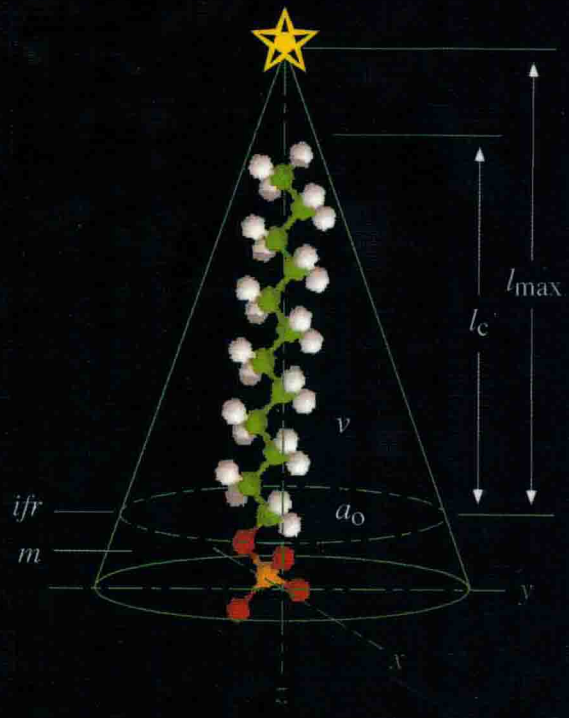
JOURNAL OF CHROMATOGRAPHY
LIBRARY - volume 63

the proteome revisited

P.G. Righetti
A.V. Stoyanov
M.Y. Zhukov



ELSEVIER

JOURNAL OF CHROMATOGRAPHY LIBRARY – volume 63

the proteome revisited
theory and practice of all relevant electrophoretic steps

Pier Giorgio Righetti
Department of Agricultural and Industrial Biotechnologies, University of Verona, Verona, Italy

Alexander V. Stoyanov
Department of Biomedical Sciences and Technologies, University of Milan, Milan, Italy

Mikhail Y. Zhukov
Department of Mechanics and Mathematics, Rostov State University, Rostov-na-Don, Russia

2001

ELSEVIER
Amsterdam – London – New York – Oxford – Paris – Shannon – Tokyo

ELSEVIER SCIENCE B.V.
Sara Burgerhartstraat 25
P.O. Box 211, 1000 AE Amsterdam, The Netherlands

First edition 2001

Library of Congress Cataloging in Publication Data
A catalog record from the Library of Congress has been applied for.

ISBN: 0 444 50526 1
ISSN: 0301-4770

♾ The paper used in this publication meets the requirements of ANSI/NISO Z39.48-1992 (Permanence of Paper).
Printed in The Netherlands.

JOURNAL OF CHROMATOGRAPHY LIBRARY – volume 63

the proteome revisited

theory and practice of all relevant electrophoretic steps

On the cover:
Parameters governing packing of dodecylsulfate anions into micelles. Geometry of the SDS micelle is effected by head group area (a_o) and volume (v). Maximum and mean hydrocarbon tail length (l_{max} and l_c) are indicated. The interfacial region (ifr) can move along the Z axis as the head group bobs in and out of the micelle surface (through the XY plane). The hydrophilic mantle region (m) is relatively constant in size due to inflexibility at the first CH_2 group. Contributed by Gary B. Smejkal, Cleveland State University and David Segrist, WebMolecules.com. Star added by Eaton Publishing.

Reprinted from BioTechniques Vol. 29, No. 6, December 2000, with permission of Eaton Publishing.

Introduction

It might be argued that electrophoresis was born as soon as Volta described the pile, *i.e.* the first power supply able to deliver continuous electricity. In fact, there are rumours that Reuss, an officer in the Czar army, was caught stealing sand on the banks of the Moskva river, instead of fighting against the Napoleon army, for filling a U-tube with which he was performing electrophoresis, utilizing as a power supply a voltaic pile composed of 92 silver roubles and an equal number of zinc plates. His was a rich man pile, indeed. In his memoirs, by the title "Notice sur un nouvel effet de l'electricité galvanique", dated 15 April 1808, he reported a curious phenomenon of water transport at the negative pole, upon passage of the current; he had discovered electroendoosmosis!

By all means, though, 1808 could hardly be labelled as the birth of modern electrophoresis. It was the twentieth century that made fundamental contributions to the field and, perhaps, it was the elegant work of Arne Tiselius, a 1948 Nobel laureate, that laid the foundations of present-day electrokinetic methodologies. Arne himself, though, realized that his instrumentation would not have carried us that far in the field. All previous work, including his own, was performed in free solution, which was anathema to any separation of macromolecules. The latter, in fact, had the nasty habit of sedimenting in the electric field, as soon as they were physically separated from each other and surrounded by zones of pure electrolyte, for the simple reason that such zones were denser than the surrounding liquid. Therefore, Tiselius' electrophoretic cell was constructed in such a way (here too a U-cell with ascending and descending limbs) that only boundary separation among the various protein zones would occur. The sample zone was thus rather large and would fill up all the bottom, as well as parts of the limbs, of the U-cell. Thus, as the current was applied, ascending and descending boundaries would be detected in a schlieren observation chamber, but no complete physical separation of pure zones could ever be achieved. Thus, Tiselius himself searched for other means for achieving a "zone separation", in which each component would be allowed to form a zone separated by others by empty regions.

A host of stabilizing media for zone electrophoresis were soon described, since the electrophoretic chamber had to be filled with a micro capillary system able to suppress convective flows, as well as to prevent protein sedimentation. Paper, fabrics, such as pure cotton, silk, potato starch, cellulose powder, glass powder and plastics, such as polyvinyl chloride, pevikon C-870 and even minerals, such as asbestos, were tested. Definitely,

though, modern zone electrophoresis was born with the advent of hydrophilic gels, such as Sephadex, agarose and polyacrylamide. For proteins, polyacrylamide turned out to be a unique zonal support, offering full transparency in the visible and near UV region, elasticity, flexibility and a full range of porosities which could be engineered at whim by altering either the total monomer content (%T) or the cross-linker value (%C), or both. In polyacrylamide support media, three major events concurred in shaping modern technologies:

- The description of disc electrophoresis by Ornstein [1] and Davies [2], a method which dramatically increased resolution by introducing in the matrix and buffers a series of discontinuities able to sharpen up the bands and form incredibly thin starting zones;
- The discovery of sodium dodecyl sulphate (SDS) electrophoresis [3], by which detergent-saturated proteins would be separated in a polyacrylamide gel mostly via their mass, rather than by combined mass and charge effects, as customary in conventional electrophoresis;
- The description of isoelectric focusing in polyacrylamide gels [4], by which proteins would be separated essentially on the basis of their net charge (at a given value of their titration curve, the isoelectric point).

Now the elements for the next major quantum leap were laid out on the table. Three groups took advantage of that and reported, simultaneously and independently, in 1975, the creation of two-dimensional maps, by combining orthogonally a first dimension, based on pure charge fractionation, with a second dimension, based on size discrimination [5–7]. With that, modern proteome analysis was born, although it took, of course, many years of developments and refinements, for bringing the technique to the present-day very sophisticated level. Instrumental to that were major contributions from the field of informatics, who had to develop new algorithms for mapping the field, acquiring the images, cleaning the background, matching different maps among themselves. Informatics has also tremendously contributed by laying out a format for protein databases, many of which today are available and which represent a formidable tool in protein characterization [8]. We biochemists owe a big tribute to them; without their contribution, present-day two-dimensional map analysis would be meaningless. Another major contribution, of course, was the introduction, in 1982, of immobilized pH gradients [9], which offered a new, most powerful view of the field, guaranteeing much increased resolution and much higher reproducibility in spot position. Just as fundamental was the introduction, in the early nineties, of mass spectrometry as a tool for sequencing small peptides and for identifying proteins. Especially its version of MALDI-TOF (matrix-assisted, laser desorption ionization, time of flight) coupled to delayed extraction, has been instrumental in protein recognition and expanding databases [10]. With this trident (2-D maps, protein databases and mass spectrometry) we can venture in the ocean, like the Greek God Triton, equipped to catch the Marlin of our life, like the old character in the famous novel of Hemingway.

At the start of the third millennium, we are now faced with a dramatic growth of proteome analysis, due also to the fact that we have reached near completion in the major undertaking of the molecular biology of the last decade, namely the sequencing of the human genome as well as of a number of other genomes. Now that the code is decoded, we are faced with the fact that the vast majority of proteins are still a *terra*

incognita, a huge field to be explored and mapped. This is perhaps the starting of a new stampede, a rush towards the new gold field, the forty-niners running after the gold of the third millennium, mining the proteome. Pharmaceutical companies, universities, venture capitals, geneticists, physicians, they are all engaged into this race, which promises a good harvest. Therefore, it is felt that a book in the field was sorely needed, to accompany us in this search. It is true, plenty of books have appeared recently in the field of proteome analysis, but they also become rapidly obsolete, as major advances appear almost every day. The present book also has a distinct flavour: it combines not only the practice, amply described in three major chapters (Chs. 12–14), but also the theory, dealt with *in extenso* in the first eleven chapters. Present-day books, most unfortunately, simply ignore all the theoretical developments, and often are reduced to mere cook books. But science is not, and cannot be, relegated to pure recipes: in order to progress, science has to be nourished by theory, by predictions, by description of basic phenomena. So, we hope that this unique combination will make the book more palatable to present-day audience. Perhaps, it might become the *vademecum*, the map of the gold fields for the new miners, proteome scientists, ready to go underground and dig. Dig you may, of course, and we hope you will also find the mother lode.

REFERENCES

1. L. Ornstein, Ann. N.Y. Acad. Sci., 121 (1964) 321–349.
2. B.J. Davis, Ann. N.Y. Acad. Sci., 121 (1964) 404–427.
3. A.L. Shapiro, E. Vinuela and J.V. Maizel, Biochem. Biophys. Res. Commun., 28 (1967) 815–820.
4. P.G. Righetti and J.W. Drysdale, Biochim. Biophys. Acta, 236 (1971) 17–24.
5. P.H. O'Farrell, J. Biol. Chem., 250 (1975) 4007–4021.
6. J. Klose, Humangenetik, 26 (1975) 231–243.
7. G.A. Scheele, J. Biol. Chem., 250 (1975) 5375–5385.
8. A. Bairoch, in M.R. Wilkins, K.L. Williams, R.D. Appel and D.F. Hochstrasser (Eds.), Proteome Research: New Frontiers in Functional Genomics, Springer, Berlin, 1997, pp. 93–148.
9. B. Bjellqvist, K. Ek, P.G. Righetti, E. Gianazza, A. Görg, W. Postel and R. Westermeier, J. Biochem. Biophys. Methods, 6 (1982) 317–339.
10. J. Godovac-Zimmermann and L.R. Brown, Mass Spectrometry Reviews, 20 (2001) 1–57.

PIER GIORGIO RIGHETTI (Verona)
ALEXANDER V. STOYANOV (Milano)
MIKHAIL Y. ZHUKOV (Rostov-na-Don)

Contents

PART I

Isoelectric Focussing: Fundamentals. Perspectives and Limits. Optimization of the Separation Process

Introduction

CONTENTS

This part of the book does not pretend to be a complete description of all aspects of isoelectric focussing. It is devoted first of all to those aspects that have still not yet found the appropriate reflection in the scientific literature. Firstly this concerns the question of the theory of polyvalent electrolyte dissociation. This is a matter of great importance since for modelling the polyelectrolyte behaviour at any process of electrophoretic separation one needs an appropriate pH-mobility relation, and it is extremely important to use a correct dissociation scheme.

The first section is devoted to the fundamentals of IEF. In the first two chapters some different aspects of the dissociation theory are considered, in the third one the kinetic aspects of acid–base equilibria are briefly discussed, but there the disadvantages of the traditional description are only emphasised. The fourth and fifth chapters concern the basic principles of the pH gradients. The steady-state IEF is discussed in Chapter 6, some aspects of the dynamics are dealt with in the following chapter (Chapter 7).

The second section deals with various topics under the title 'optimisation of electrophoretic separation'. Here, the two dimensional methods are also analysed. Chapter 11, entitled 'The limitation of the method of IEF', deals with important aspects like the resolving possibility limits for the isoelectric focussing technique, the perspectives of IEF whilst also the questions of microheterogeneity of biopolymers are discussed there.

ISOELECTRIC FOCUSSING: PRINCIPLES AND HISTORICAL ASPECTS

It is hardly possible to overemphasise the importance of isoelectric focussing for analytical biochemistry. Isoelectric focussing (IEF) is the method of electrophoretic separation of amphoteric substances based on the difference in their isoelectric points (p*I*). In the electric field any charged particle is subjected to the force $F = QE$ and in the isoelectric point, where its charge by definition equals zero, it should be immovable.

References pp. 5–6