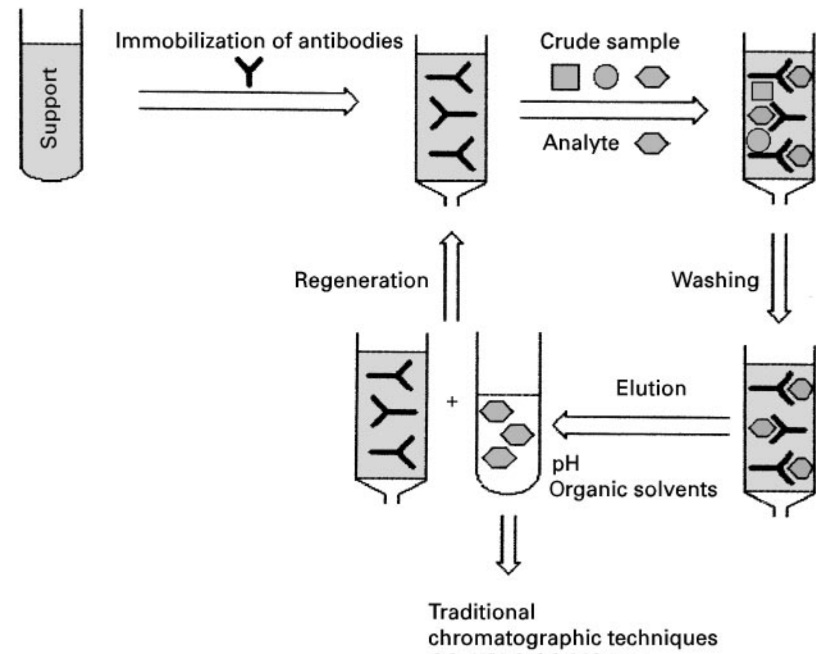

Figure 6 Schematic separation of analytes by immunoaffinity chromatrography for pesticide determination in food matrices.

can overcome some of these limitations. In the LC-MS techniques, the use of an atmospheric pressure chemical ionization interface is at present the best alternative since it offers high selectivity and sensitivity for the trace determination of carbamates.

See also: **II/Affinity Separation:** Immunoaffinity Chromatography. **Chromatography: Gas:** Detectors: Mass Spectrometry. **Chromatography: Liquid:** Detectors: Mass Spectrometry. **Extraction:** Supercritical Fluid Extraction. **III/Immunoaffinity Extraction. Multiresidue Methods: Extraction. Pesticides:** Extraction from Water; Gas Chromatography; Supercritical Fluid Chromatography; Thin-Layer (Planar) Chromatography.

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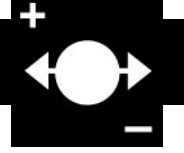
CARBOHYDRATES



Electrophoresis

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Introduction

Electrophoresis has been an important tool for carbohydrate analysis since its early stages of development. Moving with time from paper electrophoresis to polyacrylamide slab gel electrophoresis and then to the sophisticated high performance capillary

Step 1. (pH > 9)

$$B(OH)_3 + OH^- \rightleftharpoons B(OH)_4^-$$
(B) (B⁻)

Step 2. Equilibria of complexation

$$B(OH)_4^- \text{ (B}^-\text{)} + \text{(L)} \rightleftharpoons \text{(BL}^-\text{)} + 2H_2O$$

(BL^-), monodiole

$-2H_2O$

(BL_2^-), bisdiol (spirane)

Figure 1 Boric acid as a Lewis acid forms borate in water. Borate reacts with polyol compounds to mono- or bisdiol complexes.

electrophoresis (HPCE), its influence in many chemical, biochemical and biomedical fields has grown constantly. New detection techniques and buffer systems have been developed to make use of the advantages offered by HPCE, such as low sample and buffer volumes, fast separations and high efficiencies. Today, sample volumes in the range of single cell liquids can be analysed. The development of highly sensitive detectors and new derivatization procedures has constantly lowered the detection limit. In the light of these advantages, the following article will focus on capillary electrophoresis.

The Electrophoretic System

Electrolyte Systems

The presence of an ionic charge is the usual prerequisite for migration in an electrophoresis system. Only some saccharides, such as aldonic acids, uronic acids, sialic acids, aminosugars or sulfated sugars of chondroitin, dermatan, keratan and heparin, possess charged functional groups in their structures. The separation of uncharged molecules requires the conversion into charged species by complex formation or derivatization.

The most commonly used systems in planar electrophoresis are those with borate buffers, but other inorganic acids can form complexes with uncharged saccharides. In HPCE, systems are based mainly on sugar–borate complexes and to a lesser extent on sugar–metal cation complexes. Boric acid in aqueous media acts as a Lewis acid to form the tetrahedral anion $B(OH)_4^-$. At pH 8–10, the formation of mono- and diesters with the carbohydrates is most effective, but depends largely on the structure of that particular sugar (**Figure 1**).

The complexation of metal cations also depends on the structure of the carbohydrate. In addition, the ionic radius is crucial for the stability of the complex. Complexation is possible with alkaline metals, Cu^{2+}, Pb^{2+}, Zn^{2+}, Ni^{2+} and Cd^{2+}. For alkaline earth metal buffers, the separation efficiencies decrease in the following order: $Ba^{2+} > Sr^{2+} > Ca^{2+} \gg Mg^{2+}$. Another way to introduce an ionic charge is by the deprotonation of the hydroxyl functions of the sugars. For this purpose, high pH values (sodium hydroxide solutions, pH > 12) are necessary.

Carbohydrates can also be separated by micellar electrokinetic chromatography (MEKC). In this case, the separation is caused by the different distribution coefficients of the sugars between the free solution and the interior of the migrating micelles of a charged detergent (sodium dodecyl sulfate).

Detection Systems

Precolumn derivatization Most carbohydrate species do not posses strong chromophores in their structures. This condition does not allow their direct sensitive detection by UV absorption. To overcome this difficulty, carbohydrates can be tagged with suitable fluorophores or chromophores. If the tag also provides the charge necessary for electrophoresis,

uncharged carbohydrates can also be separated without the use of complexing buffer systems as well. In principle, derivatization of the hydroxyl groups should be a good approach for the tagging of carbohydrates, but the derivatization of sugars with different reactivities would lead to multiple tagging of the molecule. This would consequently cause a distribution of different products rather than a single derivative. Therefore, other functional groups of the sugar molecule must be considered. The carbonyl group in the open chain form of reducing sugars is the most widely used functional group for the attachment of the label. In amino sugars or acidic compounds, the amino group or the carboxyl group provides potential for derivatization.

Since it is required to produce only one species of derivative in precolumn derivatization, the label must not possess more than one reactive function for the atttachment to the carbohydrate. Another requirement is the high molecular absorptivity (UV detection) or photoluminescence efficiency (fluorescence detection) of the tag. Although laser-induced fluorescence detection (LIF) exhibits the lowest detection limits, it is difficult to find a suitable reagent for the respective laser system. The label has to be matched to the laser system used with respect to excitation wavelength and emission intensity. **Table 1** compares the detection limits of the different systems after derivatization.

The different labelling agents can be divided into three groups according to their set charge in the separation system: positive, negative or uncharged. **Table 2** gives examples of some commercially available derivatization agents and the wavelengths used in different detection systems. Recently, they have found their way into standard chemical catalogues (Fluka, Sigma, Aldrich, ICN). The positively charged compounds usually contain heterocyclic nitrogen functions, e.g. 2-aminopyridine or 6-aminoquinoline. They can be protonated at lower pH values and are commonly used for separation in phosphate buffer solutions (pH 2.5–4). Depending on their pK_a values, these amino compounds can also be used as uncharged labels in high pH buffers. Chromophores with strongly acidic groups, like aminonaphthalene trisulfonic acid (ANTS), remain negatively charged over a wide pH range. Multiple sulfonic acid functions introduce several negative charges to the molecules, which results in high mobility and low adsorption of the derivatives to the capillary surface. All that these compounds have in common, is that they contain a single amino function, which allows the attachment to the carbonyl function of the reducing sugar by reductive amination. In this one-pot reaction, the open chain form of the sugar reacts with the amine to a Schiff's base. To accelerate the reaction and to shift the equilibrium to the product side, the imino function is reduced by sodium cyanoborohydride to the respective secondary amine (**Figure 2**). An exception to this is the reaction of reducing carbohydrates with 1-phenyl-3-methyl-pyrazolone (PMP). In this case, the acidic hydrogens of PMP and the aldehyde functionality condense under slightly basic conditions.

A disadvantage of precolumn derivatization is the higher structural similarity of the compounds and the higher complexity of sample preparation. Another problem is the varying reactivity of carbohydrates, especially of monosaccharides, with the derivatizing agent. Optimized reaction conditions have to be determined for every sample mixture. The reproducibility of the sample preparation is crucial for the quantification of the compounds.

Indirect detection Indirect detection modes have been applied for the analysis of compounds whose structures lack the necessary physical properties for direct detection. The key element is the use of a background electrolyte, which provides a high, continuous signal. The analyte ion displaces the background electrolyte ion and leads to a change in the UV absorption signal. Therefore, the background electrolyte needs to have the same type of ionic charge as the analyte. The attainable detection limit is given by

$$C_{\text{lim}} = \frac{C_M}{DR \cdot TR}$$

C_M represents the concentration of the background electrolyte, DR is the dynamic reverse and TR is the transfer ratio. DR is defined as the ability to measure a small change on top of a large signal and is equal to the signal/noise ratio of the background signal. TR is defined as the number of molecules of the background ions displaced by the charged analyte. It can be seen,

Table 1 Detection limits of monosaccharides after derivatization

Detection	*Absolute amount (mol)*	*Concentration (mol L^{-1})*	*Weight concentration*
UV derivatization	10^{-13}–10^{-11}	10^{-6}–10^{-4}	ca 10 ppm
Fluorescence derivatization	10^{-16}–10^{-11}	10^{-9}–10^{-4}	ca 10 ppb
Derivatization for LIF	10^{-21}–10^{-17}	10^{-13}–10^{-9}	ca 1 ppt

Table 2 Examples for derivatization agents and used detection wavelengths

Structure	*Name*	λ_{ex}	λ_{em}
	2-Aminopyridine (2-AP)	240, 320	400
	6-Aminoquinoline (6-AQ)	355	550
	1-Phenyl-3-methyl-5-pyrazolone (PMP)	245	–
	5-Aminofluoresceine	257	471
	1-Aminonaphthalene-7-sulfonic acid	229	482
	7-Aminonaphthalene-1,3-disulfonic acid (ANDS)	325	520
	8-Aminonaphthalene-1,3,6-trisulfonic acid (ANTS)	223, 325	520
	8-Aminopyrene-1,3,6-trisulfonic acid (APTS)	325	520
	2-Aminoacridone (AMAC)	488	520

Figure 2 The open chain of the reducing carbohydrate reacts with the amino compound to a Schiff base, which is reduced to a secondary amine by sodium cyanoborohydride.

that C_M should be as low as possible while still generating a high *DR*. The *TR* should be close to unity.

Either indirect laser-induced fluorescence detection or UV detection can be applied to the separation of underivatized carbohydrates. Coumarin (LIF) and sorbic acid (UV), for example, have been used as background electrolytes with deprotonated carbohydrates at high pH values. In indirect detection, the concentration of the background electrolyte should be low. Thus, the use of a borate buffer (required concentrations between 0.1 and 0.25 mol L^{-1}) is excluded. Furthermore, the deprotonation of the carbohydrates at high pH values is limited by the increasing concentration of hydroxide anions, which compete with the background electrolyte ions in the displacement mechanism.

Direct detection Different modes of direct detection have been applied to HPCE separations of underivatized carbohydrates. First, low wavelength UV (below 200 nm) can be used to detect carbohydrates which have a sufficient molar absorption coefficient, especially those compounds having carboxyl or other UV-absorbing groups. Mixed oligosaccharides of heparin and heparan sulfate, chondroitin sulfate and dermatan sulfate can be detected at 232 nm. The poor absorption coefficients of many carbohydrates in the range 190 to 280 nm, limits this detection mode.

Amperometric detection Another method is pulsed amperometric detection (PAD) with gold or platinum electrodes, using a strongly alkaline buffer, which has been adapted from the HPLC-PAD systems. These systems require a specialized pulse sequence and therefore expensive instrumentation. Other systems have used ultramicroelectrodes, equipped with a 25-μm copper wire at a constant potential. These systems permit the realization of a linear range over three magnitudes and a limit of detection for mono- and disaccharides in the femtomole range. However, using PAD systems, the running buffer must not contain any electroactive species that might oxidize on the working electrode and cause a strong background signal or poison the electrode surface. Thus, amperometric detection excludes the use of borate buffers, which are essential for the separation of many neutral and native saccharides.

Refractive index detection The determination of the refractive index (RI) has shown its potential in HPLC, although it is not very sensitive and its detection limit falls below that of low wavelength UV. To overcome the problems with the low volume flow in capillary electrophoresis (CE), a sub-nanolitre laser-based refractive index detector has been developed. The detection is based on the change of interferences caused by the change of the refraction index. However, the

adaptation of RI detection of HPCE gives some problems, caused by the joule heating, which introduces changes in the temperature of the liquid and, with this, changes in the refractive index.

Capillaries with partially increased inner diameter Theoretically, the limit of detection is reduced with the increasing inner diameter of the capillary, due to the increased lightpath through the solution. This is limited by the increasing current with constant field strength, on moving to greater diameters. High currents lead to joule heating effects and contribute to additional band broadening. Capillaries, which are widened locally at the detection window, can help to decrease the detection limit. In this case, the capillary is partially etched with hydrofluoric acid at the detection window. The reaction is controlled by temperature. The diameter widens drastically at the 'hot spot' only. The reaction rate with the capillary wall at other locations is relatively low. Using this procedure, 25-μm capillaries can be widened up to 75 or 150 μm, easily. Recently, detection cells with elongated lightpath (high sensitivity cells, z cells) have become commercially available.

Applications and Separation Methods

Monosaccharides

The analysis of monosaccharides, the basic units of carbohydrates, is crucial for many areas of biochemistry, pharmacology, biotechnology and food science. Several approaches have been made to influence the resolution of the HPCE separation of monosaccharides.

The most common buffer system is based on the complexation of the carbohydrates with an alkaline borate buffer. **Figure 3** demonstrates the separation of a mixture of several aldopentoses and aldohexoses. The sugars were tagged by reductive amination with 2-aminoanthracene. A counter-electroosmotic separation with normal polarity and fluorescence detection was performed. In this case, the intrinsic mobilities of the analytes are lower than the mobility of the electroosmotic flow (EOF). The analyte molecules are transported against their migration direction to the cathodic end of the capillary. The fastest compound is detected last. Five monosaccharides were baseline separated, while one pair (arabinose/mannose) comigrate. The stability of the carbohydrate complex with the borate ion, which depends on the structure of the monosaccharide, has the dominating influence on the separation. Due to these differences, fucose (desoxyhexose) is separated from the unresolved pair mannose (hexose) and arabinose.

To underline the differences in the migration mechanisms, **Figure 4** shows a co-electroosmotic separation of the same mixture in an acidic medium. At a pH of 2.0, the amino function of the analyte is protonated and leads to a co-electroosmotic migration, i.e. in the same direction as the electroosmotic flow. The fastest compound is detected the first. Due to the low dissociation of the silanol groups at the capillary surface, the EOF is very low at this pH.

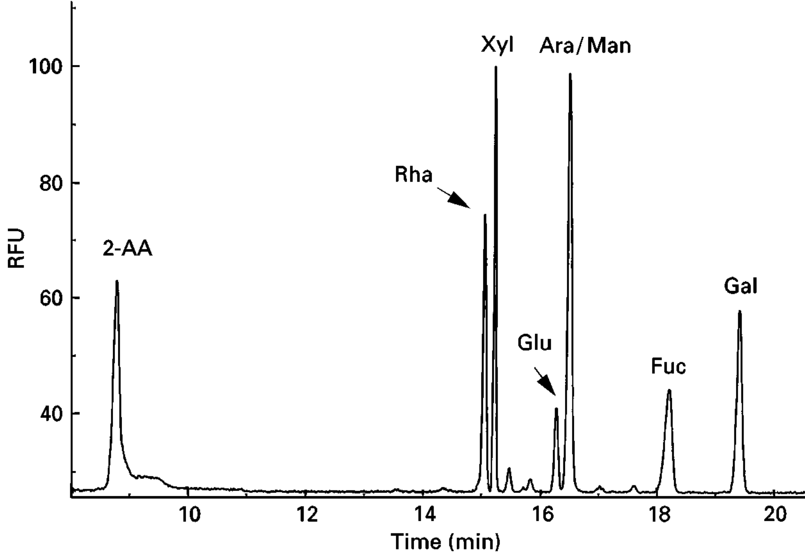

Figure 3 Counter-electroosmotic separation of 2-AA-labelled monosaccharides rhamnose (Rha), xylose (Xyl), glucose (Glu), arabinose (Ara), mannose (Man), fucose (Fuc), galactose (Gal) and 2-aminoanthracene (2-AA). Buffer: borate 250 mM, pH 10.5; $U = 30$ kV; capillary-fused silica, i.d. = 50 μm, $l = 88$–100 cm; inj. 6s 100 mbar.

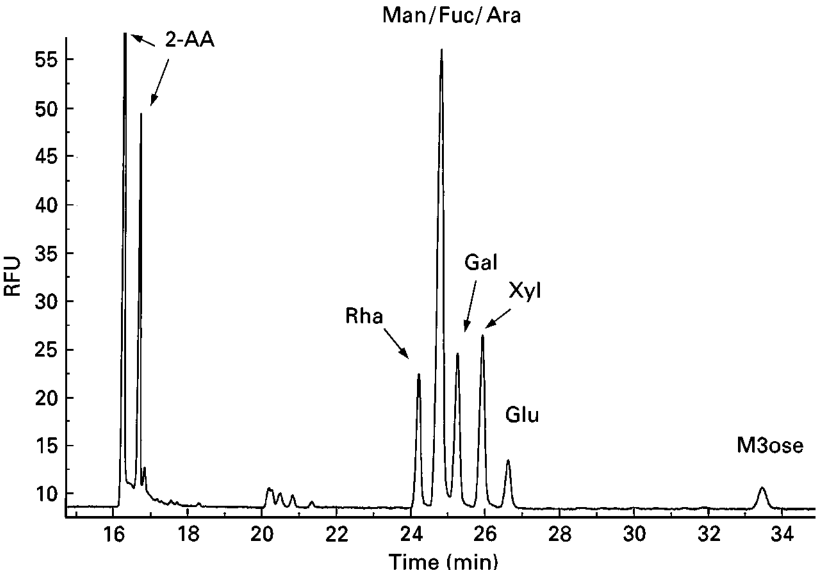

Figure 4 Co-electroosmotic separation of 2-AA-labelled monosaccharides rhamnose (Rha), xylose (Xyl), glucose (Glu), arabinose (Ara), mannose (Man), fucose (Fuc), galactose (Gal), maltotriose as standard (M3ose) and 2-aminoanthracene (2-AA). Buffer: phosphate 100 mM, 20% (v/v) iPropOH, pH 2.0; capillary fused silica, $l = 49$–60 cm, i.d. = 50 μm; U = ramp 2.5 kV min^{-1} from 5 to 30 kV; inj. 6s 100 mbar.

To enhance resolution, an organic modifier (isopropanol) was added. The co-electroosmotic separation leads to a different electropherogram. Here, other parameters, such as the hydrodynamic radius of the analytes, have the dominating influence on the migration mechanism. The differences in mobility of mannose, fucose and arabinose are too small to lead to a separation, while the resolution of the epimers glucose and mannose is increased compared to the borate buffer system.

It is also possible to separate a monosaccharide mixture by micellar electrokinetic chromatography (MEKC). In this system, the dominating mechanism is the dynamic distribution of the uncharged analytes between free solution and the migrating micelles of the detergent (often SDS). Here, other properties of the analytes, such as differences in their hydrophobicity, effect the separation.

Figure 5 shows the separation of a monosaccharide mixture in a trisborate/SDS buffer. Unlike the results of the previous separation systems, the peaks of mannose and arabinose show a sufficient resolution.

Table 3 gives some examples of the buffer systems and tags used so far to analyse monosaccharide mixtures. The use of borate buffers dominates. Other systems have also been used, but have not yet found their way into standard applications.

Linear Oligo- and Polysaccharides

Oligo- and polysaccharides can be classified either as homo- or heteropolymers, depending on whether they consist of one type or more than one type of monosaccharide unit that alternate in the repetitive sequence. High molecular weight polysaccharides are usually analysed through their degradation products (chemical or enzymatic cleavage). The systems described for the separation of monosaccharides can be easily applied to the separation of oligosaccharides. With few exceptions, the mobilities decrease with increasing molecular weight of the analytes. The use of divalent metal ions for complexation is not favourable for analysing carbohydrates, as it leads to insufficient separation efficiency.

The composition of oligosaccharide mixtures should be considered when selecting the separation system. The use of a borate buffer focuses on the structural differences of the oligosaccharides and should be used for separation of hetergeneous oligosaccharides with the same degree of polymerization. For homologous oligosaccharides, the use of permanently charged labels in a co-electroosmotic system is favourable. Here, the charge-to-mass ratio is the dominant parameter responsible for the migration differences. The influence of the electroosmotic flow should be reduced to a minimum to ensure good resolution of the compounds with higher molecular mass.

Figure 6 shows the counter-electroosmotic separation of 2-AA labelled maltooligosaccharides in the borate buffer system. As the molecular weight increases, the differences in migration time decrease very fast. This can be explained by a lower degree of

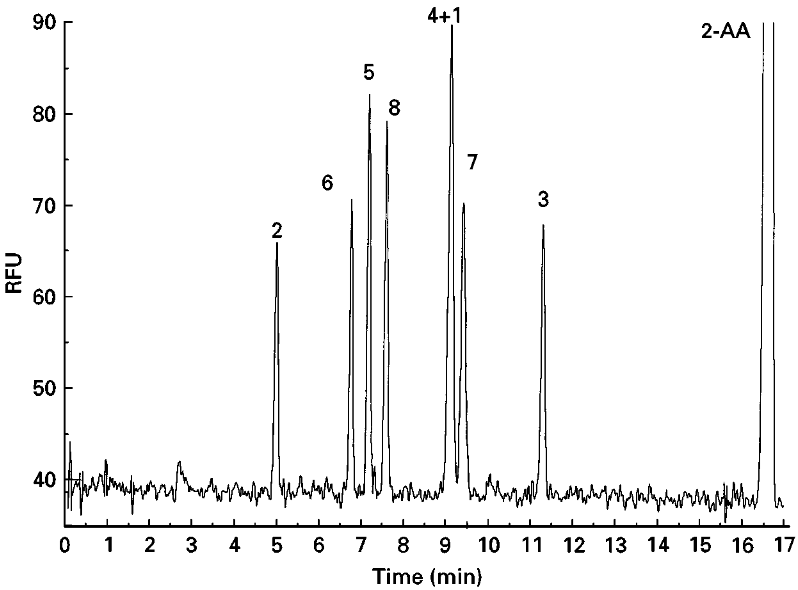

Figure 5 MEKC separation of 2-AA-labelled monosaccharides. 1, rhamnose; 2, cellobiose; 3, xylose; 4, ribose; 5, glucose; 6, mannose; 7, arabinose; 8, galactose; 2-AA, 2-aminoanthracene. Buffer: trisborate 100 mM/urea 4 M/SDS 100 mM, pH 8.3; capillary-fused silica, $l = 48$–60 cm, i.d. $= 25$ μm; $U = 30$ kV.

structural change, going step by step to higher degrees of polymerization. Also, the ratio of $\mu_{\text{analyte}}/\mu_{\text{EOF}}$ decreases with increasing degree of polymerization. With a higher degree of polymerization, the mobility differences decrease drastically and lead to insufficient resolution.

For higher degrees of polymerization (> 7), the use of charged labels at low EOF is to be preferred.

Table 3 Examples of the separation mode, buffer systems and detection methods for different carbohydrates

Analytes	*Detection*	*Separation mode*	*Derivatization*	*Buffers*
Monosaccharides	Direct UV	CZE	2-AP	200 mM borate, pH 10.5
Monosaccharides	Direct Fluorescence	CZE	2-AA	200 mM borate, pH 10.5
Monosaccharides	Indirect UV	CZE	–	6 mM sorbate, pH 12.1
Monosaccharides	Indirect LIF	CZE	–	50 μM fluoresceine, pH 12.2
Monosaccharides	Direct LIF	CZE	APTS	25 mM borate, pH 10.0
Monosaccharides	Amperometric detection	CZE	–	100 mM NaOH, pH 13
Monosaccharides	RI detection	CZE		100 mM borate, pH 9.0
Maltooligosaccharides $DP_{max} < 25$	Direct UV	CZE	2-AP	100 mM phosphate, pH 2.5
Maltooligosaccharides	Direct LIF	CZE	ANTS	50 mM phosphate, pH 2.5
Branched xyloglucans	Direct UV	CZE	2-AP	100 mM phosphate, 50 mM tetrabutylammonium bromide, pH 4.75
Dextrans $DP_{max} < 25$	Direct fluorescence	CZE	2-AA	100 mM phosphate, pH 2.0
Low molecular weight heparin	Direct UV	CZE	–	10 mM borate, 50 mM sodium dodecyl sulfate
Polygalacturonic acid $DP_{max} < 70$	Direct LIF	CGE	ANTS	100 mM Tris, 25 mM borate, pH 8.5 Polyacrylamide gel, 18% T, 3% C
Hyaluronic acid $DP_{max} < 400$	Direct LIF	CGE	APTS	25 mM citric acid, 12.5 mM tris, 0.03% aminodextran (mol. wt 10 000)
Glycoforms of ribonuclease A	Direct UV	CZE		20 mM phosphate, 50 mM sodium dodecyl sulfate, 5 mM borate, pH 7.2

DP_{max}, maximum degree of polymerization (baseline separation); CZE, capillary zone electrophoresis; CGE, capillary gel electrophoresis.

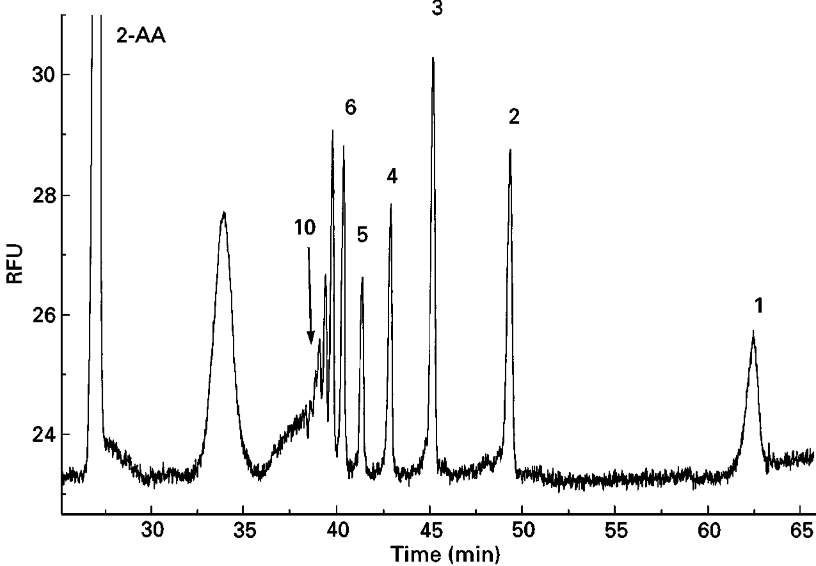

Figure 6 Counter-electroosmotic separation of the starch hydrolysate Dextrin 10 (numbers indicate the degree of polymerization). Buffer: borate 300 mM, pH 10.5; capillary fused silica, $l = 108\text{–}120$ cm, i.d. $= 50$ μm; field $= 30$ kV; inj. 6s 100 mbar.

It can be seen in **Figure 7** that protonation of the amino function of the 2-aminoanthracene-labelled aminoglycans leads to a higher resolution of the oligosaccharide peaks. With a higher number of monosaccharide units, the mobility differences decrease less than those in the borate system. Increasing charge of the label can increase the mobility of the molecules. This has proved to be the case with naphthalene di- and trisulfonic acid derivatives especially. By derivatizing with aminonaphthalene trisulfonic acid, it is possible to separate more than 30 malto-oligomers within 10 min.

To improve the resolution of homologue oligosaccharides with a higher degree of polymerization, two different approaches have been attempted. First, the use of coated capillaries (polyether, polyvinyl alcohol) showing very low or virtually no electroosmotic flow can lead to an increase in resolution. The disadvantages of these systems are the limited pH range in which they can be used and the high cost of the capillary material.

A similar approach to that for the separation of oligonucleotides involves using gel-filled capillaries. Gel-filled capillaries can have a sieving effect on the

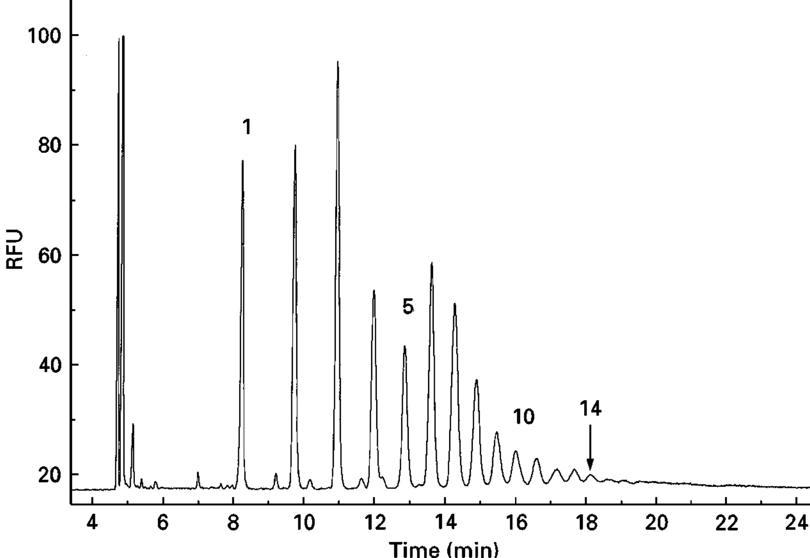

Figure 7 Co-electroosmotic separation of the starch hydrolysate Dextrin 10 (numbers indicate the degree of polymerization). Buffer: phosphate 100 mM, pH 2.0; capillary-fused silica, $l = 49\text{–}60$ cm, i.d. $= 50$ μm; $U = 25$ kV; inj. 5s 100 mbar.

analytes. This size discrimination can be used to increase the differences in mobility. Unfortunately, the use of gel-filled capillaries is accompanied by many difficulties such as poor gel-to-gel reproducibility, bubble formation, quenching effects by the matrix, and the collapse of the gel matrix buffer. Another problem is the relatively low mobility of the formerly uncharged molecules, which are tagged by a charged label. The use of gel-filled matrices can increase the migration time to an unacceptable extent.

For structures consisting of charged monosaccharide species, such as polyuronic acids, the use of a mixture of aminodextran and linear polyacrylamide (LPAA) as buffer additive is successful. In this case, the retention mechanism cannot be due to the sieving effect of the matrix only. The system is, rather, based on size-dependent electrostatic interactions between the protonated amino functions of the aminodextran and the dissociated carboxylic groups of the uronic acids.

Table 3 gives some examples of the systems used so far.

Branched Oligosaccharides

Branched oligosaccharides can be produced by enzymatic digestion of branched heteropolysaccharides, for example xyloglucan polysaccharides. In combination with a charged label, some information about the branching of the compounds can be achieved. If only the label is charged, the compounds migrate in order of increasing size, as shown before. With similar molecular weight, the less branched oligosaccharides migrate faster than the more branched ones. For branched xyloglucan oligosaccharides, a mobility index system has been introduced to describe the migration behaviour of the branched compounds with respect to their linear homologues. The equation below shows how the mobility index (MI) can be calculated:

$$MI = 100n + 100\left(\frac{\log \mu_s - \log \mu_{n+1}}{\log \mu_n - \log \mu_{n+1}}\right)$$

μ_s is the electrophoretic mobility of the branched analyte, and μ_n and μ_{n+1} are the electrophoretic mobilities of the two homologues with n and $n+1$ repetitive units, which migrated before and after the branched fragment. This index system can only be applied if the following requirements are fulfilled. First, the system must consist of homopolymers or heteropolymers with a strictly repeating sequence. Second, the logarithmic mobilities of the homologues must show a logarithmic dependence on the degree of polymerization, which is not always the case.

Glycoconjugates

Glycoproteins Glycoproteins function as enzymes, transport proteins, receptors, hormones and structural proteins. The carbohydrate content of glycoproteins can vary from less than 1% to more than 60% by weight. Protein glycosylation can occur at two or more positions in the amino acid sequence. The glycans at a single position may be heterogeneous or may be missing from some molecules. This leads to populations of glycosylated species of a single protein (glycoforms). The relative proportions of glycoforms are found to be reproducible, depending on the glycosylation conditions (environment of the reaction, physical state and type of organism), the manufacturing process and the isolation procedures.

On the one hand, glycoform separation and mapping has to cope with the problems introduced by the protein moiety, such as interactions of the protein with the capillary wall in low pH electrolyte systems. In this case, a hydrophilic coating of the capillary surface can aviod adsorption phenomena. On the other hand, the UV absorption coefficients at 200 nm are sufficient for the detection of the compounds without precolumn derivatization. Common buffer systems are phosphate, TRIS/boric acid or borate buffers, depending on the separation mode and the pH range.

Glucosaminoglycans Glucosaminoglycans (mucopolysaccharides) are unbranched polysaccharides of alternating uronic acid and hexosamine residues, for example, heparin, chondroitin sulfate, dermatan sulfate or hyaluronic acid. After exhaustive treatment with polysaccharide lyases, disaccharides can be obtained bearing unsaturated uronic acids. These compounds can be detected directly at 232 nm. To enhance sensitivity, they can also be labelled. Since the mucopolysaccharides are already charged over a wide pH range, it is not necessary to introduce a charge by a tag or complexation. The use of SDS micelles can increase the resolution.

Glycolipids Some lipids contain oligosaccharide moieties as an integral part of their structure. Since these contain a hydrophilic head and a hydrophobic tail, they form micellar systems. Temperature, concentration of the analyte and ionic strength of the electrolyte system are crucial for the size and mobility of the micelles. Thus, the separation of some glycolipids as monomeric species is impeded. The investigations made so far have been based on phosphate buffer systems and low wavelength UV detection.

Further Directions

Capillary electrophoresis has proven potential for carbohydrate analysis. High efficiencies and short analysis times are its advantages compared with other analytical separation methods. A variety of different separation modes can be applied to separate complex carbohydrate mixtures. It has been shown that, depending on the properties of the sample, different separation methodologies can be applied and optimized.

The electrophoretic analysis of carbohydrates is still under development. In particular the analysis of biochemical compounds, such as glycoconjugates, is still a challenge and can give new information about cell mechanisms, structure of antibodies, etc. This article has focused on the basic aspects of capillary electrophoresis of carbohydrates. The carbohydrates described were confined to simple model compounds. For more specific information, the Further Reading list should be consulted.

See also: **III/Ion Analyses: Capillary Electrophoresis.**

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Gas Chromatography and Gas Chromatography–Mass Spectrometry

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Overview of Derivatization of Sugars for GC, GC-MS or GC-MS-MS Analysis

Table 1 overviews many of the important events in chromatographic and mass spectrometric analysis of sugar monomers. These will be discussed at appropriate points in this review. It was not until the years 1961–1963 that gas chromatography (GC) was applied to the quantitative analysis of mixtures of neutral and amino sugar monomers, even though individual sugars had been derivatized earlier. Carbohydrate analysis by GC lagged behind the analysis of many other compounds because of the difficulty in producing volatile derivatives. Two schools of thought developed, relating to whether the anomeric centre should be retained or destroyed in derivatization. Both persist to this day. Sugars exist in equilibrium betweeen ring and straight chain forms. If the anomeric centre is not eliminated, derivatization fixes the sugars in the anomeric ring forms. Thus, two to four peaks will be produced from each L or D sugar (two from furanose and two from pyranose anomers). Interpretation of chromatograms becomes complicated and quantitation difficult. Acidic sugars (i.e. generally with carboxyl groups) require additional derivatization steps. The carboxyl moiety may be converted to a lactone, ester or reduced to an alditol.

The Alditol Acetate Procedure

The alditol acetate procedure was the first developed in which the anomeric centre is eliminated. The anomeric centre is converted by borohydride reduction (although later borodeuteride was introduced)