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Selection of Anionic Dopant for Quantifying Desialylation Reactions with MALDI-FTMS

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Alkylsulfonates are examined as anion dopants for the matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) of neutral oligosaccharides. The anion dopants allow neutral oligosaccharides to be examined in the same mixture as acidic oligosaccharides. The alkylsulfonate dopants interact strongly with the oligosaccharide to produce a quasimolecular ion composed of the oligosaccharides and the deprotonated alkylsulfonates. Compounds as small as disaccharides to large branched oligosaccharides can all be examined. The new series of alkylsulfonates works as well as HSO_4^- in terms of signal-to-noise ratio, sensitivity, and ease of preparation in the negative ion mode. The alkylsulfonates are used to construct a calibration curve for obtaining the relative amounts of varying neutral and acidic oligosaccharide mixtures. With the anion dopants, the MALDI-FTMS signals are shown to have a linear relationship with the molar ratios of the neutral and sialylated oligosaccharides. The method is also used to monitor the product of a desialylation reaction by acid hydrolysis.

The classification of oligosaccharides into neutral and anionic compounds relates as much to their analyses as their biological functions. Release of oligosaccharides is typically followed by separation into neutral and anionic components. In mass spectrometry, the analyses of neutral and anionic components proceed by two routes. Neutrals are typically examined in the positive mode while anionic or acidic oligosaccharides are analyzed in the negative mode. Neutral oligosaccharides do not typically produce strong signals in the negative mode, while acidic oligosaccharides fragment readily and incorporate alkali metal ions and other cations that distribute the intensity of the quasimolecular ion in the positive mode.

Sialic acids make up a group of acidic saccharides that contain a carboxylic acid group.[1−3] These compounds are typically found in the nonreducing end of oligosaccharides in higher animals from echinoderms up to humans. The biological functions of sialic acids are highly diverse and sometimes diametrical ranging from cell−cell recognition to the masking of cells or molecules. Many pathogenic agents including toxins, viruses, and bacteria bind host cells via sialic acid-containing receptors.[4] On the other hand, erythrocytes, i.e., red blood cells, are covered by a dense layer of sialic acids. Sialic acids are removed stepwise from the surface of the cells by the action of serum sialidase and by spontaneous chemical hydrolysis. When all the sialic acids are removed, this represents a signal for the degradation of the respective blood cell and it is removed.[5]

When a monosialylated oligosaccharide is desialylated, the molecule becomes neutral. Therefore, there is a great need for the simultaneous analysis of both neutral and acidic oligosaccharides by mass spectrometry. We recently introduced HSO_4^- as an anion dopant[6] for observing neutral oligosaccharides in the negative mode with matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS).[7,8] Observing neutrals in the negative mode has one major advantage over the positive mode. Fragmentation is minimized in the negative mode, allowing the determination of components in complex mixtures. We found that compared to alkali metal-coordinated species in the positive mode, the loss of labile residues is reduced. In the positive mode, glycosidic bond cleavage is believed to be charge induced and is facilitated by small alkali metal ions. Thus, the fragment yield of Li^+-coordinated species is significantly greater than Na^+- or K^+-coordinated species.[9,10]

In the negative mode, glycosidic bond cleavages are likely to be charge-remote processes. These fragmentation reactions generally have higher activation barriers than charge-site processes. Because the sensitivity depends on the formation of the quasimolecular ion rather than the fragments, better sensitivity may be obtained in the negative mode rather than the positive mode, albeit direct comparisons have not been reported.

Although HSO_4^- has proven to be a suitable anion dopant, it has some limitations. It does not bind strongly with small oligosaccharides such as disaccharides. Control of the laser irradiance was also found to be important as a higher laser irradiance produced derivatization of the sugar by effectively adding SO_3^-. To find a more effective and more general anion dopant, we have began to explore the use of alkylsulfonates. In this report, we present alkylsulfonates as anion dopants. We

(1) Varki, A. *Glycobiology* **1992**, *2*, 25−40.
(2) Traving, C.; Schauer, R. *Cell. Mol. Life Sci.* **1998**, *54*, 1330−49.
(3) Schauer, R.; Kelm, S.; Reuter, G.; Roggentin, P.; Shaw, L. In *Biology of Sialic Acids*; Rosenberg, A., Ed.; Plenum Press: New York, 1995; pp 7−67.
(4) Schauer, R.; Kamerling, J. P. In *Glycoproteins II*; Montreuil, J., Vliegenthart, J. F. G., Schachter, H., Eds.; Elsevier: Amsterdam, 1997; pp 241−400.
(5) Bratosin, D.; Mazurier, J.; Debray, H.; Lecocq, M.; Boilly, B.; Alonso, C.; Moisei, M.; Motas, C.; Montreuil, J. *Glycoconjugate J.* **1995**, *12*, 258−67.
(6) Wong, A. W.; Cancilla, M. T.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 205−11.
(7) Karas, M.; Hillenkamp, F. *Anal. Chem.* **1988**, *60*, 2299−301.
(8) Stahl, B.; Steup, M.; Karas, M.; Hillenkamp, F. *Anal. Chem.* **1991**, *63*, 1463−6.
(9) Cancilla, M. T.; Wong, A. W.; Voss, L. R.; Lebrilla, C. B. *Anal. Chem.* **1999**, *71*, 3206−18.
(10) Ngoka, L. C.; Gal, J. F.; Lebrilla, C. B. *Anal. Chem.* **1994**, *66*, 692−8.

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investigate the relative strength of interaction of the individual candidates. The suitable candidate is then used to construct a calibration curve to obtain quantitative information on variable concentrations of neutral and monosialylated oligosaccharides. The utility of the dopant is illustrated by monitoring the desialylation reaction of an acidic oligosaccharide.

EXPERIMENTAL SECTION

Chemicals. Monosialylated, galactosylated biantennary N-linked oligosaccharide (A1), asialo, galactosylated biantennary N-linked oligosaccharide (NA2), asialo, galactosylated tetraantennary N-linked oligosaccharide (NA4), LS-tetrasaccharide a (LSTa), lacto-*N*-tetraose (LNT), lacto-*N*-difucohexaoses I and II (LNDFH-I and -II), and 2- and 3-fucosyllactose (2- and 3-FL) were obtained from Oxford Glycosystems (Rosedale, NY). All maltose-based oligomers were obtained from Sigma Company (St. Louis, MO). All compounds were obtained in the highest purity. No further purification was performed.

Sulfanilic acid, 4-hydroxybenzenesulfonic acid, *p*-toluenesulfonic acid, benzenesulfonic acid, 4-chlorobenzenesulfonic acid, 4-sulfobenzoic acid (potassium salt), 3-nitrobenzenesulfonic acid (sodium salt), sulfosuccinic acid, 3-hydroxy-1-propanesulfonic acid (sodium salt), 1-propanesulfonic acid (sodium salt monohydrate) and 2,5-dihydroxyacetophenone (DHAP) were purchased from Aldrich Chemical Co. (Milwaukee, WI). Sulfuric acid was obtained from Fisher Scientific (Fair Lawn, NJ). All materials were used without further purification.

Analyte Preparations. Unless indicated, all neutral and acidic oligosaccharides were dissolved in water at a concentration of 1 mg/mL. The sulfonated anion dopants were prepared at concentrations of 1×10^{-3} M in water. DHAP was dissolved in ethanol until saturated.

A 1-μL aliquot of analyte solution was applied to the probe tip, followed by 1 μL of sulfonated anion dopant, and then 2 μL of matrix (DHAP). The mixture was dried in the vacuum manifold at the inlet of the ionization source.

MALDI-FTMS. All the experiments were performed on an external source FTMS instrument HiResMALDI (IonSpec Corp., Irvine, CA) equipped with a 4.7-T magnet[9,11] in the negative ion mode. Three-stages of differential pumping keeps the ion cyclotron resonance (ICR) cell at a pressure of roughly 10^{-11} Torr even when a sample probe is placed in the source. MALDI is performed using an LSI 337-nm nitrogen laser which comes standard with the instrument. When the laser is fired, the MALDI-produced ions are transported to the ion cyclotron resonance cell using radio frequency (rf)-only quadrupole rods. The rf-only quadrupole rods are activated to focus the ions through the fringing field of the magnet. The ions are trapped in an elongated ICR cell ($2 \times 2 \times 4$ in.) located in the homogeneous region of the superconducting magnet. A 2-ms pulse of argon gas is used to translationally cool the trapped ions before the ions are excited by a radio frequency sweep and detected as a transient signal.

Collision-Induced Dissociation (CID). CID experiments were performed using the method of sustained off-resonance irradiation (SORI).[12] The molecular ion was isolated by a series of both rf sweeps and bursts generated by an arbitrary waveform generator (IonSpec Corp.). The molecular ion was excited by a rf pulse for 1000 ms at 1000 Hz above the cyclotron frequency of the molecule. Four argon pulses (2 ms each) in 250-ms intervals were applied to maintain a pressure of $\sim 5 \times 10^{-6}$ Torr during the CID event.

RESULTS AND DISCUSSION

Optimization of Ionization Conditions. To obtain the best signals for the mass spectra, several physical and chemical factors (concentration, laser irradiance. choice of matrix) were studied to determine the optimal ionization conditions.

Concentration. To observe the effects of concentration on the quality of the spectra, maltohexaose (1 mg/mL) was doped with 0.05, 0.01, 0.005, and 0.001 M 4-hydroxybenzenesulfonic acid in the presence of DHAP[13] prior to MALDI. As with HSO_4^-, the ratio of the alkylsulfonate to oligosaccharide is the crucial parameter for obtaining good spectra. The ratio [acid]/[oligosaccharide] in the range between 1:1 and 50:1 provided good signal, but the best spectra were obtained when the concentrations of acid and sugar were about the same. Only the quasimolecular anion corresponding to the adduct $[M + RSO_3]^-$ is formed with 4-hydroxybenzenesulfonic acid and other alkylsulfonates.

Laser Irradiance. The degree of fragmentation in the MALDI spectra was examined using maltohexaose doped with 0.001 M 4-hydroxybenzenesulfonic acid at various laser irradiances. No change was observed in the overall appearance of the spectra when the focal length of the lens was moved over a distance of 6 mm, which corresponds to almost a 100% increase in laser irradiance (spectra not shown). This illustrates that the adduct species $[M + OHC_6H_4SO_3]^-$ is very stable and fragmentation cannot be easily induced by the laser.

Choice of Matrix. We have had good experience with both DHAP and 1-methyl-9-*H*-pyrido[3,4-*b*]indole (harmane)[14] for negative ion analysis. In a previous paper, we noted that harmane was generally superior to DHAP for the sulfate. For the alkylsufonilic acids, both harmane and DHAP produce strong signals; however, DHAP has several practical advantages. First, DHAP is able to form a very uniform crystalline layer on the probe tip and it is almost unnecessary to search for a good spot. Second, the time of the crystal formation with DHAP in the vacuum manifold is much shorter compared to drying in ambient conditions with harmane. Third, DHAP is less sensitive to pH changes than harmane.

Analysis of Neutral Oligosaccharides. LNDFH-1, a branched hexasaccharide, was doped with all alkylsulfonic acids listed in the Experimental Section to determine the efficacy of the sulfonic acids. Figure 1 shows representative mass spectra of 1 mg/mL LNDFH-1 doped with 1 mM of selected sulfonic acids including sulfanilic acid, 4-hydroxybenzenesulfonic acid, *p*-toluenesulfonic acid, benzenesulfonic acid, chlorobenzenesulfonic acid, and sulfobenzoic acid. The quasimolecular ions $[M + RSO_3]^-$ were obtained as the base peak with the presence of a small amount of anion dopant $[RSO_3]^-$ (m/z 172.99). In some cases, a small amount of hydrogen sulfate adduct $[M + HSO_4]^-$ was observed. In general, the coordinated complexes or the quasimolecular ions

(11) Cancilla, M. T.; Penn, S. G.; Lebrilla, C. B. *Anal. Chem.* **1998**, *70*, 663−72.
(12) Gauthier, J. W.; Trautman, T. R.; Jacobson, D. B. *Anal. Chim. Acta* **1991**, *246*, 211−25.
(13) Krause, J.; Stoeckli, M.; Schlunegger, U. P. *Rapid Commun. Mass Spectrom.* **1996**, *10*, 1927−33.
(14) Nonami, H.; Fukui, S.; Erra-Balsells, R. *J. Mass Spectrom.* **1997**, *32*, 287−96.

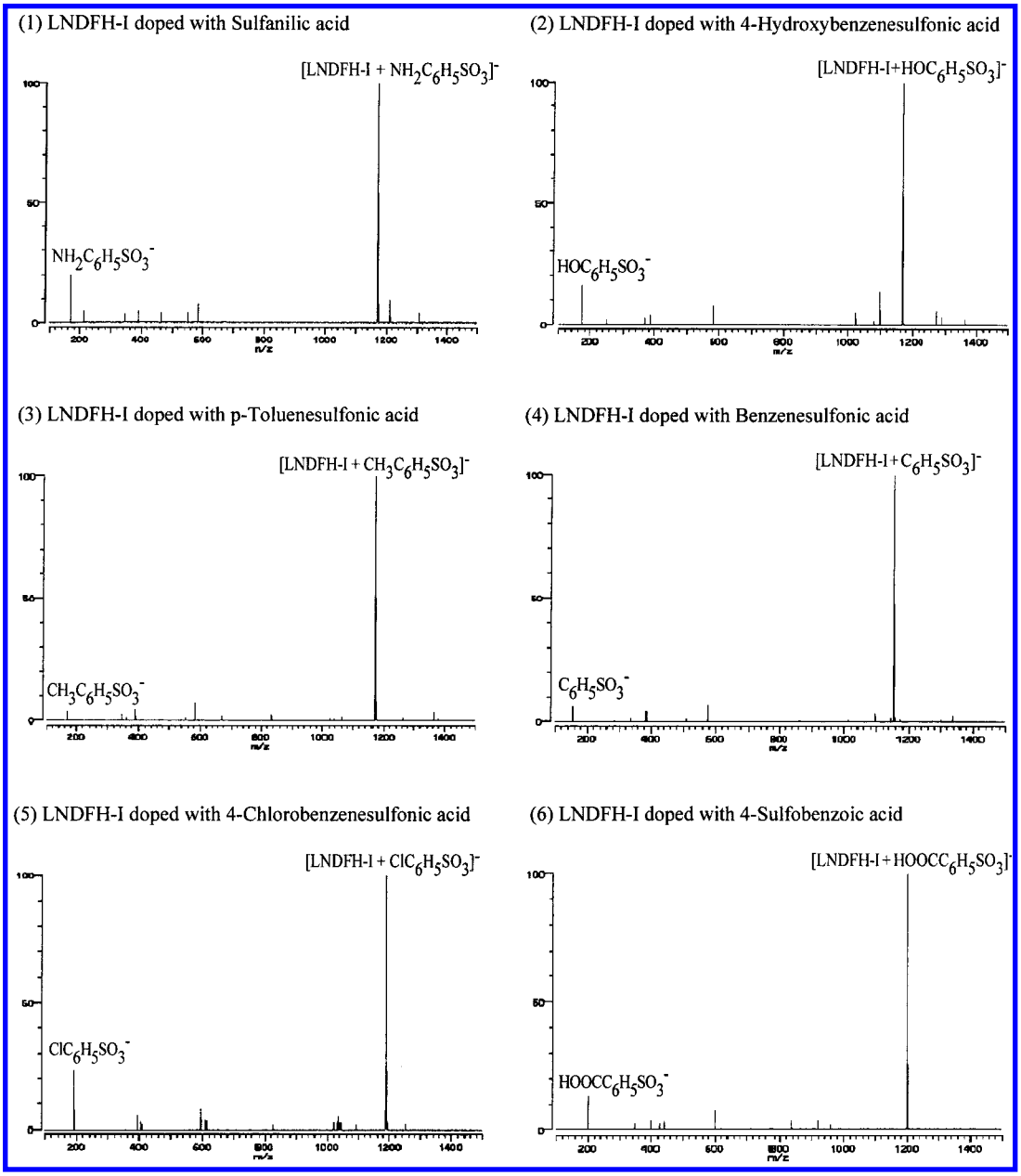


Figure 1. Negative ion MALDI-FTMS spectrum of LNDFH-I doped with 1 mM each of several alkylsulfonic acid dopants. The base peak is the quasimolecular ion corresponding to [M + RSO_3^-]. The free dopant is also observed at the lower *m*/*z* ratios.

were the base peak in all the spectra and fragmentation was found to be minimal or nonexistent. Similar experiments were performed with maltohexaose, a linear hexasaccharides (See Chart 1 for structures), to yield the same results.

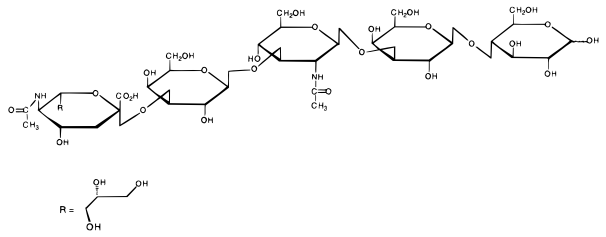
To illustrate that neither the size nor the type of oligosaccharide affects the sensitivity as it did for the hydrogen sulfate adduct,[6] a total of 15 neutral and acidic oligosaccharides were examined with sulfanilic acid to study the effect of the anion dopant on various sizes of oligosaccharides (Table 1). The quasimolecular ion of all the neutrals, except the monosaccharides, was observed corresponding to the species [M + $H_2NC_6H_4SO_3$]$^-$, where M is the mass of the oligosaccharide. Adducted species of anionic oligosaccharides such as LSTa and A1 (Chart 2) were not observed.

Binding Affinities of Sulfonated Dopants. The strength of the interaction between the oligosaccharide and the sulfonate determines the sensitivity of the method. Strong interactions allow the complex to survive the MALDI process and the long time scale of the FTMS technique. Several sulfonated dopants (benzene-containing and straight-chain sulfonic acids) were examined with lacto-*N*-difucohexaose I, a neutral hexasaccharide isolated from human milk. LNDFH-I was a good representative of a neutral oligosaccharide because it contained fucose, hexose, and *N*-acetylhexose and some degree of branching. To investigate the binding affinities of the sulfonated dopants to LNDFH-I, the dissociation threshold of the loss of the anion dopant was studied. By applying the empirical method derived from Armentrout's method,[15,16] CID was used to determine the multicollision dis-

(15) Armentrout, P. B. In *Advances in Gas-Phase Ion Chemistry*; Adams, N., Babcock, L. M., Eds.; JAI Press Inc: Greenwich, CT, 1992; Vol. 1; p 83.
(16) Dalleska, N. F.; Honma, K.; Armentrout, P. B. *J. Am. Chem. Soc.* **1993**, *115*, 12125−31.

Chart 1. Structures of LS-tetrasaccharide a (a) and Lacto-*N*-difucohexaose (b)

(a) LS-tetrasaccharide a (LST a)



(b) Lacto-N-difucohexaose I (LNDFH-I)

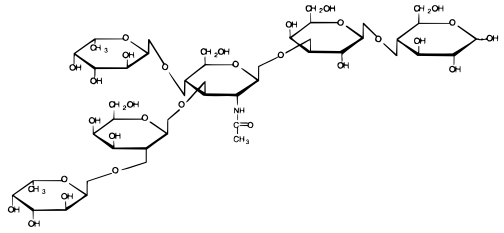


Table 1. *m*/*z* of the Adduct $[M + H_2NC_6H_4SO_3]^-$ of Different Oligosaccharides with Sulfanilic Acid as the Anion Dopant

oligosaccharides	no. of residues	neutral (N)/ acidic (A)	adduct formed (*m*/*z*)
glucose/fucose	1	N	NO[a]
maltose	2	N	514.12
maltotriose	3	N	676.17
2-fucosyllactose	3	N	660.18
3-fucosyllactose	3	N	660.18
lacto-*N*-tetraose	4	N	879.25
maltotetraose	4	N	838.23
maltopentaose	5	N	1000.27
maltohexaose	6	N	1162.38
LNDFH−I	6	N	1171.34
β-cyclodextrin	7	N	1306.40
NA2	9	N	1812.63
NA4	13	N	2543.99
LSTa	5	A	NO
A1	10	A	NO

[a] NO, not observed

sociation thresholds (MCDT). A detailed description of this method was provided in a recent publication.[9] The MCDT values allowed direct comparison of relative binding strengths. A plot of the rate constant versus the center-of-mass energy at infinite collisions ($E_{cm\infty}$) yielded the MCDT value. The method allowed the extrapolation to threshold thereby overcoming the dynamic range problem of FTMS. An example of a representative plot is provided for LNDFH-I complexed to 4-hydroxybenzenesulfonic acid (Figure 2).

Tables 2 and 3 list the MCDT values for the dissociation of the anion dopant from LNDFH-I for a series of benzene-containing and straight-chain sulfonic acids. For all the entries in which E_{com} were obtained, the threshold plots appeared similar to Figure 2. In situations in which the quasimolecular ion peak was too low, no values were obtained. Also listed in Table 2 are the Hammet−Taft parameters (σ_{para}), a relative measure of the electron-donating ability of the substituent. Because of the nature of the determi-

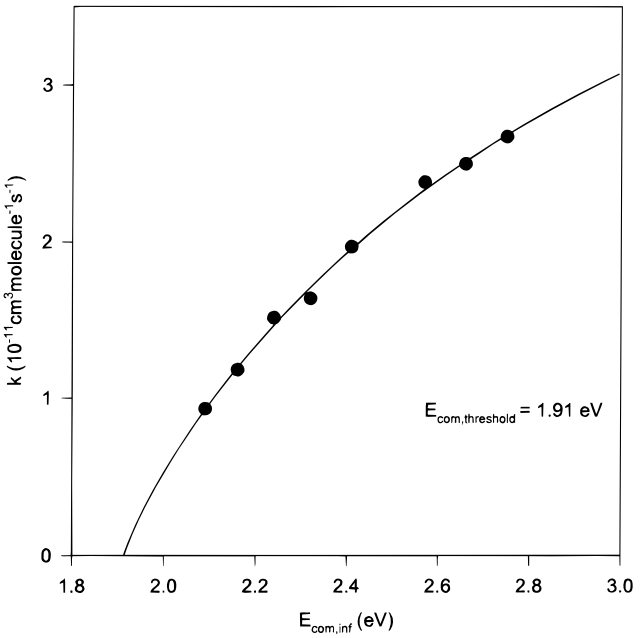
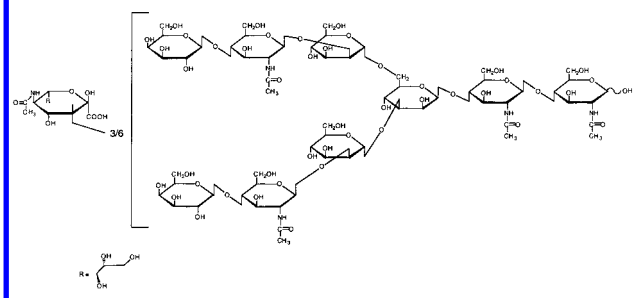


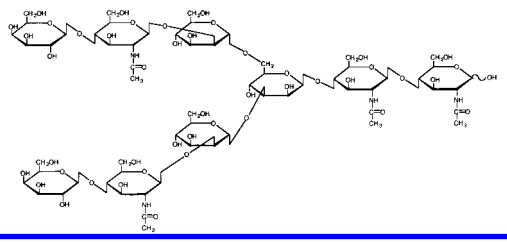
Figure 2. Dissociation rate constant *k* versus the E_{cm} for 4-hydroxybenzenesulfonic acid coordinated to LNDFH-I. The appearance of the benzenesulfonic acid was monitored as a function of the complex. The fitting curve produced a threshold corresponding to 1.9 eV.

Chart 2. Structures of Monosialylated-, Galactosylated Biantennary N-Linked Oligosaccharide (a) and Asialo, Galactosylated Biantennary N-Linked Oligosaccharide (NA2) (b)

(a) Mono-sialylated, galactosylated biantennary N-linked oligosaccharide (A1)



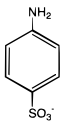
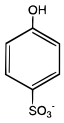
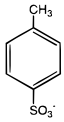
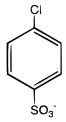
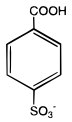
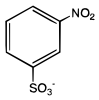
(b) Asialo-, galactosylated biantennary N-linked oligosaccharide (NA2)



nation, the absolute values of the dissociation threshold results may not have physical significance. The values should be considered in the relative manner but do reflect the relative binding affinities of the various dopants. All standard errors were ±0.15 eV based on multiple determinations.

The dopants in Table 2 were listed in order of decreasing electron-donating ability. The most notable trend was that electron-donating groups increase the strength of interaction. Thus, electron-donating substituents such as amine, hydroxyl, and

Table 2. MCDT Values for the Dissociation of the Anion Dopant from LNDFH-I for Series Benzene-Containing Sulfonic Acid

Dopant Structure	Name	σ_{para}	MCDT (eV)
	Sulfanilic acid [M + 172.01]	-0.66	2.63
	4-hydroxybenzenesulfonic acid [M + 172.99]	-0.37	1.91
	p-toluenesulfonic acid [M + 171.01]	-0.17	1.66
	Benzenesulfonic acid [M + 157.00]	0.0	1.84
	4-chlorobenzenesulfonic acid [M + 190.96]	+0.23	1.45
	4-sulfobenzoic acid, potassium salt [M + 200.99]	+0.45	1.09
	3-nitrobenzenesulfonic acid, sodium salt [M + 201.98]	N/A	weak intensity

methyl all had MCDT higher than benzenesulfonic acid. The most strongly bound species (MCDT = 2.63 eV) was the anion of sulfanilic acid. The most weakly bound was the anion of 3-nitrobenzenesulfonic acid (MCDT not determined, weak quasimolecular ion). The MCDT value of sulfuric acid is 1.63 eV (Table 3), slightly lower than benzenesulfonic acid. A plot of the σ_{para}[17–19] versus the MCDT values is provided in Figure 3. The linear regression has a correlation coefficient (r) of 0.94. The groups that were electron donating relative to hydrogen (negative σ_{para} values) increased the charge density in the phenyl ring and in the sulfonate groups. The increased electron density in the sulfonate groups further increased the strength of the interaction with the oligosaccharides as these interactions were hydrogen bonding in nature.

Determination of MCDT values for straighy-chain sulfonates could not be performed. These alkylsulfonates were too weakly bound, resulting in poor signals that made it difficult to perform CID. On the basis of the lowest value that could be determined by this method, the linear chain sulfonates are expected to have MCDT values less than 1.09 eV.

(17) Ritchie, C. D.; Sager, W. F. *Prog. Phys. Org. Chem.* **1964**, *2*, 323−400.
(18) Hansch, C.; Leo, A.; Unger, S.; Kim, K. H.; Nikaitani, D.; Liem, E. J. *J. Med. Chem.* **1973**, *16*, 1207−16.
(19) Hansch, C.; Leo, A.; Taft, R. W. *Chem. Rev.* **1991**, *91*, 165−95.

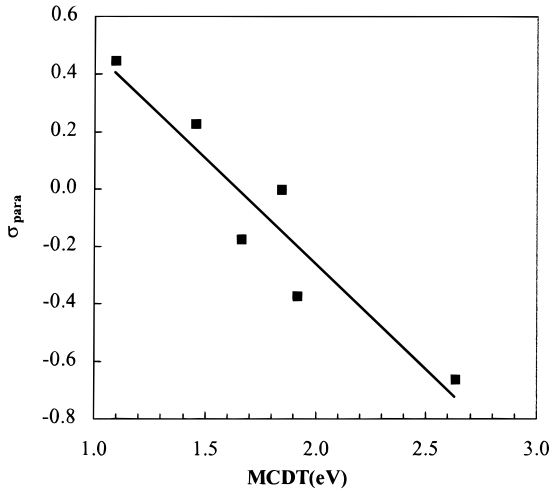


Figure 3. σ_{para} versus MCDT values. The strength of the interaction increases linearly with the electron-donating ability of the substituent. The linear regression has a correlation coefficient of 0.94.

On the basis of these studies, sulfanilic acid is the best dopant of the group and should generally provide the most abundant quasimolecular ion. In the following experiments described, sulfanilic acid was used as the general dopant.

Simultaneous Analysis of Acidic and Neutral Oligosaccharides. In biological systems, oligosaccharide moieties occur as mixtures of neutral and acidic oligosaccharides, which always makes the simultaneous analysis very difficult. Our previous paper has shown that HSO_4^- enables us to observe neutral and acidic oligosaccharides in the negative ion mode.[6] To determine the general utility of sulfanilic acid, the dopant was examined with two sets of neutral and acidic oligosaccharide mixtures. LSTa and LNDFH-I comprised one set, while A1 and NA2 comprised another (see Charts 1 and 2.).

In the mixture of LSTa and LNDFH-I, the base peak was [LSTa − H]$^-$ (m/z 997.31) and the LNDFH-I adduct [LNDFH-I + $H_2NC_6H_4SO_3$]$^-$ (m/z 1171.34) when the concentration ratio of the oligosaccharides was 1:1 **(**Figure 4). No signals corresponding to the fragmentation of LNDFH-I were present. A weak signal corresponding to the dopant $H_2NC_6H_4SO_3^-$ (m/z 172.01) was observed. For LSTa, peaks corresponding to B_1 (m/z 290.09) and B_2 (m/z 452.14) cleavages were the two most abundant fragment ions in the mass spectrum. Small amounts of C_4 (m/z 835.27) ions and [GlcNAc − H_2O − H]$^-$ (m/z 202.07) were also observed.

When equal concentrations of A1 and NA2 were present in the mixture, the base peak was [A1 − H]$^-$ (m/z 1930.72) (spectrum not shown). NA2 appeared as the sulfanilic acid adduct [NA2 + $H_2NC_6H_4SO_3$]$^-$ (m/z 1812.63). As mentioned previously, the adduct species were only observed with the neutral. The acidic species were always observed as the deprotonated forms. We have not found exceptions to this rule. The fragments from A1 were mainly [A1 − H − H_2O]$^-$ (m/z 1912.71) and [A1 − H − GlcNAc − H_2O]$^-$ (m/z 1709.65) as well as a small abundance of cross-ring cleavage from the reducing end corresponding to $^{0,2}A_7$ (m/z 1829.68). However, there were no fragment ions from NA2.

One of the most important uses of these dopants is to monitor the presence of sialic acid residues in oligosaccharides. A large degree of heterogeneity with respect to sialylation is obtained with

Table 3. MCDT Values for the Dissociation of the Anion Dopant from LNDFH-I for Straight-Chain Sulfonic Acid

LNDFH-I w/	anion name	MCDT (eV)
HSO_4^-	sulfuric acid [M + 96.96]	1.63
$HO(CH_2)_3SO_3^-$	3-hydroxy-1-propane sulfonic acid, sodium salt	weak intensity
$HO_2CCH_2CH(SO_3^-)CO_2H$	sulfosuccinic acid	weak intensity
$CH_3CH_2CH_2SO_3^-$	1-propanesulfonic acid sodium salt monohydrate	weak intensity

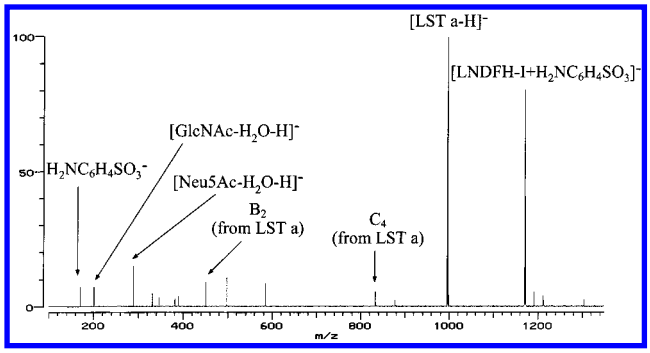


Figure 4. Negative mode MALDI-FTMS spectrum of mixture containing equimolar LSTa, an acidic oligosaccharide, and LNDFH-I, a neutral oligosaccharide. The sample is doped with sulfanilic acid. Only the neutral coordinates sulfanilic acid in the spectrum. LSTa is observed as the deprotonated species.

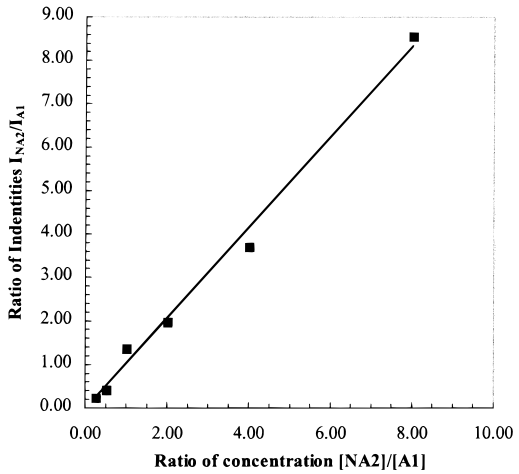


Figure 5. ratio of the intensity of A1 and NA2 obtained from MALDI-FTMS plotted as a function of the ratio of the concentration. The correlation coefficient is 0.99.

oligosaccharides due to both the biosynthesis and the biodegradation processes. The two sets of oligosaccharides were examined to determine whether varying the ratio of the components yields a similar response in their relative intensities. MALDI-MS analyses of six mixtures with varying ratios of the acidic and neutral oligosaccharides were performed. The total amount of both oligosaccharides remained constant at ~100 pmol. The conditions for MALDI were maintained for all the samples.

The first set consists of monosialylated, galactosylated biantennary N-linked oligosaccharide (A1, Chart 2a) and asialo, galactosylated biantennary N-linked oligosaccharide (NA2, Chart 2b). The two oligosaccharides are identical in structure except for the presence of sialic acid in NA2. Both were purified from human fibrinogen. The ratio of the intensity of the two components was plotted against the ratio of the amounts for the mixture (Figure 5). A linear relationship was obtained with a correlation coefficient (r) equal to 0.99. The slope (1.05) of the fit was close to unity, indicating that concentration was directly proportional to the intensity of the molecular ions. The linear relationship between the ratio of intensities versus concentration suggested that no suppression effects are present. Furthermore, the slope being nearly 1.0 suggests that both analytes produce quasi-molecular ions with the same specific intensity.

Similar experiments were performed with the set consisting of LS-tetrasaccharide a (Chart 1a) and lacto-*N*-difucohexaose I (Chart 1b). LSTa and LNDFH-I are both derived from human milk. LSTa is a five-residue sialylated oligosaccharide and LNDFH-I is a difucosylated six-residue neutral oligosaccharide. Using sulfanilic acid as the anion dopant, a straight line was obtained with a slope of 0.97. The correlation coefficient (r) also equaled 0.99. Both examples showed that this method is a quantitative tool for simultaneously measuring the relative amounts of both neutral and acidic oligosaccharides.

Monitoring Desialylation Product during Acid Hydrolysis. As a test of the method, LSTa was acid hydrolyzed to monitor the relative amount of LSTa and the neutral tetrasaccharide product. The loss of sialic acid residue due to hydrolysis is a common degradation reaction for compounds such as LSTa. The sialic acid is labile under ambient conditions and its loss is accelerated by acid hydrolysis.

LSTa (1 mg/mL, 40 μL) was mixed with an equal volume of 0.1 M TFA and the mixture was heated in a bath at 60 °C. The reaction mixture was monitored by removing 1 μL from the reaction mixture and applying the sample to the probe. The sulfanilic acid was added (1 μL, 0.011 M) to the probe followed by the matrix solution (saturated DHAP). The sample was vacuum-dried and analyzed by MALDI. A parallel analysis was performed without the anion dopant. Figure 6 shows representative spectra at three reaction times for the doped and undoped samples. In both series, the peaks were normalized to a constant scale. At the beginning of the reaction period, only the deprotonated species (m/z 997.30) was observed in both doped and undoped samples. After 45 min, the neutral tetrasaccharide product was observed as the sulfanilic acid adduct (m/z 879.24) and was ~30% of the base peak. However, the undoped sample did not show the neutral product in the negative mode. After 210 min, the doped sample showed the product as the base peak with the starting material roughly 30% of the base peak. In the undoped sample, the deprotonated starting material remained the base peak but was somewhat attenuated. No trace of the neutral product was observed in the negative ion mode.

CONCLUSION

Alkylsulfonates are effective dopants for producing negative ions of neutral oligosaccharides. We found that sulfanilic acid was the best of the alkylsulfonates, surpassing even sulfuric acid, an anion dopant proposed earlier, in terms of sensitivity. These

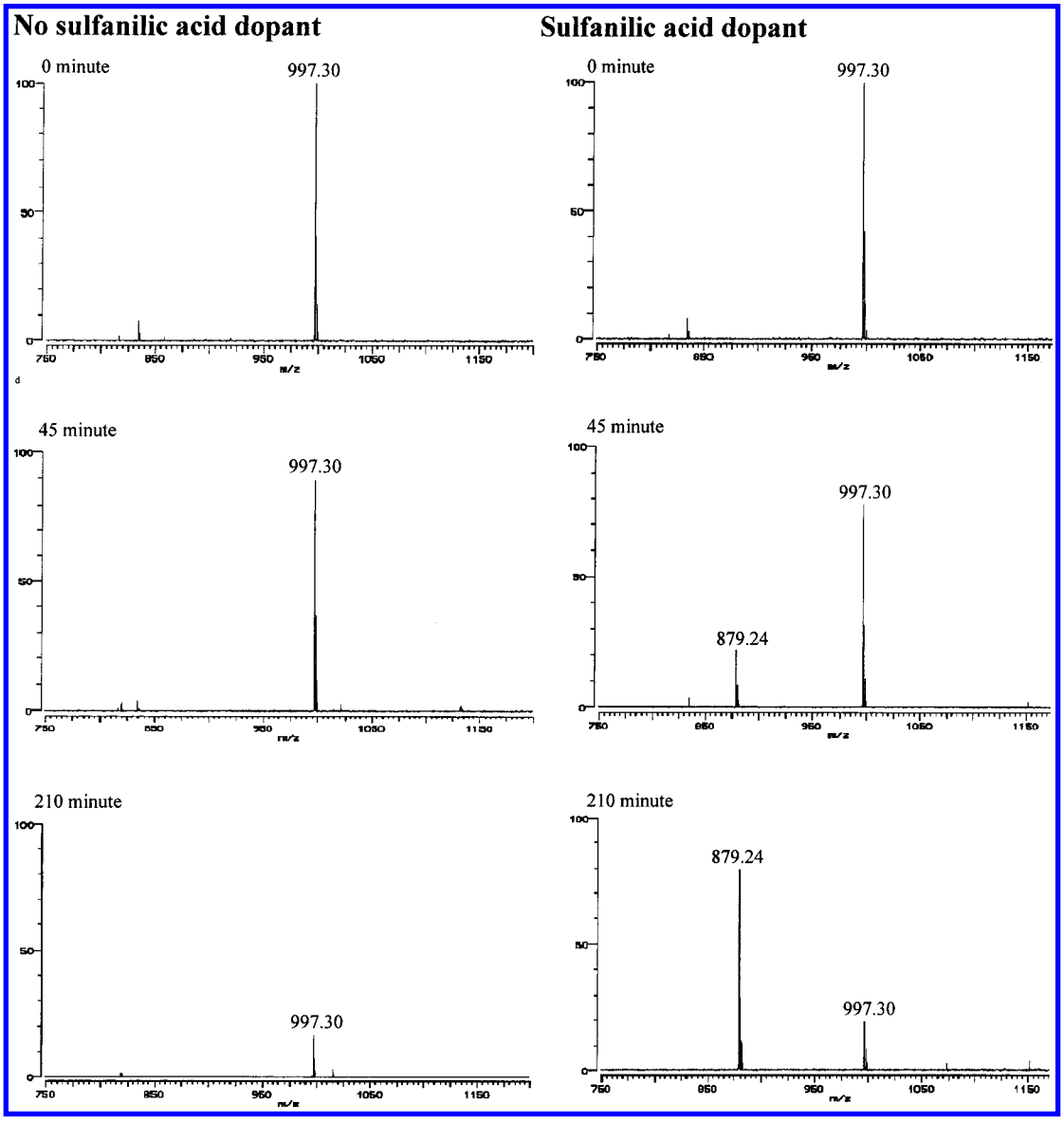


Figure 6. Negative ion MALDI-FTMS spectra of reaction mixture used to hydrolyze the acidic oligosaccharide LSTa. Conditions are chosen to cleave off the sialic acid. Spectra correspond to the beginning of the reaction (0), 45 , and 210 min. Spectra on the left are without the dopant and spectra on the right use sulfanilic acid as dopant prior to MALDI. The signal with *m*/*z* 997.3 is the deprotonated parent and *m*/*z* 879.24 is the desialylated fragment coordinated to deprotonated sulfanilic acid.

observations are supported by multicollision dissociation threshold experiments that show sulfanilic acid adducts have the highest dissociation threshold of any alkylsulfonate. Sulfanilic acid binds sufficiently strong with oligosaccharides that it produces quasi-molecular ions with compounds as small as disaccharides. The amino group is highly electron donating and increases the electron density in the sulfonate group. The high electron density, in turn, allows strong ion−dipole interactions to form with the hydroxyl groups of the oligosaccharides. These results further suggest that sulfonates are generally strong ligands for oligosaccharides.

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