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Capillary electrophoresis of sulfated molecules

Mandakini Dantuluri and Umesh R. Desai
Department of Medicinal Chemistry, Virginia Commonwealth University, Richmond VA 23298-0540, USA

Abstract

Capillary electrophoresis has been demonstrated to be useful in the analysis of virtually every class of molecule. Aided by a number of specialized modes, which fundamentally differ from each other in the bases of resolution, the power and applicability of this technique is phenomenal. Nature abounds in sulfated molecules, including the structurally complex glycosaminoglycans and sulfated metabolites. It is also expected that synthetic sulfated molecules will gain prominence as agents that can regulate natural processes. This review focuses on recent enhancement in knowledge in the area capillary electrophoresis of these natural and synthetic sulfated molecules.

Introduction

Since Hjertén's and, Jorgenson and Lucas's pioneering work on electrophoresis in small diameter tubes, capillary electrophoresis (CE) has come a long way in terms of resolution, speed, capabilities, and

Correspondence/Reprint request: Dr. Umesh R. Desai, Department of Medicinal Chemistry, School of Pharmacy, Virginia Commonwealth University, 410 N. 12th Street, #542, Richmond, VA 23298-0540, USA. Email: urdesai@vcu.edu

ease of operation.[1-3] Virtually every class of molecule, including small anions and cations,[4,5] chiral and achiral drugs,[6-8] neutral and acidic carbohydrates,[9-11] peptides and proteins,[12-14] glycoconjugates and proteoglycans,[15,16] nucleotides and deoxynucleic acids,[17] has been shown to be effectively analyzed by capillary electrophoresis. The use of the technique has not been limited to just the identification of species, although this is the direction of an overwhelming number of applications, but expands to include binding profiles,[18,19] enzyme assays,[20] single cells analysis,[21] nucleic acid sequencing,[22] DNA polymorphism,[23] high-throughput screening,[24] microorganism identification,[25] and micro-preparation of species.[26] Recently, CE has started to move in the direction of non-aqueous analysis.[27]

Capillary electrophoresis derives its major advantage from the small internal diameter (20 to 100 μm) that affords very high surface-to-volume ratio permitting extremely efficient dissipation of Joule heat generated during electrophoresis. This allows voltage gradients of the order of 800 V/cm that result in high separation efficiencies. It is not unusual to find CE methods performing at an order of magnitude better resolution than the best analysis possible with HPLC.

CE possesses many other advantages. Typically each CE run consumes as little as 2 nL sample, thus a 5 μL sample can last several repetitive runs. Most CE analyses are fast consuming less than 10 min and with a capillary regeneration time of less than 3 min, CE analyses are typically 5-fold faster than HPLC. Although a number of different types of capillaries have been generated for specific applications, most CE methods rely on uncoated fused silica capillary that can analyze a wide range of molecules, thus is relatively inexpensive.

A major disadvantage of CE is its inability to handle large amounts of analytes, thus limiting preparative applications. This limitation is being resolved through automation and novel variations in capillary design.[26]

Capillary electrophoretic methods

A number of specialized modes, which fundamentally differ from each other in the bases of resolution, are available.[28] The commonest modes include capillary zone electrophoresis (CZE), micellar electrokinetic capillary chromatography (MEKC), capillary isoelectric focussing (CIEF),[29,30] capillary gel electrophoresis (CGE)[31,32] and capillary isotachophoresis (CITP).[33]

Capillary zone electrophoresis (CZE), also called as free solution CE, is the simplest and most universally applied technique. This technique involves the resolution of charged analytes through the direct application of a high voltage across an uncoated quartz capillary filled with a buffer at desired pH (Fig. 1). The ionic species in the analyte typically migrate as a zone due to the absence (or negligible) of both thermal diffusion and interaction with the capillary wall. The mobilities of the species are dependent on the nature and strength of resident charge. Under "normal" polarity (injection at the anode (+), detection at the cathode (-)), positively charged molecules migrate fastest, followed by neutral and negatively charged species (Fig. 2A).

The reason why all species move past the detector at the cathodic end is because of the operation of electroosmotic force (EOF). Under normal polarity and alkaline pH conditions, the silanol groups of the quartz wall ionize (SiO^-) attracting cationic species of the buffer. The positively charged mono- or bimolecular layer remains essentially

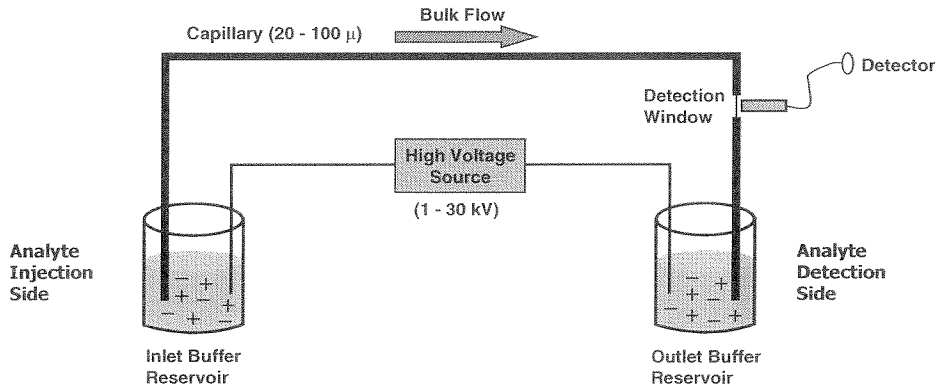


Figure 1. Schematic diagram of a typical CE instrument. The inlet and outlet buffers could be applied either positive or negative potentials each. Normal polarity has the inlet as anode (positive potential) and the outlet as cathode (negative), while the opposite is true for reverse polarity.

static, while the remaining cations move toward the cathode dragging along their shell of hydration. This phenomenon is felt by the bulk of solution resulting in a unidirectional pumping force called the EOF. As electrophoretic migration occurs, all species are eventually pushed toward the cathode. As can be predicted EOF is pH-dependent. Thus, whereas one buffer may work very well for analyses, another may completely fail. In addition, experimental variations can be large if EOF varies widely. A "relative" migration time can be calculated using a neutral marker, such as DMF or DMSO, which quantifies EOF under the experimental conditions. Changing to reverse polarity (injection at cathode, detection at anode) may eliminate or greatly reduce the EOF. Whereas reverse polarity has been exquisitely utilized for certain molecules, possibilities exist for zone broadening or wall effect under zero EOF conditions.

Micellar Electrokinetic Capillary Chromatography (MEKC) is utilized for separation of neutral compounds because under CE conditions these molecules have no net electrophoretic mobility and co-elute with the boundary of EOF. Terabe[34] introduced electrokinetic chromatography in which charged species that can interact with neutral molecules is added to the separation buffer. Resolution may occur if the charged specie differentially interacts with analyte molecules (Fig. 2B).

Micelles were the earliest charged species explored and hence the name MEKC. The resolution depends on two factors – electrophoretic mobility of the micelle in presence of the analyte and the partition co-efficient of the analyte, which intrinsically depends on its ionic and non-ionic interactions. The exquisite power of MEKC for resolving all types of molecules has led to its widespread use for both charged and uncharged analytes.

Capillary Isoelectric Focusing (CIEF) is almost exclusively used for analyses of polypeptides. In this technique, a number of ampholytes are used in open tubular capillary to form discrete stable pH zones that simulate a pH gradient (Fig. 2C), the ends of which are immersed in solutions of extreme pH values, such as 20 mM phosphoric acid and 20 mM sodium hydroxide. On electrophoresis and at steady state the analyte

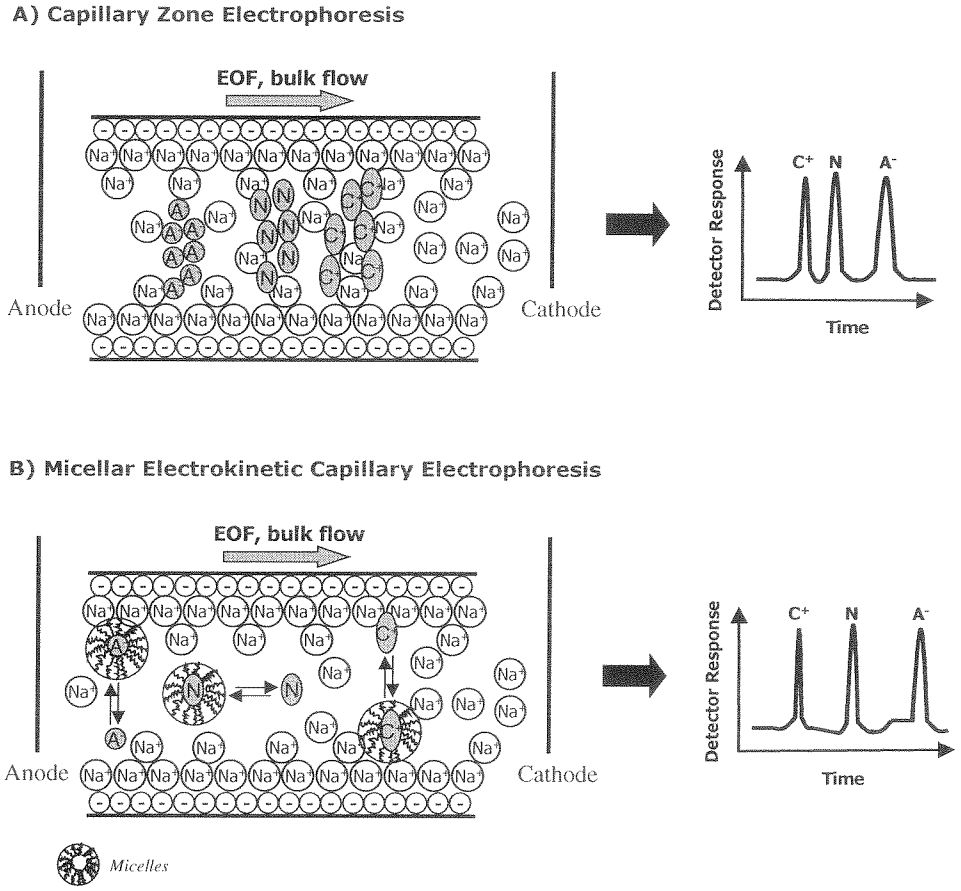


Figure 2. Two most often utilized techniques of CE. A) Capillary zone electrophoresis in which individual species, cations, neutral, and anions (C+, N, A-, respectively), migrate as zones. Shown is a normal polarity format. B) Micellar electrokinetic capillary electrophoresis in which micelles, e.g. SDS micelles, through their interaction (shown as partitioning equilibrium) affect the mobilities of analytes.

protein migrates to the zone in which the net charge on the molecule is zero. This is dependent on its isoelectric point, pI. Thus, a mixture of proteins is resolved according to the pI values resulting in separation. In a two-step technique, the system at equilibrium is dragged past the online window for detection. In a one-step technique, the focusing is performed while the zones are being dragged past the detection window.

Capillary gel electrophoresis (CGE) is a powerful technique for rapid sequencing of DNA and follows the principles of traditional slab gel electrophoresis, except for an open tubular capillary format. In this technique, a 20 to 100 μm inner diameter capillary is filled with polyacrylamide and the resolution of DNA bases is dependent primarily on the mass of the charged species. The first high-speed CGE DNA sequencing reported in 1990 was nearly 25-times faster than conventional slab gels.[35]

Sulfated molecules

Nature abounds in sulfated molecules. A vast number of structurally complex glycosaminoglycans (GAGs) exist in nature, for which presence of sulfate ($-OSO_3^-$) groups is intrinsic for origin of biological activity. Sulfated molecules are also obtained through natural metabolic pathways, as sulfation is an important phase II conjugation process. Finally, it is expected that synthetic sulfated molecules will gain prominence as agents that can regulate natural processes.

Sulfated glycosaminoglycans

Glycosaminoglycans are linear polysaccharides obtained from proteoglycans[36] and are composed of repeating disaccharide units. These disaccharide units are formed from a hexosamine (D-2-amino-2-deoxy-glucopyranose (D-GlcNp) or D-2-amino-2-deoxy-galactopyranose (D-GalNp)) residue and, a uronic acid (D-pyranosylglucuronic (D-GlcAp) or L-pyranosyliduronic acid (L-IdoAp)) residue or a neutral hexose (D-galactopyranose (D-Galp)) residue. Each of the monosaccharides may be sulfated at available 2-, 3-, 4-, and 6-positions giving rise to polyanionic polymer. The saccharide composition of a GAG defines its type. Natural GAGs are grouped into four distinct classes that include the heparin and heparan sulfate class, the chondroitin and dermatan sulfate class, the keratan sulfate class and the hyaluronan class.

Heparin and heparan sulfate

Heparin and heparan sulfate form one class of GAG and represent the two most studied molecules.[37-39] The base disaccharide structure of these GAGs contain D-GlcNp residues linked to either D-GlcAp or L-IdoAp residues in a 1→4 manner. The D-GlcNp residue may be sulfated (2-$NHSO_3^-$) or acetylated (2-$NHCOCH_3$) at the 2-position and may be sulfated at the 3- or 6-positions. In principle, the D-GlcAp and L-IdoAp residues may be sulfated at the 2- and 3-positions, however only 2-OSO_3^- derivatized iduronic acid residues are present in reasonable amounts. These structural variations in individual monosaccharides give rise to 24 disaccharide sequences in heparin and heparan sulfate chains.

A linear combination of disaccharide units, in the range of 5–40, in virtually any sequence order constitutes a heparin chain (Fig. 3A). The average length of a so-called full-length heparin chain is about 25 disaccharide units corresponding to a molecular mass of ~15,000 Da. Heparan sulfate chains are typically longer than heparin. In the past decade, low-molecular-weight heparins (LMWH) that have a chain length of ~5–15 disaccharide units have been introduced.[40] LMW heparins are obtained through chemical or enzymatic treatment of full-length heparin and hence may contain non-natural structures. These unnatural structures introduce additional structural heterogeneity in the preparations.

The ratio of L-IdoAp residues to D-GlcAp residues is about 9:1 in heparin, while it is quite variable in heparan sulfate and can be in the range of 1-4:9-6. Heparin chains have greater sulfation levels; nearly 2.4–2.7 $-OSO_3^-$ groups per disaccharide unit. Together with the presence of a carboxylate moiety in each disaccharide unit, the high sulfate content make heparin the most acidic molecule in our body. Heparan sulfate chains are sulfated to much lower extent, however may have regions of high negative charge density that is equivalent to heparin in structure and sequence. The major disaccharide sequence in heparin is →4)-β-D-GlcNp2S,6S-(1→4)-α-L-IdoAp2S-(1→ (Fig. 3B), while it is →4)-β-D-GlcNp2Ac-(1→4)-α-L-GlcAp-(1→ (Fig. 3C) in heparan sulfate.

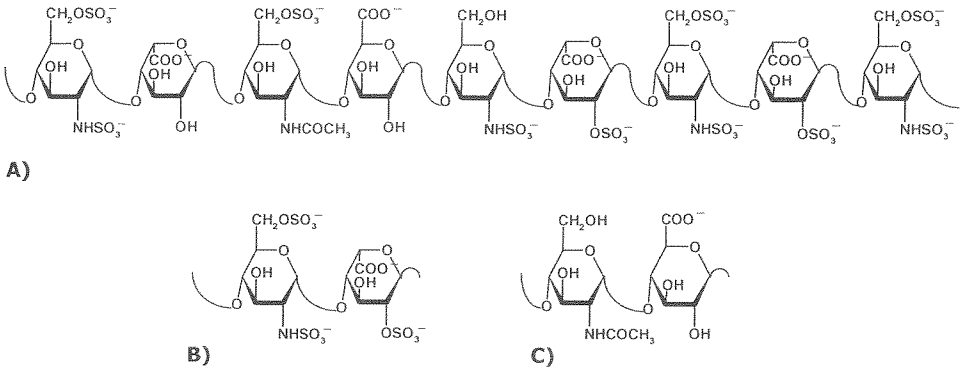


Figure 3. Structure and structural variability in heparin. A) A prototypic polysulfated heparin or low-molecular weight heparin chain. B) The major disaccharide sequence in heparin that constitutes nearly 80-90% of heparin mass. C) The major disaccharide sequence in heparan sulfate, which is devoid of sulfate moieties.

The biological activities of heparin and heparan sulfate originate from their unique structure and highly anionic character that facilitate interaction with numerous proteins.[39,41] Heparin is most well-known for its anticoagulant activity and has been clinically used for thrombotic disorders, especially deep vein thrombosis. Heparin is biosynthesized as a proteoglycan and stored in mast cells granules. Heparin becomes available to the vasculature following mast cell degranulation. Heparan sulfate, on the other hand, is an essential component of cell membranes and its primary structure is regulated in a tissue specific manner.[42,43] These heparan sulfate chains have been shown to play a dominant role in viral invasion[44-46] and angiogenesis.[47,48] In addition, heparan sulfate proteoglycans are suggested to play an important role in cell adhesion, regulation of cellular growth and differentiation, inhibition of blood coagulation, inhibition of complement activation, cell surface binding of lipoprotein lipase, and tumor metastasis.[49,50] Because glycosaminoglycan heparin and proteoglycan heparan sulfate are structurally related, heparin (and LMW heparins) may be expected to regulate many of these interactions.[51]

CE of Heparins—Capillary electrophoresis of heparin, low-molecular-weight heparins, heparin oligosaccharides and heparin disaccharides has been extensively investigated. A whole gamut of CE techniques has been explored to gather structural information on heparin samples, including CZE and MEKC, with and without chemical derivatization of samples and capillaries. Further in countless studies, CE has been used as a major technique for assessing the purity of samples obtained from heterogeneous preparations. CE has also been explored to investigate the binding properties of heparin and heparin derivatives with proteins.

Polymeric natural and synthetic heparins have been assayed by CE techniques in low pH buffer using both the normal length[52] and short-end injection configuration.[53] Typically, the main disadvantage of these polymeric species is the wide peak generally observed. The short end injection configuration enhanced efficiency, reduced analysis time and improved reproducibility. The analysis was highly sensitive to the pH of the

buffer, but less so to the ionic strength. In an alternative approach, Toida and Linhardt have analyzed these polymers as copper complexes in an acidic buffer by reversed polarity,[54] while Stefansson and Novotny have used cationic compounds to aid resolution.[55] Since the introduction of CE analyses using reverse polarity,[56] the trend has been to perform separations in either phosphate or formic acid buffers with pH in the range of 3 to 4.[57,58] Under these acidic conditions, the EOF is nearly eliminated and resolution is a direct function of the negative charge density and the structure of the analytes.

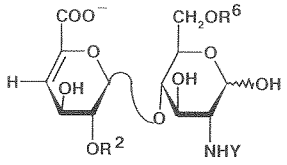
The applicability of CE to compositional analysis of heparin fragments has been most fruitful. Three major tools have contributed to the overall success – the enzymatic depolymerization of heparin with heparin lyases,[59] the biophysical structural elucidation of individual oligosaccharide isolate[60] and the resolving power of CE. Enzymatic depolymerization of intact heparin can be used to fingerprint the sample.[56,61] In a comparative study of heparin oligosaccharides, Pervin et al.[59] noted that normal polarity mode at pH 8.8 was better than reverse polarity mode at pH 3.5, especially as the size of the heparin specie increases. Recently, direct coupling of CE with ionspray mass spectrometry (IS-MS) has been optimized to enable identification of heparin oligosaccharides.[62] It is expected that this and similar techniques will greatly enhance the ability to sequence intact heparin.[63]

The use of heparin lyases introduces a chromophore, $\Delta^{(4,5)}$-double bond, useful for oligosaccharide detection, but destroys information pertaining to the uronic acid residue at the non-reducing end. To facilitate detection of an unmodified heparin specie, indirect UV detection was developed using either 5-sulfosalicyclate – or 1,2,4-tricarboxy-benzoate – based buffers under acidic conditions.[64] For heparin pentasaccharides, the CE-indirect UV detection method was found to be 10-fold more sensitive than the direct detection using $\Delta^{(4,5)}$- chromophore.

A number of CE methods are available to heparin disaccharides since the seminal reports by Ampofo et al.[65] using MEKC conditions in sodium borate buffer, pH 8.5, containing 50 mM SDS. This method relied on normal polarity mode to resolve 8 disaccharides. Scapol et al.[66] studied the resolution using triethylamine and acetonitrile as additives. Triethylamine was found to influence the migration time by altering both the electrophoretic mobility and the electroosmotic flow. Reversed polarity mode at low pH has been found to exhibit better resolution of disaccharides.[56,60,67-69] Finally, a combination of reverse polarity and pressure gradient in formic acid buffer has aided disaccharide compositional analysis of heparin.[57]

Disaccharide compositional analysis utilizing the reactivity of reducing end terminus has been explored. Several methods have been devised each principally differing in the reducing end label used for detection. Kitagawa et al.[70] have used the fluorophore 2-aminoacridone in remarkable separation was achieved between positional isomers. This method has been greatly improved through the use of laser-induced fluorescence (LIF).[71,72] All twelve non-, mono, di-, and trisulfated D-disaccharides were completely resolved in a single run, using 50 mM sodium phosphate buffer, pH 3.5 and reverse polarity at 30 kV (Fig. 4).[72] These disaccharides could be detected nearly 27–744-times better through LIF. Finally, two methods have also been developed for heparin monosaccharide compositional analysis. Both methods are based on LIF using fluorescein[73] or 8-aminopyrene-1,3,6-trisulfonate[74] (APTS) fluorophores. Where fluorescein was added to the background electrolyte, the monosaccharides were derivatized at the reducing end with APTS.

A)



No.	Formula	R^2	R^6	Y
1.	ΔUAp-(1→4)-GlcNp2Ac	H	H	Ac
2.	ΔUAp-(1→4)-GlcNp	H	H	H
3.	ΔUAp-(1→4)-GlcNp2Ac,6S	H	SO_3^-	Ac
4.	ΔUAp-(1→4)-GlcNp6S	H	SO_3^-	H
5.	ΔUAp2S-(1→4)-GlcNp2Ac	SO_3^-	H	Ac
6.	ΔUAp2S-(1→4)-GlcNp	SO_3^-	H	H
7.	ΔUAp-(1→4)-GlcNp2S	H	H	SO_3^-
8.	ΔUAp2S-(1→4)-GlcNp2Ac,6S	SO_3^-	SO_3^-	Ac
9.	ΔUAp2S-(1→4)-GlcNp6S	SO_3^-	SO_3^-	H
10.	ΔUAp2S-(1→4)-GlcNp2S	SO_3^-	H	SO_3^-
11.	ΔUAp-(1→4)-GlcNp2S,6S	H	SO_3^-	SO_3^-
12.	ΔUAp2S-(1→4)-GlcNp2S,6S	SO_3^-	SO_3^-	SO_3^-

B)

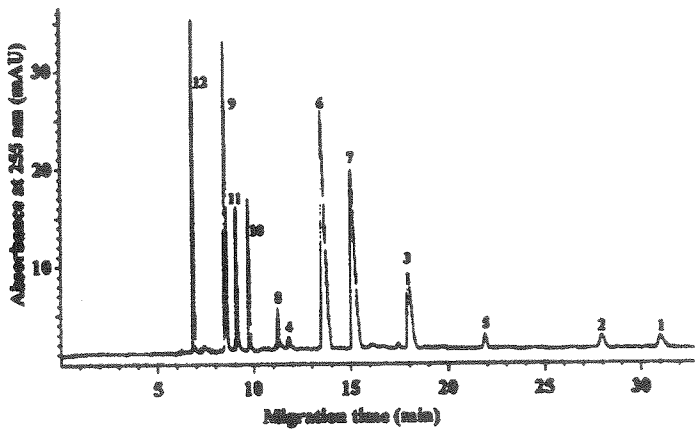


Figure 4. CE of heparin disaccharides. A) Chemical structures of twelve heparin/heparan sulfate disaccharides. B) Electrophoretic profile of the above 12 disaccharides obtained by Militsopoulou et al.[72] at 25 ^{O}C in 50 mM sodium phosphate buffer, pH 3.5, at -30 kV. [Reproduced with permission from Wiley-VCH Verlag GmbH.]

Chondroitin and dermatan sulfate

Chondroitin and dermatan sulfate (CS and DS) form the second most studied glycosaminoglcans, and differ from heparin and heparan sulfate in being constituted of galactoamino sugars instead of glucosamino residues. Both chondroitin and dermatan sulfate are biosynthesized as structurally complex, highly sulfated, polydisperse, micro-heterogeneous linear polysaccharides.[75-77] Chondroiti0n sulfate proteoglycans and

glycosaminoglycans are involved in a wide variety of biological processes including cellular proliferation, differentiation, and wound healing.[75,77,78] These are cell surface molecules and are designated as "ground substances" controlling filtration through basement membranes and binding of growth factors and protease inhibitors.[79]

Three chondroitin sulfates, labeled as A, B, and C,[78,80] are known to occur in nature more often, while chondroitin sulfates D and E[81-84] can be found in smaller proportions. The core disaccharide unit of CS contains galactosamine (GalNp) residue linked to a uronic acid residue in 1→4 manner. The GalNp residue is almost exclusively N-acetylated, in contrast to GlcNp residues in heparin/heparan sulfate which are mostly N-sulfated. In addition, whereas the interglycosidic linkage in heparin/heparan sulfate is 1→4 throughout the polymeric chain, in CS polymer, the linkage is 1→4 between GalNp and uronic acid residues, and is 1→3 between uronic acid and GalNp residues.

Chondroitin sulfates A and C consist of glucuronic acid residue, while chondroitin sulfate B, better known as dermatan sulfate, contains both glucuronic and iduronic acid residues. The proportion of iduronic acid residues in DS may range from 1 to 90% of the total uronic acid residues.[85] CS containing 4-sulfated galactosamine residues is referred to as chondroitin sulfate A, while that containing 6-sulfated GalNp residues is called condroitin sulfate C. Thus, the major disaccharide repeating units of CS A, B, and C are →3)-β-D-GalNp2Ac,4S-(1→4)-β-D-GlcAp-(1→, →3)-β-D-GalNp2Ac,4/6S-(1→4)-α-L-IdoAp-(1→, and →3)-β-D-GalNp2Ac,6S-(1→4)-β-D-GlcAp-(1→, respectively (Fig. 5). Chondroitin sulfates D and E are typically more sulfated than their other counterparts and have unusual 2-*O*-sulfated and 3-*O*-sulfated glucuronic acid residues, respectively.[81-84] These fundamental backbone differences result in considerable structural and functional differences.

CE of Chondroitin and Dermatan Sulfates—The structural complexity of chondroitin sulfates, similar to heparins, makes CE a powerful analytical and sequencing tool. The polyanionic CS polysaccharides have been analyzed in an open-tube and gel-filled format. CGE of CS resolves according the size of the polysaccharides and is especially useful for deducing the molecular weight.[58] In the open-tube format, two groups have used reversed polarity under acidic conditions (pH <3.5) to analyze CS and DS.[54,86] While Toida and Linhardt have used complexation with Cu^{+2} for detection,[54] Stefansson and Novotny have utilized the fluorescence of reducing end label CBQCA at 550 nm.[86] Recently, CE in normal polarity under high pH conditions was utilized to demonstrate the polydispersity of a natural CS isolate.[87]

CE of CS disaccharides has been extensively investigated. Since the first nearly simultaneous reports on resolution of 8 CS disaccharides under normal polarity conditions in sodium borate buffer, pH 8.8, by Al-Hakim and Linhardt,[88] and Carney and Osborne,[89] a multitude of conditions have been reported by a number of groups. These include normal and reverse polarity, pre-column derivatization, used of additives, and coated capillaries. SDS has been used under normal polarity and MEKC conditions to sharpen peaks,[90] while additives including tetrabutylammonium phosphate,[91] cetyltrimetylamonium bromide,[92] and triethylamine[93] have been used to enhance resolution. Analysis at low pH and using reverse polarity yields complementary resolution of all CS disaccharides.[56,94] While the detection of disaccharides containing unsaturation in their non-reducing end is satisfactory for most purposes, pre-column derivatization with a fluorophore, especially AMAC or ANDSA, results in greater sensitivity (Fig. 6).[70,95-97] Detection of Δ-disaccharides derivatized with ANDSA by a

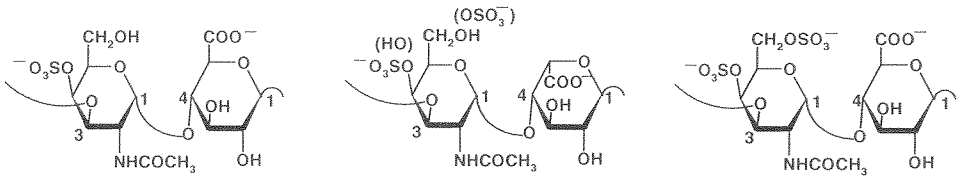
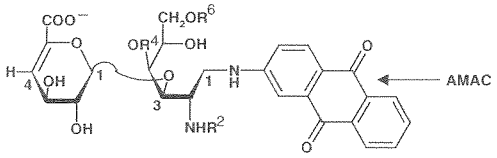


Figure 5. Structure of major disaccharide sequences of chondroitin sulfates. Positions 1, 3, and 4 are marked.

A)



No.	Formula	R^2	R^4	R^6
1.	ΔUAp-(1→3)-GlcNp2S,4S,6S-AMAC	SO_3^-	SO_3^-	SO_3^-
2.	ΔUAp-(1→3)-GlcNp2S,4S-AMAC	SO_3^-	SO_3^-	H
3.	ΔUAp-(1→3)-GlcNp2S,6S-AMAC	SO_3^-	H	SO_3^-
4.	ΔUAp-(1→3)-GlcNp4S,6S-AMAC	H	SO_3^-	SO_3^-
5.	ΔUAp-(1→3)-GlcNp2S-AMAC	SO_3^-	H	H
6.	ΔUAp-(1→3)-GlcNp6S-AMAC	H	H	SO_3^-
7.	ΔUAp-(1→3)-GlcNp4S-AMAC	H	SO_3^-	H
8.	ΔUAp-(1→3)-GlcNp-AMAC	H	H	H

B)

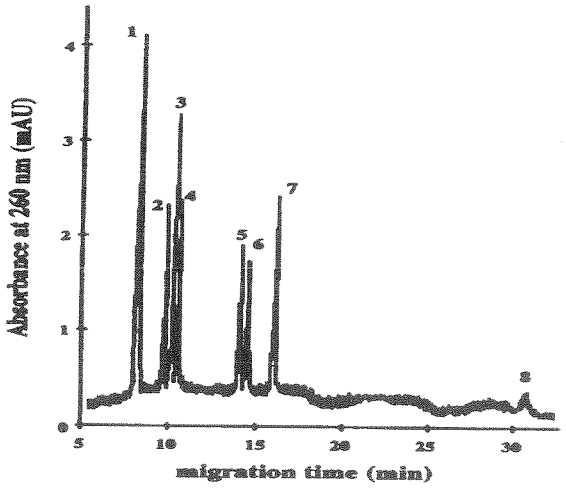


Figure 6. CE of chondroitin sulfate disaccharides. A) Chemical structures of eight disaccharides chemically derivatized through reductive amination with chromophore AMAC. B) Typical electropherogram showing the separation of all known non-, mono-, di- and tri-sulfated D-disaccharides labeled with AMAC.[97] CE was performed in 15 mM sodium phosphate buffer, pH 3.0, at –30 kV with detection at 260 nm. Numbers correspond to the structures above. [Reproduced with permission from Wiley-VCH Verlag GmbH.]

He-CD LIF detector gives three-orders of magnitude lower limit of detection than detection of underivatized Δ-disaccharides.[95] A microemulsion electrokinetic capillary chromatography technique in which neutral and ionized species can be resolved according to their partition coefficient in oil-water mixture has also been reported for complete separation of CS disaccharides.[98]

CE has been used as a powerful tool to perform compositional analysis of CS and DS polymers in normal and pathological states. Enzymatic digestion with chondroitinases combined with a rapid CE disaccharide analysis affords high-sensitivity protocol for detecting compositional changes natural CS and DS isolates from normal or infected tissues. Thus CS disaccharide compositional analysis of atherosclerosis and aneurysmal dilatation of the human abdominal aorta,[99] spontaneous osteoarthritis,[100] intervertebral disk chondrocyte,[101] uterine leiomyoma and normal myometrium,[102] normal and arthritic synovial fluid,[103] has been attempted.

Sulfated non-glycosaminoglycans

The use of CE for separation and detection of small organic molecules including pharmaceutical drugs, xenobiotics, and fine chemical intermediates, is becoming increasingly popular. Fueling this application is CE's ability to analyze all molecules, neutral, charged, hydrophobic, and polar, simultaneously with high resolving power. The introduction of a number of detector systems, including UV diode array, LIF, MS, and conductivity, have greatly aided CE based methods for quantification of drug metabolites.[104-107] Metabolic profiling is further made easy because CE tolerates the direct application of biological fluids.

Metabolic profiling

Sulfation is an important phase II conjugation reaction that modifies drugs with hydroxy and amino groups to their more water soluble sulfate conjugates. A number of sulfate metabolites, chiral and achiral, have been detected and quantified using CE techniques. In one of the first reports, a urinary excretion product, (+)-cicletanine sulfate, was found to be present in 5-fold more amount than its enantiomer using MEKC method with normal polarity in 10 mM sodium borate buffer, pH 8.6, containing 100 mM SDS, 25 mM γ-CD and 10% acetonitrile as modifiers.[108,109] Sulfated cicletanine is the major metabolite of newly found anti-hypertensive compound cicletanine.

The metabolic profile of paracetamol was identified in urine using diode-array and MS detectors. A CZE method based on 50 mM borax at high pH and 20 kV under normal polarity led to simultaneous quantification of major, sulfate and glucuronide, and minor, cysteinate, mercapturate, and 3-hydroxyl, metabolites of paracetamol.[110] This CE method is especially useful for checking patient's glucuronidation and sulfation capacity.

The consumption of herbal medicinal products is on the rise and extracts from bearberry leaves are used as disinfectants in the therapy of lower urinary tract infections. Arbutin is the principle active constituent of these leaves, which undergoes hydrolysis and releases hydroquinone. Use of CE led to the detection of hydroquinone metabolites, sulfate and glucuronide conjugates, of arbutin. Hydroquinone sulfate was detected and quantified at 30 kV in 200 mM borate buffer under high pH in normal polarity mode.[111,112]

Two novel serotonin catabolites, serotonin-*O*-sulfate and γ-Glu-serotonin-*O*-sulfate, have been identified using capillary electrophoresis coupled with EI-MS. This is the first demonstration of this new pathway for serotonin and is projected to contribute to serotonergic response.[113]

Vitamin C sulfate and phosphate are two main ascorbic acid derivatives used for supplementation of fish and shrimp feed because of their better stability compared to the free acids. Several phosphates, mono, di, and tri, derivatives are possible. An analytical CZE-based procedure that rapidly assays the presence of these variously charged species has been developed. Separation was achieved using 80 mM tricine buffer at high pH and quantification of individual components through direct injection of extracts of various tissue samples was tested.[114]

Synthetic sulfates

CE methods have been used for assaying a number of biologically relevant sulfated molecules that are organic or polymeric. Direct monitoring of enzymatic hydrolysis of sulfated organics, e.g., β-glucuronidases, was achieved using MEKC under normal polarity conditions,[115] while the synthesis of pentosan polysulfate, a mixture of multiply charged polysaccharide, was monitored under reverse polarity conditions with inverse detection.[116] It is likely that the ease of CE for analyses of these polyanionic polysulfates will extend the applicability to routine monitoring of enzymatic activity and purity testing.

Future

Sulfated molecules, synthetic and natural, are likely to become more important given the widespread distribution and functional roles of glycosaminoglycans in nature. The wide structural, compositional, and size differences in these natural products suggests that large number of synthetic sulfates will be needed to potentially fully mimic the functional roles of natural parents. In addition, sulfated metabolic products are likely to increase in number. As the review indicates, the diverse advantages of CE will enhance its applicability to analyze sulfated molecules. Technological advances, especially on the design of capillaries and detectors, are likely to make analysis of fewer than hundred molecules feasible.

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Abbreviations

CE, capillary electrophoresis; HPLC, high performance liquid chromatography, CZE, capillary zone electrophoresis; MEKC, micellar electrokinetic capillary chromatography; CIEF, capillary isoelectric focussing; CGE, capillary gel electrophoresis; CITP, capillary isotachophoresis; EOF, electroosmotic force; DMF, dimethyl formamide; DMSO, dimethyl sulfoxide; GAG, glycosaminoglycan; D-GlcNp, D-2-amino-2-deoxy-glucopyranose; D-GalNp, D-2-amino-2-deoxy-galactopyranose; D-GlcAp, D-pyranosylglucuronic; L-IdoAp, L-pyranosyliduronic acid; D-Galp, D-

galactopyranose; LMWH, low-molecular-weight heparins; LIF, laser-induced fluorescence; APTS, 8-aminopyrene-1,3,6-trisulfonate; AMAC, 2-amino-acridone; ANDSA, 7-amino-napththalene-1,3-disulfonic acid; LIF, laser-induced fluorescence; MS, mass spectrometry; EI-MS, electrospray-mass spectrometry; IS-MS, ionspray mass spectrometry; γ-CD, gamma – cyclodextrin; SDS, sodium dodecyl sulfate

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