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Department of Chemistry, Indiana-Purdue University, 1125 East 38th Street, P.O. Box 647, Indianapolis, IN 46223, U.S.A.

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PREFACE

Size-exclusion chromatography (SEC) has developed along at least two paths. Following the pioneering work of Flodin, Porath and others in Uppsala, polysaccharide-based gels - Sephadex, Agarose and the like - came to be fundamental tools for protein separation in the biochemical laboratory. However, such "gel filtration" methods, being slow and ill-suited to organic-soluble macromolecules, failed to attract the attention of industrial polymer chemists. These practitioners became actively involved in SEC only after Waters Associates commercialized the use of semi-rigid porous styrene/divinyl-benzene resins (developed by Moore in 1964) for "gel permeation chromatography" (GPC). Two separate lines of development consequently ensued. In the industrial laboratory, where the molecular weight distributions of synthetic polymers was of primary concern, GPC flourished and benefited from the general technological progress of high-performance liquid chromatography, manifested in high-efficiency columns, which - coupled with high-pressure pumps and injectors and low-volume fittings - provided short analysis times. At the same time, "soft" polysaccharide gels were being employed in biologically oriented laboratories, where the focus on biopolymers and the attendant absence of concern about molecular weight distributions (MWD) made the low efficiency of these packings more acceptable.

Synthetic water-soluble polymers were the orphans in this situation, finding their home neither with the organic solvent-compatible polystyrene GPC methodology, nor with the slow, low-efficiency gel filtration systems. In some ways, controlled-pore glass (CPG) developed by Haller in 1965, answered this need, inasmuch as CPG is water-compatible and, because of its mechanical strength, permits high flow rates. But CPG strongly absorbs proteins, cationic polymers, and some nonionic polymers, and its large particle size precluded efficient column packing.

The aforementioned state of affairs persisted for over a decade. Separate bodies of literature dealt, on the one hand, with gel filtration - usually meaning protein separation on soft gels - and, on the other, with gel permeation - commonly referring to the analysis of the MWD of synthetic organic-soluble polymers on semi-rigid polystyrene gels. The relatively unpopular porous glass was situated between these two camps, finding occasional applications to both biopolymers and water-soluble synthetic macromolecules.

This artificial dichotomy has been slowly eroding since around 1980 when high-efficiency, high-speed columns for aqueous SEC started to be commercialized, most notably by Toyo Soda Co. Toyo Soda offered both surface-derivatized porous silica (SW) and also crosslinked synthetic hydrophilic polymer gel (PW) packings. At least six other companies now produce columns of one of these two types for "high-performance" aqueous SEC. In addition, Pharmacia has recently introduced a high-efficiency agarose-based packing (Superose). All these "modern" packed columns offer in excess of 5,000 theoretical plates meter^{-1} and can operate at 300 or more p.s.i.

In addition to these technological developments, the range of applications of aqueous SEC has increased dramatically. Size and size distributions can be determined for colloidal systems, pre-eminently micellar aggregates of either synthetic or natural surfactants. In principle, one may also obtain information about the association equilibrium in such systems. The use of aqueous SEC to evaluate equilibrium constants for the binding of smaller molecules to larger ones by the Hummel-Dryer method and related techniques is well documented. Ligand/macromolecular systems studied in this way include small ion/protein, substrate/enzyme, and protein/polyelectrolyte mixtures. The perturbation of ionic concentrations by polyelectrolytes, i.e. the Donnan effect, is also susceptible to a similar approach.

The commercialization of high-resolution derivatized porous silica packings for aqueous SEC dramatically captured the attention of protein chromatographers. Without derivatization, proteins would characteristically exhibit total adsorption on siliceous substrates. Complete elimination of adsorption, however, is in practice not necessarily desirable, inasmuch as chromatographic separation factors may - in specific cases - be enhanced by differential adsorption. Indeed, protein separation as currently practised consists in large part of mixed-mode chromatography, in which hydrophobic, electrostatic and active site (affinity) interactions may be combined in various ways and superimposed on size exclusion. Mixed eluants and gradient chromatography can be used to further enhance these separations. The chromatographic process may also involve partial or reversible protein unfolding. This superposition of complex effects, while pragmatically valuable, can obscure the roles of the various phenomena. Therefore, despite the dramatic progress currently being made in protein separations by mixed-mode chromatography, fundamental studies with less complex substrates may be required to develop guiding principles for protein chromatography.

The intent of this volume is threefold. First, we wish to provide a thorough description of the scope of aqueous SEC. Second, we intend to discuss several areas of technological progress. Last, some fundamental

aspects of the separation process are considered in detail. The reader will find a strong bias toward the elucidation of principles with a correspondingly reduced emphasis on instrumentation per se. This is not to dispute the importance of technological developments such as improved software for SEC data analysis and in-line viscometric detection. Information on these devices, being relevant to SEC in general, is easily found in one of the several books and symposium volumes dedicated to GPC, and is also available from the instrument companies.

Several chapters illustrate how the range of applications of aqueous SEC extends beyond the measurement of MWD of water-soluble polymers. The separation of inorganic salts is discussed in Chapter 4. Chapter 15 describes the analysis of surfactant micelles by SEC. Further explorations of the behavior of associating and aggregating systems appear in Chapter 13 which reviews the Hummel-Dryer method and in Chapter 14 which describes frontal zone chromatography. The chromatographic analysis of pore size distributions by inverse SEC is covered in Chapter 6. The important subject of protein chromatography is dealt with explicitly in Chapters 9-11 as well as in several other sections.

Technological developments of various sorts are presented. Chapter 8 deals with new packings based on derivatized silica. Modern polymer-based stationary phases especially designed for biopolymer separations are described in Chapter 9. The application of on-line light-scattering detectors to such separations is described in Chapter 12.

A number of fundamental questions related to chromatographic equilibria and dynamics have been explicated. The fundamental issue of the nature of the macromolecular dimensions that determine K_{SEC} is the subject of Chapter 1. The resolving power of aqueous SEC columns is considered from the perspective of column pore size distributions in Chapter 6. These and other aspects of efficiency are explored in detail in Chapter 7. Interactions between macromolecules and stationary phases are covered in both Chapter 2 (hydrophobic effects) and Chapter 3 (electrostatic effects).

To the extent that an edited volume is a team effort, the editor is its temporary foreman. This role is an honor when the participants have the scientific repute of the present contributors, whose combined bibliographies in this field would number in excess of 500, and whose collegiality and cooperation in this joint task have been on a par with their scientific competence. The professionalism of the publisher also deserves high praise. Having worked with other publishers and their acquisition editors, the editor is able to particularly appreciate the guidance, assistance and responsiveness of Mr. Robert Marx of Elsevier Science Publishers. Finally, a very

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Indiana-Purdue University
Indianapolis, IN 46223

PAUL DUBIN

LIST OF CONTRIBUTORS

G.W. BECKER, Department of Biochemical Research, Lilly Research Laboratories, Building 88, Eli Lilly & Co., Indianapolis, IN 46285, U.S.A.

K.S. BIRDI, Department of Physical Chemistry, Danish Technical University, DK-2800 Lyngby, Denmark

P.L. DUBIN, Department of Chemistry, Indiana-Purdue University, 1125 East 38th Street, P.O. Box 647, Indianapolis, IN 46223, U.S.A.

L. HAGEL, Department of Quality Control, Pharmacia AB, Biotechnology, S-75182 Uppsala, Sweden

I. HARA, Scientific Instrument Division, Toyo Soda Mfg. Co., Hayakawa, Ayaseshi, Kanagawa 252, Japan

H. HATANO, Department of Chemistry, Faculty of Science, Kyoto University, Kitashirakawa, Sakyo-ku, Kyoto, Japan

J.-P. Himanen, Department of Biochemistry, University of Turku, SF-20500 Turku 50, Finland

M.E. HIMMEL, Biotechnology Branch, Solar Energy Research Institute, 1617 Cole Blvd., Golden, CO 80401, U.S.A.

M. JANADO, Department of Food Science, Kyoto Women's University, Higashiyama-ku, Kyoto 605, Japan

J.N. KINKEL, Chemische Forschung, E. Merck, D-6100 Darmstadt, F.R.G.

K. KONISHI, Department of Biophysical Chemistry, Dokkyo University, School of Medicine, Mibu, Tochigi 321-01, Japan

T.K. KORPELA, Department of Biochemistry, University of Turku, SF-20500 Turku 50, Finland

S. KUGA, Department of Forest Products, Faculty of Agriculture, The University of Tokyo, Yayoi, Bunkyo-ku, Tokyo 113, Japan

K. MAKINO, Department of Polymer Science & Engineering, Faculty of Textile Science, Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606, Japan

R.C. MONTELARO, Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803, U.S.A.

S. MORI, Department of Industrial Chemistry, Faculty of Engineering, Mie University, Tsu, Mie 514, Japan

N. OHTA, Department of Chemistry, St. Marianna University, School of Medicine, Sugao, Miyamae-ku, Kawasaki 213, Japan

M. OKAZAKI, Laboratory of Chemistry, Department of General Education, Tokyo Medical and Dental School, Kohnodai, Ichikawa-shi, Shiba 272, Japan

M. SHIBUKAWA, Department of Chemistry, St. Marianna University, School of Medicine, Sugao, Miyamae-ku, Kawasaki 213, Japan

P.G. SQUIRE, Department of Biochemistry, Colorado State University, Fort Collins, CO 80523, U.S.A.

K.K. UNGER, Institut für Anorganische Chemie und Analytische Chemie, Johannes Gutenberg-Universität, Postfach 39 80, D-6500 Mainz, F.R.G.

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