Ion Mobility Studies of Carbohydrates as Group I Adducts: Isomer Specific Collisional Cross Section Dependence on Metal Ion Radius

Yuting Huang and Eric D. Dodds*

Department of Chemistry, University of Nebraska—Lincoln, Lincoln, Nebraska 68588-0304, United States

Supporting Information

ABSTRACT: Carbohydrates play numerous critical roles in biological systems. Characterization of oligosaccharide structures is essential to a complete understanding of their functions in biological processes; nevertheless, their structural determination remains challenging in part due to isomerism. Ion mobility spectrometry provides the means to resolve gas phase ions on the basis of their shape-to-charge ratios, thus providing significant potential for separation and differentiation of carbohydrate isomers. Here, we report on the determination of collisional cross sections for four groups of isomeric carbohydrates (including five isomeric disaccharides, four isomeric trisaccharides, two isomeric pentasaccharides, and two isomeric hexasaccharides) as their group I metal ion adducts (i.e., [M + Li]⁺, [M + Na]⁺, [M + K]⁺, [M + Rb]⁺, and [M + Cs]⁺). In all, 65 collisional cross sections were measured, the great majority of which have not been previously reported. As anticipated, the collisional cross sections of the carbohydrate metal ion adducts generally increase with increasing metal ion radius; however, the collisional cross sections were found to scale with the group I cation size in isomer specific manners. Such measurements are of substantial analytical value, as they illustrate how the selection of charge carrier influences carbohydrate ion mobility determinations. For example, certain pairs of isomeric carbohydrates assume unique collisional cross sections upon binding one metal ion, but not another. On the whole, these data suggest a role for the charge carrier as a probe of carbohydrate structure and thus have significant implications for the continued development and application of ion mobility spectrometry for the distinction and resolution of isomeric carbohydrates.

It would be quite difficult to overstate the diversity and centrality of the many biological functions mediated by carbohydrates.¹,² Beyond their important roles as structural components and energy sources, carbohydrates are involved in many kinds of intermolecular recognition (e.g., in infection and immunity),³,⁴ have strong influence on overall protein structure (e.g., overall structure and solubility of glycosylated proteins),⁵,⁶ and relate to human health and illness in a host of additional respects (e.g., as disease biomarkers and in congenital disorders of glycosylation).⁷–¹¹ While elucidating the structures of carbohydrates is essential to understanding their functions at the molecular level, the diversity of the constituent monosaccharides and their ability to form branched structures make this task analytically demanding.¹²–¹⁴ This is particularly relevant in the analysis of carbohydrates in biological mixtures, where isomeric structures must be distinguished and assigned in order to achieve complete characterization.

Many separation methods have been applied to carbohydrate analysis, often with the goal of resolving or distinguishing isomers. These include gas chromatography, high-performance liquid chromatography, and capillary electrophoresis, among others.¹⁵–²¹ An important strength of each of these separation methods is their compatibility with mass spectrometry (MS). MS provides an indispensable platform for carbohydrate analysis,²²–²⁷ either alone or in synergy with other complementary carbohydrate structural analysis methods such as nuclear magnetic resonance spectroscopy.²⁸,²⁹ In recent years, ion mobility (IM) has been increasingly applied to separation and analysis of biomolecules, including carbohydrates.³⁰–³³ In IM, ions are separated on the basis of their mobilities in a buffer gas under the influence of an electric field. The mobility of a given ion is directly proportional to the ion charge state and inversely proportional to the orientationally averaged ionic-neutral collisional cross section (CCS). Thus, IM is able to measure ion “shape-to-charge ratios”, which can be useful for differentiation of isomers or detection of multiple conformations. When coupled to MS, the information provided by IM can be highly complementary to the mass-to-charge ratio information provided by the mass analyzer. Because of the time scale of IM separations, the use of ion mobility mass spectrometry (IM-MS) need not be exclusive of online chromatographic separations. For example, IM separations (typically requiring on the order of 10⁻³–10⁻¹ s) can readily be performed as an intermediate analytical dimension linking a chromatographic separation (typically requiring on the order of...
10^2−10^3 s) and time-of-flight mass spectrometry (TOF-MS; typically requiring on the order of 10^−5−10^−4 s per spectrum). IM can also be coupled to other types of mass spectrometers (e.g., ion trap instruments and Fourier transform ion cyclotron resonance instruments). Under appropriate experimental conditions, IM drift times can be used to calculate ion-neutral collisional cross sections, thus providing an additional dimension of structural information which is not accessible by mass analysis alone. In all, these characteristics render IM-MS an attractive platform for carbohydrate analysis. To date, IM-MS has been used for the characterization and separation of saccharide isomers, profiling and analysis of glycans released from glycoproteins, conformational study and separation of heparin oligosaccharides, and some studies of various types of glycoconjugates. Carbohydrates have been studied by IM primarily as sodium ion adducts; indeed, there have been relatively few reports of carbohydrate IM measurements involving other metal ion adducts. Nevertheless, it has been demonstrated that cation adduction has an influence on the conformation and separation characteristics of carbohydrate isomers in IM. These observations have motivated the present study.

In this work, IM-MS has been used to systematically study the influence of group I metal cations (Li+, Na+, K+, Rb+, and Cs+) on the CCSs of carbohydrate metal ion adducts in several groups of carbohydrate isomers (five disaccharide isomers, four trisaccharide isomers, two pentasaccharide isomers, and two hexasaccharide isomers). In comparing the 65 CCSs thus obtained, it was found that the CCSs of the carbohydrate metal ion adducts often do not simply scale in proportion to the size of the metal cation. In many cases, the CCSs were found to have carbohydrate isomer specific dependencies on the radius of the coordinated metal ion. Such observations have immediate and significant analytical consequences. For example, in multiple instances a given pair of isomeric carbohydrates could be readily distinguished on the basis of CCS as one metal ion adduct, but not another. Overall, these results illustrate that the binding of metal ions of appropriate radius can induce conformational differences in isomeric carbohydrates. This suggests that charge carriers can play an analytical role as structural probes for improving the resolution and distinction of isomeric carbohydrates and their corresponding glycoconjugates by IM-MS.

## EXPERIMENTAL SECTION

**Chemicals.** Disaccharides (cellobiose, maltose, melibiose, sucrose, and trehalose), trisaccharides (isomaltotriose, maltotriose, melezitose, and raffinose), and lacto-N-fucopentaose I (LNFP I) were purchased from Sigma-Aldrich (St. Louis, MO). Lacto-N-fucopentaose V (LNFP V) was purchased from Accurate Chemical and Scientific (Westbury, NY). Lacto-N-fucopentaose V (LNFP V) was purchased from Santa Cruz.
Biotechnology (Dallas, TX). Lacto-N-difucohexaose I (LNDFH I) was purchased from Fisher Scientific (Pittsburgh, PA). The structures of these carbohydrates are shown in Scheme 1. Polyalanine and the salts of group I cations (LiCl, NaCl, KCl, RbI, and CsCl) were also purchased from Sigma-Aldrich. Solutions of polyalanine, each carbohydrate, and each salt were individually prepared in 50% aqueous acetonitrile (v/v) with 0.1% formic acid (v/v). Equal volumes of 50 μM carbohydrate and 100 μM salt were combined and mixed before analysis, resulting in a sugar concentration of 25 μM and a carbohydrate-to-salt mole ratio of 1:2. A working solution of polyalanine was prepared at a concentration of 12.5 μg/mL.

**Mass Spectrometry and Ion Mobility.** All mass spectrometry and ion mobility experiments were performed using a Waters Synapt G2 HDMS quadrupole time-of-flight hybrid mass spectrometer (Q-TOF-MS) with traveling wave ion mobility (TWIM) capabilities (Waters, Manchester, U.K.). The instrument was equipped with a custom nanoelectrospray ionization (nESI) source, which made use of home-pulled borosilicate emitters fabricated from Corning Pyrex melting point capillaries (100 mm length and 1.5–1.8 mm inner diameter; Corning, NY) with the use of a vertical micropipet pipet puller (David Kopf Instruments, Tujunga, CA). The emitters were filled with several microliters of analyte solution, and they were then fitted onto an electrode holder such that the solution was placed directly in contact with a platinum electrode, which delivered the nESI capillary potential. The capillary voltage was optimized for each individual emitter in order to establish continuous and stable spray. This was usually accomplished at a capillary potential of 1.00–1.50 kV. The ion source temperature was set at 80 °C. Sampling cone and extraction cone voltages were set at 30.0 and 2.0 V, respectively.

The major experimental parameters affecting TWIM measurements are the drift gas pressure, the TWIM DC traveling wave height, and the TWIM DC traveling wave velocity. For all IM experiments, the helium cell gas pressure, the TWIM DC traveling wave measurements are the drift gas pressure, the TWIM DC traveling wave height, and the TWIM DC traveling wave velocity. For each calibration standard or analyte of interest, the ion drift velocity ($v_d$) depends on the ion mobility ($K$) according to:

$$v_d = KE = \frac{d_d}{t_d}$$

where $E$ is the magnitude of the electric field, $d_d$ is the drift distance, and $t_d$ is the drift time. In an IM drift tube having a static, uniform, and low electric field, $K$ is given by:

$$K = \frac{3ze}{16N\Omega} \frac{2\pi}{k_BT\mu}$$

where $z$ is the ion charge state, $e$ is the elementary charge, $N$ is the number density of the drift gas, $\Omega$ is the ion-neutral CCS, $k_B$ is the Boltzmann constant, $T$ is the absolute temperature, and $\mu$ is the ion-neutral reduced mass. For ion-neutral collision partners having masses of $m_1$ and $m_2$, respectively, the reduced mass is defined as:

$$\mu = \frac{m_1m_2}{m_1 + m_2}$$

This normalization is useful because it provides a parameter that encompasses the three major analyte characteristics which relate to drift time: analyte ion CCS, analyte ion charge, and the reduced mass of the analyte ion and the neutral collision partner.

For each calibration standard or analyte of interest, the experimentally observed TWIM arrival time distribution (ATD) was centroided in order to obtain the corresponding apparent drift time ($t_{ATD}$). Apparent drift times were corrected for the dead time associated with ion transit through the TWIM cell, as well as the time required for ions to traverse the ion transfer and TOF interface regions of the instrument, which lies between the IM cell exit and the TOF pusher stack. The mobility dead time ($t_{m}$) was approximated as the time required for ions to pass through the TWIM drift region solely under the influence of the DC traveling wave (i.e., in the absence of ion-neutral collisions). This depends on the distance traveled through the mobility cell ($d_{m}$ in Synapt G2 HDMS instruments, $d_{m} = 25.4$ cm), and the mobility cell DC traveling wave velocity ($v_m$):

$$t_m = \frac{d_m}{v_m} = \frac{0.254 m}{v_m}$$

Correcting the apparent drift times for $t_{m}$ is analogous to correcting retention times for the void time of an unretained species in chromatography. The apparent drift times were further corrected for the ion transit time through the transfer stacked ring ion guide ($t_t$), which depends upon the distance traveled through the transfer guide ($d_t$) in Synapt G2 HDMS
instruments, $d_i = 13.5$ cm) and the transport cell DC traveling wave velocity ($v_i$):

$$t_i = \frac{d_i}{v_i} = \frac{0.135 \text{ m}}{v_i} \quad (6)$$

Finally, the apparent drift times were corrected for the ion transit time through the TOF interface region ($t_d$), which is composed of several static DC ion optic elements. Unlike $t_m$ and $t_i$, $t_d$ is mass-to-charge-dependent and must be calculated for each individual ion of interest. The value of $t_i$ was calculated according to:

$$t_i = k_i \sqrt{\frac{m}{z}} = k_{\text{EDC}} \left(\frac{m}{10^9}\right)^{1/2} \quad (7)$$

where $k_i$ is the TOF interface flight time constant, which is derived from the “enhanced duty cycle” constant ($k_{\text{EDC}}$) that is empirically determined for each individual instrument during commissioning. Importantly, $k_{\text{EDC}}$ includes dimensions of microseconds, and it has thus been corrected in eq 7 to yield a $k_i$ value consistent with units of seconds. With the drift time corrections afforded by eqs 5–7, the corrected TWIM drift times ($t_d'$) were ultimately calculated according to:

$$t_d' = t_d - t_m - t_i$$

Collisional cross section calibration curves were generated by plotting $\Omega^d$ as a function of $t_d'$ and fitting a power function of the form $\Omega^d = a t_d'^b$. Example calibration curves are shown in Figures S4–S6 of the Supporting Information. Calibration curves constructed in this manner were then used to calculate unknown $\Omega_i^d$ values given experimentally determined $t_d'$ values. Analyte $\Omega_i^d$ values (mass- and charge-normalized CCSs) were then converted to $\Omega$ values (absolute CCSs) by recasting eq 4. The CCS values for all analytes measured in this study fell within the calibrated range provided by the polyalanine standards. Whenever TWIM parameters were optimized for a different series of analytes, a new calibration was constructed by analyzing the polyalanine calibrants using the same experimental parameters. While this calibration approach is subject to some caveats (e.g., drift gas polarizability is not expressly accounted for, temperature is assumed to remain constant, among others), there is a precedent for agreement between DTIM and TWIM CCSs for carbohydrate ions to within a few percent.\(^{45}\)

**RESULTS**

**Arrival Time Distributions of Cationized Carbohydrate Isomers.** The arrival time distributions for all carbohydrate group I metal ion adducts studied here are presented in Figures S7–S19 of the Supporting Information. As expected, the size of the metal ion exerted a significant influence on the drift times of the carbohydrate metal ion adducts. As a general trend, drift times of these ions increased as the size of metal ions increased. For example, the drift time of trehalose varied from $t_d = 5.00$ ms for the [M + Li]$^+$ ion to $t_d = 5.62$ ms for the [M + Cs]$^+$ ion. Importantly, though, the size of the metal ion was not the only factor affecting the drift times. For a given set of carbohydrate isomers, it became immediately apparent that the drift times of isomers may exhibit strikingly different dependencies upon the identity of the bound cation.

As illustrated by the ATDs presented in Figure 1, the drift times of the two disaccharide isomers sucrose and trehalose were essentially indistinguishable as the [M + Li]$^+$ adducts ($t_d = 4.95$ ms for [sucrose + Li]$^+$ versus $t_d = 5.00$ ms for [trehalose + Li]$^+$); however, the drift times of their [M + K]$^+$ adducts differed by 0.25 ms ($t_d = 5.17$ ms for [sucrose + K]$^+$ versus $t_d = 5.42$ ms for [trehalose + K]$^+$). In this case, an increase in the size of the metal ion allowed the two isomers to assume conformations which were more readily distinguished by their drift times.

Isomer specific dependencies of drift time upon cation size were also manifest in other intriguing and potentially useful ways, as shown in Figure 2. In this example, the drift times of the trisaccharide isomers maltotriose and isomaltotriose as their [M + Li]$^+$ adducts differed by 0.14 ms ($t_d = 5.73$ ms for [Maltotriose + Li]$^+$ versus $t_d = 5.87$ ms for [Isomaltotriose + Li]$^+$), sodium ion adducts (center traces), and cesium ion adducts (lower traces) of sucrose (solid blue traces) and trehalose (dashed red traces). Each arrival time distribution is labeled with the corresponding centroid drift time.

![Image](dx.doi.org/10.1021/ac402133f)
[maltotriose + Li]+ versus \( t_d = 5.87 \) ms for [isomaltotriose + Li]+), while the drift times of their \([M + Na]^+\) adducts were practically identical (\( t_d = 5.99 \) ms for [maltotriose + Na]+ versus \( t_d = 5.96 \) ms for [isomaltotriose + Na]+). This convergence of drift times is in contrast to the previous examples involving disaccharides, in which adduction of a larger cation caused the drift times of two isomers to diverge. Interestingly, the drift times of the \([M + Cs]^+\) ions of maltotriose and isomaltotriose differed substantially (\( t_d = 6.28 \) and 6.51 ms for the two ATD features of [maltotriose + Cs]+ versus \( t_d = 6.12 \) ms for [isomaltotriose + Cs]+). Thus, the coordination of the cesium ion re-establishes distinct drift times of these two carbohydrate isomers. In addition, cesium ion adduction had the effect of reversing the order of drift times relative to the lithium ion adducts. This dramatically reiterates that the drift times of carbohydrate isomers can scale as a function of cation size in very unique ways. Finally, the presence of two major features in the ATD of [maltotriose + Cs]+ suggests that the presence of multiple ATD features should be cautiously interpreted when working with unknown mixtures. For example, such peaks might be interpreted as arising due to the presence of multiple structural isomers. In this specific case, the presence of multiple ATD features is evidently due to the presence of distinct ion binding conformations (since the pure standard contains only a single isomer). These considerations suggest that results from different metal ion adducts should be considered and also argue that experimental data should be complemented by and compared to theoretical calculated structures.

In addition to the interesting variations in drift times among group I adducts of carbohydrate isomers highlighted above, there were also some instances in which drift times changed only modestly among adducts involving successively larger cations. For example, the drift times of the \([M + Li]^+\) and \([M + Na]^+\) adducts of LNFP V were \( t_d = 8.25 \) ms and \( t_d = 8.30 \) ms, respectively. The drift times of the same metal ion adducts of the LNFP I were much more distinct than those of the LNFP V isomer, from \( t_d = 8.30 \) ms to \( t_d = 8.54 \) ms (Figure 3). This suggests that LNFP V assumed or maintained a comparatively more compact structure when coordinating a sodium ion relative to the lithium ion adduct, while the LNFP I sodium ion adduct was significantly larger than the corresponding lithium ion adduct. This furnishes yet another example of how the drift times of isomeric carbohydrates can respond to changes in metal ion adduction in markedly dissimilar manners.

**Collisional Cross Sections of Cationized Carbohydrate Isomers.** To more quantitatively and exhaustively address the IM behavior of isomeric carbohydrates as their group I metal ion adducts, the CCSs of the 13 carbohydrates studied here were measured as their \([M + Li]^+\), \([M + Na]^+\), \([M + K]^+\), \([M + Rb]^+\), and \([M + Cs]^+\) adducts. The 65 resulting CCSs are reported in Table 1. In order to more directly illustrate the dependence of the CCSs upon on the size of the bound cation, the carbohydrate metal ion adduct CCSs were plotted as a function of the cross sectional areas of the bound cations treated as hard spheres. The cation cross sectional areas were calculated as \( \Omega r^2 \), where \( r \) is the Shannon effective ionic radius of the cation. If changes in CCSs of carbohydrate metal ion complexes were solely due to changes in the size of the bound cation, then, to a first approximation, the cross section of the sugar metal adduct should scale with the metal ion cross section. Deviations from this expected general trend can be interpreted as evidence for significant conformational changes occurring concomitantly with changes in metal ion size.

Figure 4 shows the CCSs of four disaccharide adducts plotted as a function of the cross sectional area of the bound cation (from left to right along the horizontal ordinate: Li+, Na+, K+, Rb+, and Cs+). For the sake of clarity, only four of the five disaccharides studied were plotted in Figure 4; however, the CCS data for all disaccharide adducts are included in Table 1, and the corresponding ATDs are presented in the Supporting Information, as noted above. As illustrated in the plot, the CCS of each disaccharide isomer changes with cation size, albeit with varied dependencies. For example, the CCSs of the lithium adducts of sucrose and trehalose were similar (\( \Omega = 107.1 \pm 0.3 \) Å² for [sucrose + Li]+ versus \( \Omega = 107.9 \pm 0.3 \) Å² for [trehalose + Li]+), as expected due to their corresponding ATDs (cf. Figure 1). As the size of the metal cation was increased, the CCSs of trehalose adducts exhibited much greater sensitivity to the cation size as compared to those of sucrose adducts, culminating in considerably different CCSs of their cesium adducts (\( \Omega = 112.9 \pm 0.3 \) Å² for [sucrose + Cs]+ versus \( \Omega = 116.5 \pm 0.4 \) Å² for [trehalose + Cs]+). Comparing melibiose to trehalose reveals a nearly opposite trend, with the isomers having different CCSs as their lithium adducts (\( \Omega = 107.9 \pm 0.3 \) Å² for [melibiose + Li]+ versus \( \Omega = 110.1 \pm 0.2 \) Å² for [trehalose + Li]+) but essentially the same cross sections as their cesium adducts (\( \Omega = 116.5 \pm 0.4 \) Å² for [trehalose + Cs]+ and \( \Omega = 116.3 \pm 0.2 \) Å² for [melibiose + Cs]+).

Beyond these specific examples, some additional general observations can be made. For instance, the CCS of sucrose (glucose-α[1→2]-fructose) was always the smallest among the disaccharides studied, regardless of the bound cation. This is consistent with the pyranosyl furanose structure of sucrose as compared to the other disaccharides, which were all pyranosyl pyranoses. Conversely, maltose (glucose-α[1→4]-glucose) generally had the largest CCS among the disaccharides studied, again regardless of the bound cation. Interestingly, cellobiose (glucose-β[1→4]-glucose) was found to have essentially the same CCS as maltose as their corresponding \([M + Li]^+\) and \([M + Na]^+\) adducts, but the CCSs of their remaining adducts diverged increasingly with increasing cation size (cf. Table 1). Cellobiose and maltose differ only in the configuration of their glycosidic linkages: β[1→4] versus α[1→4], respectively.

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**Figure 3.** Arrival time distributions of the lithium ion adducts (upper traces) and sodium ion adducts (lower traces) of LNFP V (solid blue traces) and LNFP I (dashed red traces). Each arrival time distribution is labeled with corresponding centroid drift time.
Evidently, the adoption of conformations that allow these isomers to be distinguished on the basis of CCS requires the binding of larger metal ions. Trehalose (glucose-α[1→1]-glucose) also exhibited some interesting behavior. As noted above, trehalose and sucrose had similar CCSs as their lithiated glucose) also exhibited some interesting behavior. As noted above, trehalose and sucrose had similar CCSs as their lithiated isomers to be distinguished on the basis of CCS requires the adoption of conformations that allow these isomers to be distinguished on the basis of CCS. Where visible, error bars represent the standard error of the mean for four replicate measurements.

In Figure 5, trisaccharide adduct CCSs are plotted as a function of the metal ion cross sectional area (increasing from left to right in the order lithium, sodium, potassium, rubidium, cesium). Where visible, error bars represent the standard error of the mean for four replicate measurements.

cation sizes were again observed, with even greater variability than those of disaccharides. Among the trisaccharides studied here, the CCS of melezitose was the least sensitive to the size of the bound cation, with CCSs remaining relatively unchanged for the [M + Li]+ through [M + Rb]+ adducts (all four CCS values were within 1.2 Å² of each other). Melezitose also exhibited the smallest CCS among the trisaccharides studied here regardless of the bound cation, perhaps owing to the vicinal positions of the pyranose substituents on the central furanose ring (glucose-α[1→3]-fructose-β[2→1]-glucose). At the opposite extreme, the CCSs of maltotriose adducts exhibited the greatest sensitivity to the metal ion radius, increasing steadily from \( \Omega = 138.6 \pm 0.2 \text{ Å}^2 \) for the [M + Li]+ ion to \( \Omega = 150.8 \pm 0.2 \text{ Å}^2 \) for the [M + Cs]+ species (a difference of 12.2 Å²). Maltotriose had the largest CCSs among the trisaccharides studied here as the [M + Rb]+ and [M + Cs]+ adducts. Among the [M + Na]+ and [M + K]+ adducts, maltotriose and isomaltotriose had nearly the same cross sections. Finally, among the [M + Li]+ adducts, isomaltotriose exhibited the largest CCS. This further elaborates on the inversion of drift time order for maltotriose and isomaltotriose, which was noted in Figure 2. As each group I adduct, raffinose...
had the second smallest CCS, being larger than only melezitose. Both melezitose and raffinose contain one furanose and two pyranose rings, so it is perhaps unsurprising that these have consistently smaller CCSs than the other trisaccharides examined here, which were each composed of three pyranose monosaccharides. The relative CCSs of melezitose and raffinose likely stem from their differences in linkage positions (cf. structures shown in Scheme 1). Again, these findings in sum serve to indicate that certain group I adducts are more suitable for resolution or distinction of a given pair of trisaccharide isomers by IM. For example, sodium ion adduction would be a poor choice for analysis of isomaltooltriose and maltotriose, while our indentification of the cesium ion adducts would allow clear distinction of these isomers on the basis of CCS. This particular comparison is rather provocative considering that carbohydrates are very commonly analyzed by MS as their sodiated adducts.

The metal ion size dependence of CCSs for group I adducts of LNFP I, LNF F V, LNDFH I, and LNDFH II are plotted in Figure 6. In these cases, the adduct CCSs generally showed less dependence upon the identity of the charge carrier. This may be a consequence of the larger sizes of these pentasaccharides and hexasaccharides as compared to the bound cation; however, these findings also indicate a greater difference in CCS among the two hexasaccharides than the two pentasaccharides. This perhaps counterintuitive result suggests that the potential of IM to distinguish or potentially resolve carbohydrate isomers as their group I adducts is not solely a matter of relative sugar and cation size. Moreover, preliminary data involving some larger oligosaccharides suggests that multiple cation adduction may in some cases be advantageous (data not shown).

Finally, we note that despite the challenges associated with TWIM calibration to obtain CCS values, several of the results obtained here were in quite good agreement with CCS values measured by McLean and co-workers and Bowers and co-workers using DTIM instruments. As highlighted in Table S1 of the Supporting Information, CCSs measured for the \([M + Na]^+\) ions of LNFP I, LNF F V, LNDFH I, and LNDFH II indicate a between-study relative standard deviation of 3% among the three sets of measurements.

![Figure 6. Average collisional cross sections of LNDFH I, LNDFH II, LNFP I, and LNF F V metal ion adducts plotted as a function of the metal ion cross sectional area (increasing from left to right in the order lithium, sodium, potassium, rubidium, cesium). Where visible, error bars represent the standard error of the mean for four replicate measurements.](image-url)

### CONCLUSIONS

In this work, TWIM-MS has been applied to measure the CCSs of four groups of isomeric carbohydrates, each as their group I metal ion adducts from Li\(^+\) to Cs\(^+\). This collection of data demonstrates that conformations assumed by carbohydrates upon metal ion binding exhibit significant dependencies upon both the carbohydrate structure and ionic radius of the bound cation. Furthermore, changing metal ion sizes have distinct effects upon the CCS of different isomers. Overall, the results highlight a role for the charge carrier as a structural probe of carbohydrate isomers studied by IM, with appropriate selection of metal ion binders providing opportunities to optimize the resolution and distinction of such isomers on the basis of CCS. We note that sodium ion adducts—which are perhaps the most common carbohydrate ions analyzed by MS—are often not the preferred charge carrier from the standpoint of IM separation of isomeric carbohydrates. Findings such as these will likely play a significant role in the continued development and application of IM-MS to analysis of isomeric carbohydrates and their corresponding glycoconjugates.

### ASSOCIATED CONTENT

#### Supporting Information

Supporting Information includes data on the optimization of TWIM resolution, collisional cross section calibration curves, arrival time distributions for all carbohydrate metal ion adducts studied here, and comparison of selected TWIM CCS values from the present study with DTIM CCS values from the literature. This material is available free of charge via the Internet at http://pubs.acs.org.

### AUTHOR INFORMATION

**Corresponding Author**

*E-mail: edodds2@unl.edu. Tel.: 1-402-472-3592. Fax: 1-402-472-9402.*

**Notes**

The authors declare no competing financial interest.

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### REFERENCES


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