CRC

HANDBOOK of HPLC FOR THE SEPARATION of AMINO ACIDS, PEPTIDES, and PROTEINS

Volume I

William S. Hancock

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PREFACE

The growth in application of high performance liquid chromatography (HPLC) to the life sciences can be judged from the massive increase in papers applied to the separation of macromolecules that were published recently in the *Journal of Chromatography, Analytical Biochemistry,* or the *Journal of Liquid Chromatography*. In addition more specialist journals such as *Brain Research, Journal of Endocrinology,* and *Biochemistry* are now publishing reports that make extensive use of HPLC separations. The study of neuropeptides provides a powerful example of the potential of the technique when it is extensively applied to a new area of research. Unfortunately many other areas of research in the life sciences have been slow to follow this lead. A major difficulty is often that polypeptide separations require careful optimization, which can be a daunting prospect to the researcher uninitiated in the subtilities of liquid chromatography. Another difficulty is a lack of knowledge of the biologist in the properties of siliconaceous supports and in the instrumentation required for a high efficiency separation. This Handbook was planned with these difficulties in mind, with a range of chapters that will both introduce the technique and then lead on to the detailed optimization of the chromatographic parameters required for a given separation.

At the same time even the experienced chromatographer is faced with a difficulty, that is with the rapid advances in instrumentation and separation conditions. For example, recent advances in the understanding and preparation of reversed phases means that dramatically improved separations can be achieved by the correct choice of the parent silica used to manufacture the reversed phase. It is a goal of the Handbook to present initial examples of promising new applications in HPLC in the hope that it will stimulate further studies. An example of such an application can be found in ligand-exchange chromatography, which as the reader will find in the later chapters, is a technique of great potential.

At Massey University our interest in HPLC has always been problem-orientated, so that currently we are studying the role of apolipoproteins in heart disease, and using HPLC as an analytical and preparative technique. I hope that the Handbook will reflect this practical orientation and that it will be of value to other researchers in the biological fields, who like us, find their studies are inextricably linked to the joys of chromatography.

I would like to acknowledge my gratitude for the assistance given by my research colleague and friend, Dr. David Harding in this task. Also the continued support by Professors Dick Batt and Geoff Malcolm of my research activities has made possible my interest in high performance liquid chromatography and its application to protein chemistry. I am grateful for the strong support from the members of the Advisory Board and for the many hours of careful preparation that individual researchers put into the preparation of their reports. The finished Handbook bears strong testimony to the skills of the secretaries at Massey University and the publishing staff at CRC Press. Most of all I am indebted to my wife Elizabeth for her encouragement and patient tolerance of my labors. To her this Handbook is affectionately dedicated.

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THE EDITOR

Dr. William Hancock graduated in Organic Chemistry and Biochemistry from the Adelaide University in South Australia and then continued with a Ph.D. in Natural Product Chemistry at the same University. This work was supervised by Drs. Massy-Westropp and Mander and involved synthetic organic chemistry. Even then chromatography on silica acid and alumina played a vital role in characterization and purification of the reaction products.

After graduation in 1970 he worked as a post-doctoral fellow in the laboratories of Professors Vagelos and Marshall at the Washington University School of Medicine in St. Louis. The research involved the total chemical synthesis of Acyl Carrier Protein by the Merrifield solid phase method. Again the synthetic prducts required extensive purification and this time gel filtration, ion-exchange chromatography, and affinity chromatography techniques were used.

In 1972 he was appointed Lecturer in Chemistry in the joint department of Chemistry, Biochemistry, and Biophysics at Massey University with the goal of establishing a peptide synthesis group. At that time, New Zealand Universities were undergoing a period of rapid expansion and it was decided to recruit researchers in different areas of protein chemistry in this department. As it was found in St. Louis, the purification of synthetic peptides was an important challenge as traditional chromatographic methods based on polysaccharide matrices were inefficient. On July 23, 1973, Joel Morrisett a colleague from Houston, Texas noted in a letter that his department had just purchased a liquid chromatograph for purification of their peptides. He then made prophetic statement "We feel LC is going to provide the ultimate criteria of purity". The editor was impressed by this information and with a grant of $9,800 from a New Zealand Scientific Research Committee (funded by a very popular local lottery known as the "Golden Kiwi") purchased an HPLC in late 1974. The equipment then traveled by slow boat from Milford, USA to the antipodies and was installed in mid-1975. Research in the laboratory soon demonstrated that peptides were extremely difficult to chromatograph on reversed-phase columns, often with long and irreproducible retention times. In the following year Dr. Hancock chanced on a stray comment by Reg Adams (then of the Perkin Elmer Corporation) that phosphoric acid could suppress the active sites on the silica and thus allow the more efficient chromatography of peptides. Although the reason for use of phosphoric acid did not allow for the complex ionic structures of peptides, it was nonetheless a key suggestion which allowed the laboratory to rapidly chromatograph a variety of peptide and protein samples by reversed-phase HPLC. A gratifying feature of this development was that the stray comment was made at the Lord Mayor's banquet held in honor of an IUPAC congress held in Dunedin, New Zealand (August, 1976). Thus social functions can also have a useful scientific function!

The next 6 years allowed a rapid development of the technique with some 50 papers on the subject. During this time Dr. Hancock became interested in the study of the role of the protein components of lipoproteins in heart disease. Therefore current research is directed at the synthesis and study of the interaction of model lipid binding peptides with reversed-phase columns. In addition he has co-authored with Dr. Sparrow of Houston, Texas a review on "The Separation of Proteins by Reversed Phase HPLC" in *High Performance Liquid Chromatography, Advances and Perspectives*, Volume 3, (C. Horvath, Editor). With the same co-author he has written *A Laboratory Manual on the Separation of Biological Materials by HPLC*, which is currently in press. In fact both of these publications arose from a most profitable sabbatical spent in 1980 at the Baylor College of Medicine in the department where the prophetic statement was made about the potential of reversed-phase HPLC. It is a tribute to the durability of modern liquid chromatographs that the original system mentioned in the 1973 letter was still functional and was used extensively by Dr. Hancock.

Other career details about Dr. Hancock include promotion to Senior Lecturer in 1977 and Reader in Chemistry in 1982. Also he is a member of the New Zealand Institutes of Chemistry and Biochemistry, Endocrinology and Immunology Societies, and a Fellow of the American Heart Association.

In addition to an interest in lipoproteins, he has studied the separation by LC of small peptides such as enkephalins, releasing factors and angiotensin and of protein hormones such as insulin and growth hormone. Another recent area of research has been the development of preparative separations (multigram amount) of peptides and proteins with volatile mobile phases such as perfluoroalkanoic acids or ammonium bicarbonate. A related interest has been the development of highly specific matrices for affinity chromatography based on the use of 1,1′-carbonyldiimidazole rather than cyanogen bromide as the activating reagent.

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