Contemporary Topics in Analytical and Clinical Chemistry

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Contemporary Topics in Analytical and Clinical Chemistry

Volume 1

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Volume 1

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Preface

Any addition to the ever-expanding list of scientific publications requires careful consideration and justification. There are already numerous journals in analytical and clinical chemistry adequate for the publication of research results. There does remain a need for a series focused on analytical and clinical chemistry, to provide an overview of instrumental developments relevant to the needs of analytical and clinical chemists. This is the role intended for the present series.

Although the title specifically indicates that the series will deal with analytical and clinical chemistry, our intention is that it will deal with analytical chemistry as related to other areas, such as air and water pollution, oceanography, earth sciences, and various aspects of biomedical science and technology.

It seems appropriate to publish two types of articles in the series. First, we will provide a forum for authoritative, critical reviews for the expert, to enable him to cope with the ever-growing problem of keeping abreast of rapid developments in his own and immediately related fields. In this way we hope the series will stimulate new ideas for research by being at the cutting edge of science. Second, we will publish articles written by experts in the fields being covered but primarily intended for the nonexpert, thereby providing him with some overview of the area. Thus we hope to cross-fertilize research areas, while at the same time serving an educational function. In this sense the series will include articles not only on currently fashionable analytical techniques but on state-of-the-art advances in other areas certain to assume analytical importance. Here special emphasis will be placed on making the topics useful to the practicing experimentalist and indicating why projected analytical impact is predicted.

We will seek to have an international authorship so that the series will accurately reflect the global status of analytical chemistry.

The general format of articles will include a review of the fundamentals of a topic, a description of instrumentation, critical presentation of

some interesting applications, and educated speculation as to where and how future improvements of the technique or amplification of its applications may develop. In a series such as this dealing with analytical and clinical chemistry there is always the danger that it may become too narrowly directed or too diffuse. We believe that a balance between these two extremes will best serve the analytical chemistry community. The value of the series will be directly reflected in how close we have come to achieving this goal.

The Editors

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High-Speed Liquid Chromatography of Proteins

Fred E. Regnier, Karen M. Gooding, and Shung-Ho Chang

1. Introduction

1.1. Classical Chromatography of Proteins

A discussion of protein separation should be prefaced with a review of our general knowledge of protein structure. Proteins are biopolymers composed of an ordered sequence of acidic, basic, neutral, and hydrophobic amino acids coupled via peptide bonds. The bending and folding of this primary chain results in a secondary structure that is maintained primarily by internal hydrogen bonding. Further stabilization of the protein occurs by hydrogen, ionic, hydrophobic, covalent, and van der Waals linkages between amino acid side chains. The net result of these secondary and tertiary modifications along the polypeptide chain is a three-dimensional matrix with some amino acids buried in the interior of the protein and others exposed at the surface. As a result, proteins have specific shapes and sizes; in addition, they have areas that may be anionic, cationic, and/or hydrophobic depending on the amino acid sequence. These differences in properties make possible the chromatographic resolution of proteins.

Classical separations of proteins have been achieved on supports of the gel type that are usually composed of carbohydrates or polyacrylamide with a stationary phase immobilized in the gel matrix.[1] It is important that the support matrix be hydrophilic, neutral, and have sufficient porosity to allow the penetration of macromolecules. Carbohydrates, because they are generally neutral and imbibe large quantities of water,

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have been used extensively as a support matrix for the chromatography of proteins. These matrices are both hydrophilic and macroporous. The ease with which carbohydrates can be substituted with different ion exchange groups or ligands, in addition to their chemical stability under chromatographic conditions, has also contributed to their popularity for protein separations.

When a hydrophilic support material is cross-linked into a macroporous matrix of controlled porosity without further chemical substitution, a support suitable for gel or exclusion chromatography is generated. Both the polydextran-based Sephadex and polyacrylamide-based Bio-Gel resins are examples of such size-separation supports that can be used in the fractionation of compounds with molecular weights 10^2–10^6. The polar functional groups in either of these support materials cause it to adsorb large quantities of water and swell to many times its dry volume. The volume of this swollen matrix is unfortunately sensitive to pressure and changes in pH, ionic strength, and solvent compositions, which severely limits the use of the material in practice. Thus the high pressures necessary for fast and efficient separations are precluded, and the range of useful solvent compositions is limited.

Substitution of the above gel permeation materials or cellulose with an ionic species such as diethylaminoethanol (DEAE), diethylmethylaminoethanol (QAE), glycolic acid (CM), or propanesulfonic acid (SP) yields ion exchange supports which have been used extensively in the resolution of proteins. However, volume sensitivity is an even greater limitation for ion exchange packings, where, for example, gradient elution is restricted to a narrow range of conditions.

1.2. Inorganic Supports

The need for a rigid, nongel type of support was obvious. The invention by Haller[2,3] of controlled-porosity glass (CPG) supports with pore diameters ranging from 100 to 1500 Å provided a permeable support that answered this need. These supports could be sterilized and cleaned with strong acid without deleterious effects and their pore diameters corresponded closely to the molecular diameters of viruses, proteins, and polysaccharides. It appeared that CPG would be a valuable matrix for gel chromatography. In fact, the purification of large quantities of plant[4] and animal viruses[5] and proteins[6] has been achieved with these supports.

Unfortunately, the glass and silica supports have some severe limitations in the chromatographic separation of biopolymers. As more researchers began using the CPG supports, it was found that their polar surfaces were responsible for the adsorption and denaturation of some