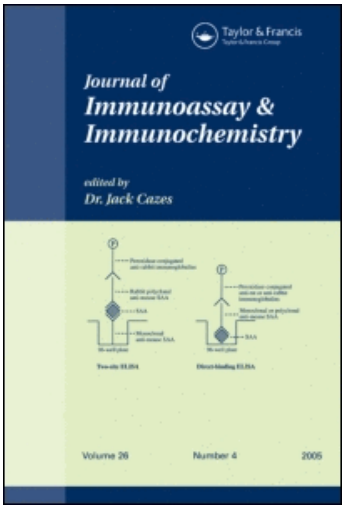

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The Book Corner

Capillary Electrophoresis of Carbohydrates, Pierre Thibault and Susumu Honda, Eds., Humana Press, Totowa, New Jersey, 2003, 318 pages. Price: $99.50.

Recent developments in high-resolution separation techniques based on capillary-scale chromatography and electrophoresis have transformed the analysis of free and conjugated mono- and oligosaccharides. In *Capillary Electrophoresis of Carbohydrates*, hands-on experts describe cutting-edge techniques in capillary electrophoresis (CE) for the analysis of complex carbohydrates. Written in step-by-step detail to ensure successful experimental results, these readily reproducible protocols provide methods for sample preparation and analysis of mono- and oligosaccharides, glycoproteins, and glycoconjugates.

Glycoconjugates, such as glycoproteins and glycolipids, play important roles in cell–cell interaction events, including development, differentiation, morphogenesis, fertilization, inflammation, and metastasis. A number of reports have documented the association of unique oligosaccharide sequences to protein targeting and folding, and in mechanisms of infection, inflammation, and immunity. For glycoproteins, these glycan appendages are the result of extensive co- or post-translational modifications of the nascent proteins in the endoplasmic reticulum and in the Golgi apparatus. Although nucleic acids and proteins are copied from a template in a repeated series of identical steps using the same enzymes, complex carbohydrates are formed by the sequential actions of cellular glycosyltransferases that specifically recognize unique substrates. The molecular biology of these transferases and other carbohydrate-modifying enzymes is providing important insights

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on oligosaccharide recognition events. While it is acknowledged that the definition of the protein complement of cells and tissues (the so-called proteome) remains an enormous task in this postgenomic era, the characterization of all glycans produced by individual organisms (referred to as the glycome) presents an equally important challenge. This task is further complicated by the fact that oligosaccharides cannot presently be cloned.

These complex carbohydrates exist in a staggering diversity of structures, linkages, and branching, thus providing an exquisite molecular repertoire for cellular interactions. In view of the challenges facing the carbohydrate chemists, the further understanding of the structure–function relationship of these glycoconjugates begins with the availability of analytical tools enabling their identification and quantitation. Obviously, progress in this area has been impeded by the structural resemblance existing between isomeric carbohydrate residues and closely related variants conferring on them similar physical and chemical properties. The recent developments in high-resolution separation techniques based on capillary-scale chromatography and electrophoresis have played a pivotal role in deciphering the structural intricacies of these complex biomolecules. Currently, CE is one of the most efficient methods for the separation of complex carbohydrates, and excellent procedures exist for the analysis of free and conjugated mono- and oligosaccharides. This field of research has matured significantly over the past two decades and it is, thus, timely that a volume describing protocols for their analysis by CE be presented in this series of *Methods for Molecular Biology*.

All contributors to *Capillary Electrophoresis of Carbohydrates* are well-experienced scientists working in the field of glycoanalysis, and the volume is designed to be a practical companion not only to well-trained glycobiologists, but also to beginners in this field. This volume is separated into five parts, with an introductory chapter describing the structural and functional diversity of glycoconjugates. In Part II, protocols for sample preparation prior to CE separation are described in Chapters 2 through 4. Cell membranes are typically composed of glycoproteins and glycolipids, two types of complex carbohydrates in which sugars are covalently bound to proteins and fatty acids, respectively. The use of endoglycopeptidases (Chapter 2) offers a convenient approach to the release of glycans from their corresponding glycoproteins, while preserving their structural integrity. These enzymatic products can be subsequently derivatized with reagents to introduce a charge and a chromophore on neutral oligosaccharides in order to facilitate their CE separation and detection with visible or fluorescence detectors. A list of common derivatization reagents, together with reliable procedures, are presented in Chapter 3. Proteoglycans, which are important extracellular matrix components and chemical-signaling molecules of animal cells, are composed of glycoaminoglycans (GAG), unbranched polysaccharide chains comprising repeating

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units of disaccharide residues. Although the release of these polysaccharides is often difficult to obtain in high yields, some eliminases cleave specific linkages of GAG residues, resulting in unsaturated oligosaccharides that provide valuable structural information about the original glycoconjugate. Chapter 4 describes a series of procedures for the preparation of such oligosaccharides.

The third part of *Capillary Electrophoresis of Carbohydrates* summarizes the separation of mono- and oligosaccharide by CE. Different separation formats are available in CE, including capillary zone electrophoresis (CZE), capillary isoelectric focusing (CIEF), capillary isotachophoresis (CITP), capillary gel electrophoresis (CGE), and micellar electrokinetic chromatography (MEKC). CZE is one of the most common separation formats used for the analysis of carbohydrate derivatives, and several applications of this technique are presented in Chapters 5 and 7. The analysis of carbohydrates as borate complexes using CZE and MEKC separation modes are given in Chapter 6. Chapter 8 demonstrates the practical use of affinity electrophoresis using lectins for the separation of oligosaccharides. The analysis of the unsaturated anionic oligosaccharides derived from GAG of proteoglycans is presented in Chapter 9.

The analysis of glycoconjugates in their native state or following minimal chemical or enzymatic treatment is described in Part IV of this volume. Most glycoproteins show microheterogeneity in the *N*- or *O*-linked glycan chains appended to the peptide backbone. This glycoform distribution can be monitored using high-resolution separation techniques such as CIEF and CZE with buffer modifiers. The analysis of biologically relevant glycoproteins using these separation formats is described in Chapters 10 through 12. The on-line coupling of CE to mass spectrometry (CE–MS) has also played an important role in the separation and characterization of glycoconjugates. Chapter 13 presents the application of this technique for the monitoring of intact protein glycoforms and for probing the site of glycan attachment in tryptic glycopeptides using specific mass spectral scanning functions. In Chapter 14, a unique application of CE–MS is demonstrated for the separation of closely related glycoform and isoform families in bacterial glycolipids, based on their unique molecular conformation and ionic charge distribution.

CE can also be applied to other challenging analytical problems such as the characterization of enzymatic activities of glycosyltransferases as presented in Chapter 15. Another example of application of CE is its use for the determination of association constants (Chapter 16). Since diverse biological functions of carbohydrates can be ascribed to the specific binding of these ligands to proteins, accurate measurements of association constants provide insights toward the further understanding of their structure–function relationships. The last chapter of this volume is dedicated to this important topic. Finally, the appendix describes the structures of the most commonly

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encountered carbohydrate residues and oligosaccharides from mammalian and bacterial origins.

Capillary Electrophoresis of Carbohydrates provides both novice and experienced CE analysts with powerful tools for the successful separation of complex carbohydrates by capillary electrophoresis, and presents the following features:

a. Cutting-edge techniques for using CE to analyze complex carbohydrates.
b. Valuable comments and notes on reagents, apparatus, and experimental protocols.
c. Analysis of carbohydrates using different separation formats: CZE, CIEF, CITP, CGE, and MEKC.
d. Appendix of the most commonly encountered carbohydrate residues and oligosaccharides.

The editors should be commended for an excellent reference, which is offered at a reasonable price.

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Bioseparations Science and Engineering, Roger G. Harrison, Paul Todd, Scott R. Rudge, and Demetri P. Petrides, Oxford University Press, New York; Oxford, 2003; hardbound, pp. xix + 406; US$95.00; ISBN 0-19-512340-9.

The 12 chapters of this work treat: (1) Introduction to bioproducts and bioseparations; (2) Analytical methods; (3) Cell lysis and flocculation; (4) Filtration; (5) Sedimentation; (6) Extraction; (7) Liquid chromatography and adsorption; (8) Precipitation; (9) Crystallization; (10) Drying; (11) Bioprocess design; and (12) Laboratory exercises in bioseparations. These are followed by an Appendix comprising a table of units and constants, and by a Subject Index.

I know how hard it is to put together a comprehensive work on any subject comparable to the one under discussion here and I also know that it is virtually unavoidable to leave some minor errors uncorrected. In the full expectation that this text is bound to go through further editions, I'll advance what are intended as a few constructive suggestions toward the correction of some minor flaws or omissions.

1. Hamaker constants: p. 97. The constant (A) pertaining to London–van der Waals interactions was first defined by H. C. Hamaker,[1] in the macroscopic sense, where the attractive London–van der Waals energies between two equal macroscopic spheres decay in simple proportion to the distance between the spheres. Hamaker also published two precursors[2,3] of what ultimately became known as the DLVO theory;[4,5] he learned only decades later that his constant (A), which plays an important role in the DLVO approach, had been designated as the Hamaker constant. This "constant" is not a constant in the sense of, e.g., Avogadro's constant (cited on p. 393 of this work), but a physical property of atoms and molecules, varying about 670-fold, between 5.4×10^{-22} J for helium and 3.6×10^{-19} for mercury, see, e.g., Ref.[6] As the range of Hamaker constants of all the elements is about 2.8 times more widely spread than the range of their atomic weights, it is an oversimplification to state that Hamaker constants do not vary greatly from one compound to another. Fortunately, the Hamaker constant of a given condensed-phase material is, at a given temperature, proportional to the Lifshitz–van der Waals (LW) component of its surface tension, which is easy to measure.[6] Indeed, relatively small differences in the LW components of the surface tension (and, thus, of the Hamaker constants) among different polymer pairs, dissolved in, e.g., benzene, can give rise to mutual miscibility in that solvent (through a van der Waals attraction), or to a phase separation (caused by a net van der Waals repulsion); see, e.g., Ref.[7]

2. Flocculation: p. 97. The reasoning behind the critical coagulation concentrations of neutral "indifferent" electrolytes (the active agents of

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which are the counterions, of opposite sign of charge to that of the charged particles that have to be flocculated) is flawed, because the classical DLVO theory (i.e., attractive van der Waals forces vs. repulsive electrical double layer interactions) *does not work in water*. In water, one has, *in addition*, to contend with the predominant influence of hydrogen-bonding and other Lewis acid–base interactions. This also impinges on the century-old Schulze–Hardy phenomenon. By adding neutral salts, e.g., with di- or trivalent counterions, to an aqueous medium containing a stable suspension of electrically charged hydrophilic particles, one decreases the zeta-potential of such particles, typically by about 60–70%, which then causes the particles *to change from hydrophilic to hydrophobic*, which is the real cause of the ensuing flocculation (see, e.g., Ref.[8]). The decrease in zeta-potential sufficient to start flocculation usually does *not*, by itself, suffice to allow the van der Waals attraction to overcome the electrical double layer repulsion: it is the much stronger hydrophobic attraction concomitant to the (relatively modest) decrease in zeta-potential that causes the flocculation. Thus, attempts to derive some particles' Hamaker constant from just the concentration of a given electrolyte needed to cause their flocculation, cannot be successful and, if such an attempt appeared to yield a right-sounding answer, this most likely was purely accidental.

3. Analytical ultracentrifugation: pp. 156–157. Having worked with Beckman-Spinco "Models E" for more than 25 years, I much appreciated seeing the entry on p. 157 on the determination of (weight-average) molecular weights (Mw) via sedimentation coefficient measurements with the analytical ultracentrifuge, using Svedberg's equation. Unfortunately actually working Model E's have now become exceedingly rare and their more recent replacement models have not caught on to any large extent, so that Mw determinations by analytical ultracentrifugation are practically on the way of becoming a lost art. This is to be regretted because modern Mw determination approaches of, e.g., biopolymers, are mainly based on a variety of liquid chromatography such as gel exclusion chromatography, or on an electrophoretic method such as SDS polyacrylamide gel electrophoresis, neither of which gives much of a clue about polymer shape (e.g., expressed as its asymmetry factor). Biopolymer shape, on the other hand, can be determined via the molecule's friction factor ratio (f/fo). For instance, f/fo ratios can be determined once one knows a biopolymer's sedimentation coefficient, s, as well as its diffusion coefficient, D. But the sedimentation coefficient is not the only molecular datum which, combined with D, can yield the f/fo ratio: the combination of a molecule's Mw and D also works. Thus, with Mw determined by one of the liquid chromatography or gel electrophoresis approaches, together with D, one can also find the f/fo ratio, and from this the asymmetry

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factor of a biopolymer molecule (see, e.g., Refs.[9,10]). Without any significant instrumentation, D can easily be determined by the angle a gel double diffusion immunoprecipitation line makes in a gel with two troughs placed perpendicular to one another, where one trough is filled with the (not necessarily very pure) biopolymer (B) solution and the other trough with an IgG isotype antibody (A) (which may be polyclonal), elicited against B. The angle, α, which the precipitate line makes with the antibody (A) trough allows the diffusion coefficient of B to be found through:

$$\tan(\alpha) = \text{square root of}\left(\frac{DA}{DB}\right)$$

where DA for an IgG isotype antibody $= 6 \times 10^{-7}\,cm^2/sec$ (cf. Ref.[11] pp. 737–758).

4. Hydration model: Fig. 8.1, p. 245. The water molecules of the hydration layers of proteins do not so much prevent two protein molecules to approach one another closely by just being there like little round beads but, rather, by having their O-atoms oriented distally, pointing away from the surfaces of the opposing protein molecules. This results in a mutual hydration orientation-driven repulsion between two protein molecules, while immersed in water (cf. van Oss and Good, 1988; Refs.[11,12] pp. 212–216).

This very thorough and up-to-date work on bioseparation methodology is highly recommended for use in undergraduate and graduate courses for chemical engineers and also for those advanced students and practitioners of molecular biology who not only prepare various recombinant peptides and proteins, but are, in addition, involved in their extraction and purification.

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