESIS

(i) A number of chemicals commonly used for gel electrophoresis are toxic whilst the status of others remains unknown. It is very important that experimenters acquaint themselves with the precautions required for handling all chemicals mentioned in this text. Particular care should be taken when handling acrylamide since this is a known potent neurotoxin. Polyacrylamide gel is not toxic unless it contains unpolymerised monomer.

(ii) Care should be taken when using gel electrophoresis apparatus that no electrical safety hazard exists. Particular care should be taken when using apparatus not obtained from commercial sources since this may not meet the usual required safety standards. It is recommended that all apparatus is checked by a competent electrician before use.

Abbreviations

A	amps
ACES	*N*-2-acetamido-2-aminoethanesulphonic acid
AEPD	2-amino,2-ethyl,1,3-propanediol (≡ Ammediol)
AMP	2-amino-2-methyl-propanol
ANS	1-anilino-8-naphthalene sulphonate
BAC	*N,N'*-bisacrylylcystamine
BES	*N,N*-bis(2-hydroxyethyl)-2-aminoethanesulphonic acid
Bicine	*N,N*-bis(2-hydroxyethyl)glycine
Bisacrylamide	*N,N'*-methylene bisacrylamide
Bistris	[bis(2-hydroxyethyl)-amino]tris(hydroxymethyl)methane
Bistrispropan	1,3-bis[tris(hydroxymethyl)methylamino]propane
% C	percentage crosslinker (as a percentage of the total monomer)
% C_{Bis}	percentage bisacrylamide crosslinker
% C_{DATD}	percentage DATD crosslinker
CTAB	cetyltrimethylammonium bromide
CZE	continuous zone electrophoresis (zone electrophoresis using a continuous buffer system)
DATD	*N,N'*-diallyltartardiamide
D.C.	direct current
DMAPN	3-dimethylamino-propionitrile
DMSO	dimethylsulphoxide
EDTA	ethylenediaminetetra-acetate
EF	electrofocusing
EPPS	*N*-2-hydroxyethylpiperazine-*N'*-3-propanesulphonic acid
g	gram(me)
xg	centrifugal force (x unit gravitational field)
GABA	γ-aminobutyric acid
GACA	γ-aminocaproic acid
HEPES	*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulphonic acid
I.D.	internal diameter
ITP	isotachophoresis
K_R	retardation coefficient (slope of the Ferguson plot); a measure of molecular size
M	molarity
M	mobility
M_o	free electrophoretic mobility ($cm^2/s/V$)
mA	milliamps
MDPF	2-methoxy-2,4-diphenyl-3(2H)-furanone
MES	2-(*N*-morpholino)ethanesulphonic acid
MOPS	3-(*N*-morpholino)propanesulphonic acid
MTT	methyl thiazolyl tetrazolium
MW	molecular weight
MZE	multiphasic zone electrophoresis (zone electrophoresis using a multiphasic buffer system)

NBT	nitroblue tetrazolium
O.D.	outside (external) diameter
PAGE	polyacrylamide gel electrophoresis
PCA	perchloric acid
pI	isoelectric point
pI′	apparent isoelectric point
PITC	phenylisothiocyanate
pK	− log dissociation constant (the pH at half dissociation)
PMS	phenazine methosulphate
PMSF	phenylmethylsulphonyl fluoride
POPOP	1,4-bis[2-(5-phenyloxazolyl)]benzene
PPO	2,5-diphenyloxazole
$\bar{R}$	geometric mean radius
R_f	relative electrophoretic mobility (e.g. relative to a dye front or to a moving boundary 'front')
SDS	sodium dodecyl sulphate (sodium lauryl sulphate)
SDS-PAGE	polyacrylamide gel electrophoresis in the presence of SDS
SSS	steady-state stacking
%T	polyacrylamide gel concentration defined as percentage total monomers (i.e. acrylamide + crosslinking agent, g/100 ml)
T_{max}	gel concentration for maximum separation between two proteins
T_{opt}	gel concentration for maximum resolution between two proteins
TAPS	3-{[tris(hydroxymethyl)methyl]amino}propanesulphonic acid
TCA	trichloroacetic acid
TEMED	*N,N,N′,N′*-tetramethylethylenediamine
TES	2-{[tris(hydroxymethyl)methyl]amino}ethanesulphonic acid
Tricine	*N*-[tris(hydroxymethyl)methyl]glycine
U.V.	ultraviolet
V	volts
V	molecular valence (net protons/molecule)
W	watt
Y_o	y intercept on the Ferguson plot; a measure of molecular net charge

Contents

CHAPTER 1

An Introduction to Polyacrylamide Gel Electrophoresis

B.DAVID HAMES

INTRODUCTION

Any charged ion or group will migrate when placed in an electric field. Since proteins carry a net charge at any pH other than their isoelectric point, they too will migrate and their rate of migration will depend upon the charge density (the ratio of charge to mass) of the proteins concerned; the higher the ratio of charge to mass the faster the molecule will migrate. The application of an electric field to a protein mixture in solution will therefore result in different proteins migrating at different rates towards one of the electrodes. However, since all proteins were originally present throughout the whole solution, the separation achieved is minimal. Zone electrophoresis is a modification of this procedure whereby the mixture of molecules to be separated is placed as a narrow zone or band at a suitable distance from the electrodes such that, during electrophoresis, proteins of different mobilities travel as discrete zones which gradually separate from each other as electrophoresis proceeds. In theory, separation of different proteins as discrete zones is therefore readily achieved provided their relative mobilities are sufficiently different and the distance allowed for migration is sufficiently large. However, in practice there are disadvantages to zone electrophoresis in free solution. Firstly, any heating effects caused by electrophoresis can result in convective disturbance of the liquid column and disruption of the separating protein zones. Secondly, the effect of diffusion is to constantly broaden the protein zones and this continues after electrophoresis has been terminated. To minimise these effects, zone electrophoresis of proteins is rarely carried out in free solution but instead is performed in a solution stabilised within a supporting medium. As well as reducing the deleterious effects of convection and diffusion during electrophoresis, the supporting medium allows the investigator to fix the separated proteins at their final positions immediately after electrophoresis and thus avoid the loss of resolution which results from post-electrophoretic diffusion. The fixation process employed varies with the supporting medium chosen.

Many supporting media are in current use, the most popular being sheets of paper or cellulose acetate, materials such as silica gel, alumina, or cellulose which are spread as a thin layer on glass or plastic plates, and gels of agarose, starch, or polyacrylamide. These media fall into two main classes. Paper, cellulose acetate, and thin-layer materials are relatively inert and serve mainly for support and to minimise convection. Hence separation of proteins using these materials is based largely upon the

charge density of the proteins at the pH selected, as with electrophoresis in free solution. In contrast, the various gels not only prevent convection and minimise diffusion but in some cases they also actively participate in the separation process by interacting with the migrating particles. These gels can be considered as porous media in which the pore size is the same order as the size of the protein molecules such that a molecular sieving effect occurs and the separation is dependent on both charge density and size. Thus two proteins of different sizes but identical charge densities would probably not be well separated by paper electrophoresis, whereas, provided the size difference is large enough, they could be separated by polyacrylamide gel electrophoresis since the molecular sieving effect would slow down the migration rate of the larger protein relative to that of the smaller protein.

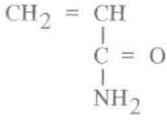
The extent of molecular sieving depends on how close the gel pore size approximates the size of the migrating particle. The pore size of agarose gels is sufficiently large that molecular sieving of most protein molecules is minimal and separation is based mainly on charge density. In contrast, starch and polyacrylamide gels have pores of the same order of size as protein molecules and so these do contribute a molecular sieving effect. However, the success of starch gel electrophoresis is highly dependent on the quality of the starch gel itself, which, being prepared from a biological product, is not reproducibly good and may contain contaminants which can adversely affect the quality of the results obtained. On the other hand, polyacrylamide gel, as a synthetic polymer of acrylamide monomer, can always be prepared from highly purified reagents in a reproducible manner provided that the polymerisation conditions are standardised. The basic components for the polymerisation reaction are commercially available at reasonable cost and high purity although for some purposes extra purification may be required. In addition, polyacrylamide gel has the advantages of being chemically inert, stable over a wide range of pH, temperature, and ionic strength, and is transparent. Finally, polyacrylamide is better suited to a size fractionation of proteins since gels with a wide range of pore sizes can be readily made whereas the range of pore sizes obtainable with starch gels is strictly limited. For these and other reasons, polyacrylamide gels have become the medium of choice for zone electrophoresis of most proteins although starch gels have been widely used for the analysis of isoenzymes. Starch gel electrophoresis has been reviewed by Gordon (1) and Smith (2). Agarose gels are used for the fractionation of molecules or complexes larger than can be handled by polyacrylamide gels, especially certain nucleic acids and nucleoproteins. In addition, agarose is widely used in immunoelectrophoresis where zone electrophoresis of proteins is coupled to immunological detection and quantitation (Chapter 7).

This chapter is concerned with the basic techniques of analytical zone electrophoresis of proteins in polyacrylamide gels plus modifications which allow small-scale preparations of proteins of interest. A more advanced text dealing with detailed quantitative approaches to analytical zone electrophoresis (including the determination of optimum gel pore size for maximum separation of two proteins) plus special techniques for large-scale preparation of proteins by zone electrophoresis is given in Chapter 2.

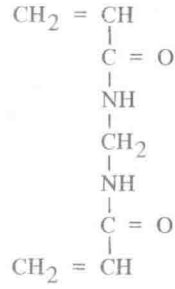
PROPERTIES OF POLYACRYLAMIDE GEL

Chemical Structure

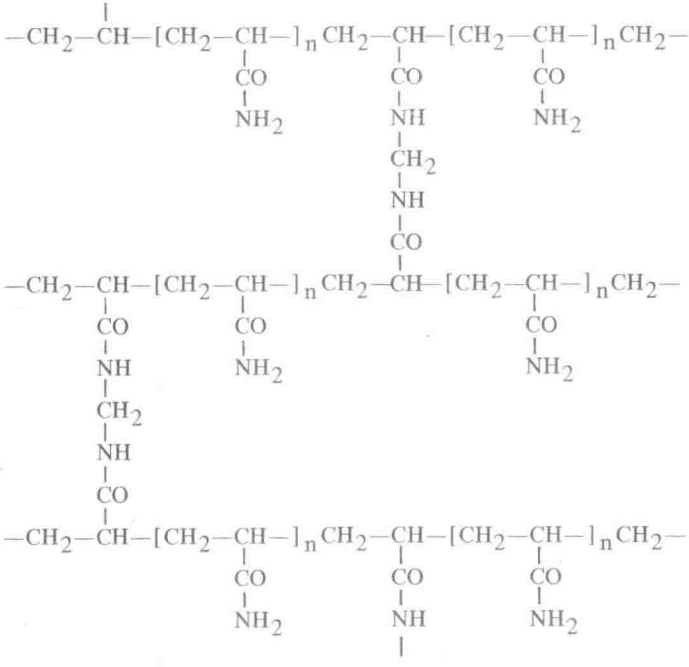
Polyacrylamide gel results from the polymerisation of acrylamide monomer into long chains and the crosslinking of these by bifunctional compounds such as *N,N'*-methylene bisacrylamide (usually abbreviated to bisacrylamide) reacting with free functional groups at chain termini. Other crosslinking reagents have also been used to impart particular solubilisation characteristics to the gel for special purposes (p. 58). The structure of the monomers and the final gel structure are shown in *Figure 1*.



Acrylamide



N,N'—methylene bisacrylamide



Polyacrylamide gel

Figure 1. The chemical structure of acrylamide, *N,N'*-methylene bisacrylamide, and polyacrylamide gel.