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*aqueous
size-exclusion
chromatography*

*edited by
P.L. Dubin*

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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/divinylbenzene 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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CONTENTS

Preface	XIII
List of Contributors	XVII
PART I: SEPARATION MECHANISMS	
Chapter 1. Size exclusion parameters (M.E. Himmel and P.G. Squire) . . .	3
1. Introduction	3
2. Solute retention mechanism in SEC	4
2.1. Column compartmentalization	4
2.2. Elution of small molecules	4
2.3. Historical aspects of solute partitioning effects in SEC . .	5
2.4. Universal calibration	10
2.5. Reconciliation of size parameters of proteins and random coils: The Himmel-Squire approach	14
3. Acknowledgements	20
References	20
Chapter 2. Partitioning: Hydrophobic interactions (M. Janado)	23
1. Introduction	23
1.1. Hydrophobic interaction	23
1.2. Nature of hydrophobicity of hydrophilic gels	24
2. Hydrophobic nature of sugars as an origin of hydrophobic properties of polysaccharide gels	24
2.1. Cosolvent effect of sugars on aqueous solubilities of hydro- carbons	25
2.2. Relative magnitude of nonpolar sugar-hydrocarbon interaction	27
2.3. Specific interaction sites of sugar molecules	30
2.4. Water structure and cavity formation in concentrated sugar solutions	34
2.5. Hydrophobicity of sugars as evidenced by their affinity for polystyrene gel in aqueous media	35
2.6. Factors determining individual hydrophobicity of sugars . . .	40
3. Hydrophobic interactions in hydrophilic gels	41
3.1. Apparent transfer parameters relevant to the static equilib- rium experiment and gel chromatography	42
3.2. Solution of hydrophobic solutes in Sephadex gels and Bio-Gel P-2	44

3.3. Thermodynamic parameters pertaining to gel chromatography of alkanols	46
3.4. Differential scanning calorimetry of water in various gels . . .	48
4. Conclusion	52
References	53
Chapter 3. Electrostatic effects (P.L. Dubin)	55
1. Introduction	55
2. SEC of low MW salts	56
2.1. Sephadex	56
2.2. Underivatized glass and silica	58
2.3. Derivatized or surface treated packings	59
3. SEC of polyelectrolytes (polyanions)	60
3.1. Polyanions on polysaccharide gels	61
3.2. Polyanions on siliceous packings	62
4. SEC of polycations	63
4.1. Polycations on derivatized glass or silica	63
4.2. Polycations on semi-rigid gels	65
5. Electrostatic effects in SEC of proteins	66
5.1. Controlled pore glass	67
5.2. Surface-derivatized silica	67
5.3. Soft and semi-rigid gels	70
6. Models for electrostatic effects	70
References	73
Chapter 4. Exclusion chromatography of inorganic compounds (M. Shibukawa and N. Ohta)	77
1. Introduction	77
2. Distribution processes of inorganic salts	77
2.1. Effect of electrolytic nature of gel	78
2.2. Effect of background electrolytes	84
2.3. Selectivity of hydrophilic gel for inorganic ions	96
3. Inorganic polymers	98
3.1. Oxo anions of phosphorus	98
3.2. Hydrolysis polymers of metal ions	99
3.3. Metal oxo anions	101
3.4. Metal hexacyanoferrate	101
3.5. Silicic acids	102
3.6. High performance exclusion chromatography of inorganic colloids	103
4. Metal complexes	104
4.1. Exclusion chromatography of metal chelates	104
4.2. Studies on metal-ligand binding	106
References	111

PART II: CHARACTERIZATION OF STATIONARY PHASES

Chapter 5. Pore size distributions (L. Hagel)	119
1. Introduction	119
2. Pore structure of supports for size exclusion chromatography	120
2.1. Gel-forming natural polymers	121
2.2. Crosslinked polymer networks	122
2.3. Silica based materials	123
3. Characterization of pore size	124
3.1. Electron microscopy	125
3.2. Gas adsorption	129
3.3. Mercury porosimetry	131
3.4. Size exclusion chromatography	133
3.5. Comparison of pore size data from the various methods	145
4. Impact of pore dimensions on size exclusion parameters	146
4.1. Effect of average pore size	146
4.2. Effect of pore size distribution	148
4.3. Effect of pore volume	149
Acknowledgements	150
References	150
Chapter 6. Structural analysis of porous materials by measurement of size exclusion (S. Kuga)	157
1. Introduction - Principle and general view of chromatographic analysis for solid-solute interaction	157
2. Size exclusion chromatography for porous structure analysis	158
2.1. Porous structure of gels	158
2.2. Solute exclusion method and size exclusion chromatography in gel studies	158
2.3. Interrelation between solute exclusion behavior and pore size distribution of gels	162
2.4. Molecular size parameter	166
2.5. Models of gel structure	167
3. Concluding remarks	169
References	169
Chapter 7. Column efficiency (S. Mori)	171
1. Introduction	171
2. Definition of column efficiency	173
2.1. The theoretical plate number	173
2.2. Height equivalent to a theoretical plate	174
3. Measurement of column efficiency	176

4. Operational variables of column efficiency	178
4.1. The velocity of the mobile phase	178
4.2. Sample load	181
4.3. Other variables	183
5. Problems associated with aqueous SEC	184
5.1. Flow rate of mobile phase	184
5.2. Other variables	186
References	189

PART III: NEW PACKINGS

Chapter 8. Native and bonded silicas in aqueous SEC (K.K. Unger and J.N.

Kinkel)	193
1. Introduction	193
2. The physical properties of support and column and their role in SEC separations	194
2.1. Control of V_e , k'' and K_{SEC}	199
2.2. Fractionation range and linearity of the log-linear calibration curve	203
2.3. Resolution and analysis time	206
3. The surface chemistry of silica SEC packings	208
3.1. Silicas with adsorbed layers or coatings	209
3.2. Bonded silicas	210
4. Commercial native and bonded silica SEC packings and columns and their operation	219
4.1. Packing characteristics	219
4.2. Comparison of SEC packings	228
4.3. Column operation	230
5. Conclusion	231
References	232

Chapter 9. Rigid polymer gels for SEC and their application to biopolymers

(K. Makino and H. Hatano)	235
1. Introduction	235
2. Currently available size exclusion chromatographic columns	236
2.1. Hydrophilic alkylchain-grafted silica	236
2.2. Acrylate polymer-based SEC columns	239
2.3. Polyvinyl alcohol gel columns	242
2.4. Dextran-based SEC columns	244
2.5. Other polymer-based SEC columns	244
3. General separation data on SEC columns	244

4. Application of SEC to biological systems	251
4.1. Proteins and peptides	251
4.2. Lipoproteins	258
4.3. Nucleic acids	258
4.4. Poly- and oligo-saccharides	261
4.5. Synthetic hydrophilic polymers	262
5. Acknowledgment	263
References	263

PART IV: BIOPOLYMERS

Chapter 10. Biopolymers. I. Protein chromatography in denaturing and non-denaturing solvents (R.C. Montelaro)	269
1. Introduction	269
2. Packings for SEC of proteins	270
2.1. Conventional SEC	270
2.2. High performance SEC	275
3. Eluant systems	278
3.1. Nondenaturing aqueous buffers	278
3.2. Nondenaturing detergent eluants	283
3.3. Denaturing eluants	284
3.4. Organic solvents	291
4. Sample preparation	292
4.1. Denaturing vs nondenaturing systems	292
4.2. Sample viscosity	293
4.3. Sample volume	293
4.4. Sample load	294
5. Column geometry	295
References	295
Chapter 11. Biopolymers. II. Serum lipoproteins (M. Okazaki and I. Hara)	297
1. Introduction	297
2. Analytical methods of serum lipoproteins by HPLC	299
2.1. Column	299
2.2. Apparatus	300
2.3. Samples	300
2.4. Loaded volume	301
2.5. Eluent and flow rate	301
2.6. Detection of serum lipoproteins by HPLC	301
2.7. Preparation of the enzyme solution and reaction conditions of HPLC	303

3. Separation of serum lipoproteins by HPLC	304
3.1. Column selection and calibration	304
3.2. Eluent	306
3.3. Flow rate	307
3.4. Separation of major proteins	307
4. Analysis of serum lipoproteins by HPLC	309
4.1. Detection of protein moiety	309
4.2. Selective detection of serum lipid components	310
5. Quantitation of lipids in major lipoprotein classes	315
6. Precision and sensitivity of the HPLC method	317
7. Correlation of lipoprotein analysis between HPLC and ultracentrifugation	318
8. Application of the HPLC method	319
8.1. Detection of abnormal lipoproteins	319
8.2. Subfractions of high density lipoproteins	321
8.3. Clinical applications	322
9. Conclusion	325
References	325
Chapter 12. Application of SEC/LALLS to biopolymer assemblies (K. Konishi)	327
1. Introduction	327
2. Low angle laser light scattering	327
3. Outline of low angle laser light scattering	328
4. Arrangement of instruments in SEC/LALLS system	329
5. Application of SEC/LALLS technique to simple proteins	330
6. Application of SEC/LALLS technique to the study of molecular weight of native membrane proteins	333
6.1. Application to acetylcholine receptor	333
6.2. Application to porin	335
6.3. Application to Na,K-ATPase	337
7. Conclusion	341
Acknowledgements	341
References	342

PART V: ASSOCIATING SYSTEMS

Chapter 13. Measurement of equilibrium constants by exclusion methods (T.K. Korpela and J.-P. Himanen)	345
1. Introduction	345
2. Description of the interacting system	347
2.1. Definition of equilibrium reactions	347
2.2. Effects of temperature	347
2.3. Preliminary tests of the system	348

3. Static equilibrium methods	349
3.1. The batch method	349
3.2. Brumbaugh-Ackers method	351
3.3. Recycling gel partition method	353
4. Dynamic methods	356
4.1. Retention data methods	356
4.2. Hummel and Dreyer method	358
4.3. Frontal analysis method	366
5. Effects of Donnan equilibrium on binding studies	369
References	371
Chapter 14. Frontal boundary analysis in size exclusion chromatography of self-associating proteins (G.W. Becker)	
1. Introduction	375
1.1. Purpose	375
1.2. Self-association of proteins	375
1.3. Methods for studying self-association	376
1.4. Large zone size exclusion chromatography	377
2. Theory of large zone SEC	379
3. Experimental approach	382
3.1. Choice of column	382
3.2. Solvent delivery	383
3.3. Detection	383
3.4. Choice of buffer	383
3.5. Sample preparation	384
3.6. Calibration of column	384
3.7. Data collection and calculations	385
3.8. Temperature studies	387
4. Specific examples of protein systems studied by large zone SEC . . .	387
4.1. Human growth hormone	388
4.2. Human proinsulin	390
4.3. Phosphofructokinase	391
4.4. Sarcoplasmic reticulum ATPase	392
4.5. Enzyme I of the Salmonella typhimurium phosphoenolpyruvate:glu- cose phosphotransferase system	393
5. Conclusions	396
References	396