Electrophoresis '84

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Preface

The analytical and preparative potential of electrophoretic separation was recognized at an early date, and not least in the biological sciences, where the introduction of electrophoretic methods of separation using different supporting media constituted a major breakthrough. These methods have helped to solve many scientific problems, and their development is still going on, as can be seen from the success and wide circulation of *Electrophoresis*, a young journal devoted solely to the subject. Nowadays, a wide variety of such methods is available, clearly demonstrating that no one method in itself is capable of solving all the separation problems for the many types of biological materials. This means that the experimentalist must have a whole repertoire of electrophoretic techniques at his disposal, or at least be fully aware of the latest trends and developments relevant to his particular area of research.

Meetings of national electrophoresis societies and the International Electrophoresis Society are now being convened at regular intervals so as to promote the exchange of information and to give experts the chance of discussing their problems with other experts working in the same field. In this connection it is interesting to note that the national electrophoresis societies were founded only recently and that their meetings are being well attended. Several books covering the papers presented at previous national and international meetings have already been published, any they all have been well received by the scientific community.

Electrophoresis '84 is unique in that it constitutes a collection of all the invited speakers' papers and all poster abstracts submitted to the fourth meeting of the International Electrophoresis Society that is available in book form prior to the convening of the congress. This has been achieved through the thoughtful cooperation of all the authors and through Verlag Chemie's readiness to print this volume at only a few weeks notice. Such a timely publication of all the latest trends and developments, new methods, and recent results in the field of electrophoresis is regarded as a further attempt not only to prepare a firm foundation for the discussion and exchange of scientific facts during the congress, but also to promote and stimulate the development of new ideas, however vague they may initially be. For this reason the present program committee, consisting of R. C. Allen (USA), M. Dunn (UK), K. Felgenhauer (FRG), T. Klose (FRG), V. Neuhoff (FRG), and B. T. Radola (FRG) – planned only a limited number of invited lectures, dispensing with the parallel sessions commonly included in the programs at comparable meetings, so as to ensure that participants will actually be able to assimilate all new information relevant to their particular fields of interest, yet leaving them time to explor other fields.

Further progress in the broad field of electrophoresis will either be inhibited by egocentric seclusion or be stimulated by the open exchange of information on new developed methods and the crucial "tricks" involved therein. It is my sincere wish that *Electrophoresis '84*, as the proceedings of the fourth meeting of the International Electrophoresis Society, will serve to further international collaboration. Obviously, the sub-

jects chosen for *Electrophoresis '84* in no way cover all aspects of electrophoresis. They were expressly selected by the members of the program committee responsible for this particular congress. Further subjects are bound to be selected by future program committees, thus ensuring a continuity of purpose in our proceedings.

I take great pleasure in thanking the members of the present program committee and all my coworkers for their cooperative efforts and sustained help. Without financial support from the Deutsche Forschungsgemeinschaft and from the Niedersächsische Minister for Wissenschaft and Kunst, as well as from numerous German firms through the Stiftverband für die Deutsche Wissenschaft, the convening of this congress would have been impossible. On behalf of the International Electrophoresis Society, the program committee and all participants I extend my sincere gratitude to these sponsors. Their funds will be productively invested; they will not only help the dissemination of information about new methods and techniques but also foster their application in many different fields, including basic research as well as clinical analysis.

Göttingen, July 1984 Volker Neuhoff

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RECENT DEVELOPMENTS IN BUFFER ELECTROFOCUSING

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SUMMARY

Buffer electrofocusing (BEF) presently provides a) a limited selection of practical pH gradients at low cost; b) computer simulation of pH gradients composed of 13 acidic, 16 basic or any higher number of constituents within negligible computer time, using a moving boundary model, which avoids the workload of empirical pH gradient engineering; c) a theory-aided selection of measures for pH gradient stabilization; d) an electrofocusing system relatively free from artifactual interaction products with proteins; e) easy separation of isolated proteins from carrier constituents. Problems associated with the present rudimentary method which lie in the way of its development to a widely useful separation method are outlined and remedies for these problems are suggested. The pro's and con's of BEF against the background of competing electrofocusing methods are outlined.

1 INTRODUCTION

Electrofocusing in mixtures of simple buffers preceded the synthesis of carrier ampholyte mixtures by Vesterberg (1), and was supplanted by it since the pH gradients formed by synthetic carrier ampholyte mixtures (designated SCAMs) were more linear, comprised wider pH ranges and were more effortlessly produced since SCAMs became commercially available. A literature review of these early buffer electrofocusing separations is provided by (2). Of importance is that all of these early BEF experiments were conducted with buffer mixtures consisting at least in part of amphoteric buffers, and could

therefore be reconciled with the concept that pH gradients form by an alignment of isoelectric ampholytes in the order of their pI values. This concept is erroneously ascribed to the theoretical treatment of electrophoretic migration and condensation (focusing) of ampholytes in a pre-existing pH gradient developed by Rilbe (3).

2 THE IMPACT OF BEF ON THE THEORETICAL UNDERSTANDING OF PH GRADIENT FORMATION

In a meaningful sense, BEF dates from 1976. In that year, Nguyen and the author described a pH gradient formed naturally from a mixture of 10 non-amphoteric organic bases (Fig.1, right) at 3 concentrations, 0.001 to 0.1 M each, and followed the genesis and maintenance of this gradient as a function of time (4). It was an incredibly poor-looking pH 4 to 10 gradient, wiggly and even violating the law of pH monotony, but it had formed nonetheless naturally, without any doubt, with time in the electric field and necessarily by a mechanism which did not involve an isoelectric condensation. Parenthetically, the worst cosmetic features of this pH gradient could remedied later once it was realized that the basic carrier constituents gave rise to polyacrylamide with a low average chain length and therefore little mechanical stability, and that this effect could be counteracted by coating of the glass walls of the gel container with linear polyacrylamide (5,6).

3 EMPIRICAL BUFFER PH GRADIENTS - AN UNPROMISING APPROACH

Viewed in 1976, the promise of BEF for practical separations did not lie with the curious non-amphoteric pH gradient, but rather with the far better-behaved 9-component pH gradient made mostly of amphoteric constituents (Fig.1,left). This promise appeared fourfold: i) There was a biochemical advantage in exposing the species of interest to chemically defined compounds with known functional groups, at known concentrations, compared to the inconcisely defined components of SCAMs. ii) The cost of buffer mixtures compared to the price of SCAMs was lower by two orders of magnitude or more, thus enabling economically disadvantaged laboratories to electrofocus. iii) It appeared likely that small molecular weight buffers would exhibit fewer interactions with proteins than larger and multivalent synthetic carrier ampholytes. iv) It appeared readily possible to tailor pH gradients to one's needs by the mere addition or subtraction of buffer constituents from the mixture. It is this part of the promise that empirical BEF was unable to fulfill with sufficient ease and precision. The simple fact is that from a knowledge of the pKs alone it is possible to design acidic, basic and neutral pH gradients (Fig.2) but it is not possible to arrive at a particular desired pH range without a great many