Application note

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Separation of isomeric disaccharides by traveling wave ion mobility mass spectrometry using CO_2 as drift gas

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The use of CO_2 as a massive and polarizable drift gas is shown to greatly improve peak-to-peak resolution ($R_{p\text{-}p}$), as compared with N_2, for the separation of disaccharides in a Synapt G2 traveling wave ion mobility cell. Near or baseline $R_{p\text{-}p}$ was achieved for three pairs of sodiated molecules of disaccharide isomers, that is, cellobiose and sucrose ($R_{p\text{-}p} = 0.76$), maltose and sucrose ($R_{p\text{-}p} = 1.04$), and maltose and lactose ($R_{p\text{-}p} = 0.74$). Ion mobility mass spectrometry using CO_2 as the drift gas offers therefore an attractive alternative for fast and efficient separation of isomeric disaccharides.

Keywords: isomer resolution; oligosaccharides; traveling wave ion mobility mass spectrometry; polarizable drift gases

Introduction

Oligosaccharides are present in nature in a great diversity of structures differing in monosaccharide residues (e.g. lactose and cellobiose), ring size (e.g. sucrose and trehalose), connectivity (e.g. maltose and isomaltose) and anomeric configuration (e.g. maltose and cellobiose).[1] In addition, oligosaccharides containing more than one type of monosaccharide unit can differ in sugar sequences, for instance, GlcNAc-GlcN *versus* GlcN-GlcNAc.

Mass spectrometry (MS) is a powerful tool for structural analysis, and the identification of the many complex oligosaccharides and their isomers have been based mostly on MS/MS experiments using collision induced dissociation.[2] However, these isomers may display sometimes too similar CID chemistry, and additional methods such as selective enzymatic digestion[3] may be required. The coupling of ion mobility with mass spectrometry (IM-MS) has also offered a powerful tool to separate and characterize oligosaccharide isomers,[4] and the diverse structural features of these isomers often lead to contrasting gas phase shapes allowing their differentiation by IM-MS. Separation on home-built instruments of a few disaccharides, trisaccharides and tetrasaccharides, often in form of derivatives, such as alditols, methyl glycosides or boronic acid esters, has been reported.[5] Field-asymmetric waveform ion mobility spectrometry, drift-time ion mobility mass spectrometry, traveling wave ion mobility mass spectrometry (TWIM-MS)[6–8] and extractive fragment ion mobility mass spectrometry[9], as well as classical separation techniques such as liquid chromatography and electrophoresis,[10,11] have been used to separate oligosaccharides obtained from a wide range of biological samples.

In IMS, ion separation occurs in a drift cell on the basis of parameters such as collision cross-section (shape), charge, mass, drift gas polarizability and lifetimes of ion-neutral gaseous complexes.[12] Nitrogen (N_2) and helium are the most commonly used drift gases, but it has been shown that more polarizable and massive molecules (such as CO_2) may improve IMS resolution.[13] The development of TWIM cells with a new form of ion propulsion based on 'traveling waves' (continuous sequences of transient voltages) allowed the use of quite small drift cells with much improved ion transmission compared with classic IM cells based on uniform electrical fields. The TWIM-MS coupling enabled the launching of the first commercial IMS instruments.[14] Recently, major improvements have been implemented in the second generation of this instrument (Synapt G2), increasing the resolving power of TWIMS approximately fourfold,[15] and further improvements in resolving power of TWIM and other types of compact IM cells will likely occur in the future.

Herein, we compared the ability of the first generation Synapt TWIM cell (herein referred simply as 'G1') and the second generation G2 cell to resolve a representative set of four isomeric disaccharides, i.e. maltose, lactose, sucrose and cellobiose (Scheme 1). The mobilities of the isomers in the form of sodiated molecules were also evaluated using N_2 and CO_2 as drift gases.

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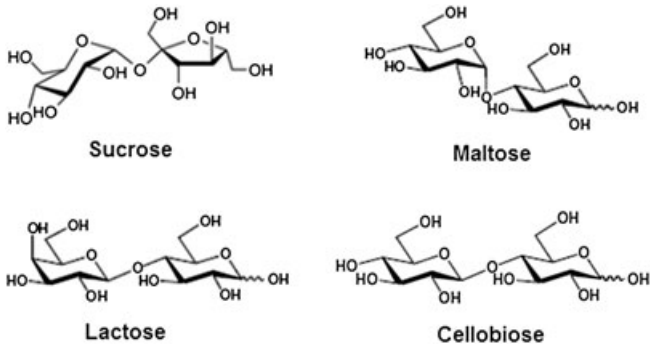

Scheme 1. Structures of the set of disaccharides.

Materials and methods

Chemicals and samples

Maltose, lactose, sucrose (saccharose) and cellobiose were purchased from Sigma-Aldrich (St. Louis, MO). Solutions in methanol (HPLC grade, Honeywell Burdick & Jackson, MI, EUA) were doped with sodium acetate to favor the formation of $[M+Na]^+$ ions.

Traveling wave ion mobility mass spectrometry

Methanolic solutions were directly infused into the electrospray ionization source of a Synapt G1 mass spectrometer (Waters, Manchester, UK) and in the Synapt G2 (Waters, Manchester, UK), operated in the positive ion mode. The mobilities of the $[M+Na]^+$ ions of *m/z* 365 were monitored. Table 1 summarizes optimized ionization and ion mobility separation conditions for both instruments. N_2 and CO_2 were used as drift gases, both at 2.00 mbar. Product ion scan TWIM-MS/MS experiments of the $[M+Na]^+$ ions were performed using 1.0×10^{-2} mbar of argon as collision gas at the post-TWIM transfer cell of the instrument and a collision energy of 100 eV.

Results and discussion

Figure 1 shows the drift-time plots using CO_2 for the $[M+Na]^+$ ions of each of the four disaccharides measured with both the G1 and G2 cells. Note the major differences in the performance and the greater resolving power (R_p) of the G2 cell due to the much sharper peaks [Fig. 1(b)] when compared with the quite broad peaks obtained under optimized conditions with the G1 cell [Fig. 1(a)]. The ability of a mobility cell to resolve two ions (see, e.g., Tabrizchi and Rouholahnejad[16]) can be measured by the peak-to-peak resolution ($R_{p\text{-}p}$), which is defined as

$$R_{p\text{-}p} = \frac{2(t_{d2} - t_{d1})}{W_{b1} + W_{b2}}$$

where t_d is the drift time of the two peaks and W_b is the peak width at base. $R_{p\text{-}p}$ depends, however, both on peak separation as measured by the separation factor (α) and peak width as measured by the resolving power (R_p), which are defined as

$$\alpha = \frac{t_{d2}}{t_{d2}} \qquad R_p = \frac{t_d}{W_{1/2}}$$

where $W_{1/2}$ is the peak width at half of the maximum intensity. $R_{p\text{-}p}$ relates to α and R_p as follows:

$$R_{p\text{-}p} = 0.589 R_p \frac{\alpha - 1}{\alpha}.$$

Note also in Figure 1 that lactose and sucrose, as well as cellobiose and maltose, display very close drift times in both cells, but the two pairs of isomeric disaccharides are quite properly resolved using CO_2 as the drift gas particularly in the G2 cell. CO_2 increases $R_{p\text{-}p}$ likely because the $[M+Na]^+$ ions with uneven charge distribution and higher polarity may form stronger ion-neutral complexes with CO_2 with a lifetime long enough to survive over several mean free paths, whereas ions with more delocalized charge are more largely free between collisions.[17] Proper resolution is observed therefore for the following pairs: cellobiose/sucrose, maltose/sucrose and maltose/lactose.

Table 2 shows the R_p of both TWIM cells, as calculated from Fig. 1. As previously reported for other separations,[14] the results show that R_p of Synapt G2 (not necessarily $R_{p\text{-}p}$,) is almost four times better than that of Synapt G1 ($R_p = 22.4$ and 5.6, respectively).

Table 3 also presents the peak-to-peak resolutions ($R_{p\text{-}p}$)[16] measured for each possible isomeric pair. Although some of the most challenging isomeric pairs could not be properly resolved, the G2 cell offers near or baseline $R_{p\text{-}p}$ for the separation of cellobiose and sucrose (0.76), maltose and sucrose (1.04), and maltose and lactose (0.74). This resolution opens new applications of TWIM(CO_2)-MS in disaccharide analysis using commercial instrumentation. Note in Table 3 that the separation factor (α) is almost

Table 1. Optimized conditions for electrospray(+) ionization and traveling wave ion mobility separation

Parameter	G1	G2
Capillary voltage (kV)	2.5	0.6
Sampling cone voltage (V)	20.0	14.0
Extraction cone voltage (V)	3.0	5.0
Source temperature (°C)	150	120
Desolvation temperature (°C)	200	299
Cone gas flow (L/h)	20.0	28.0
IMS Wave velocity (m/s)	100	550
IMS Wave height (V)	30.0	40.0
Drift gas pressure (mbar)	2.00	2.00

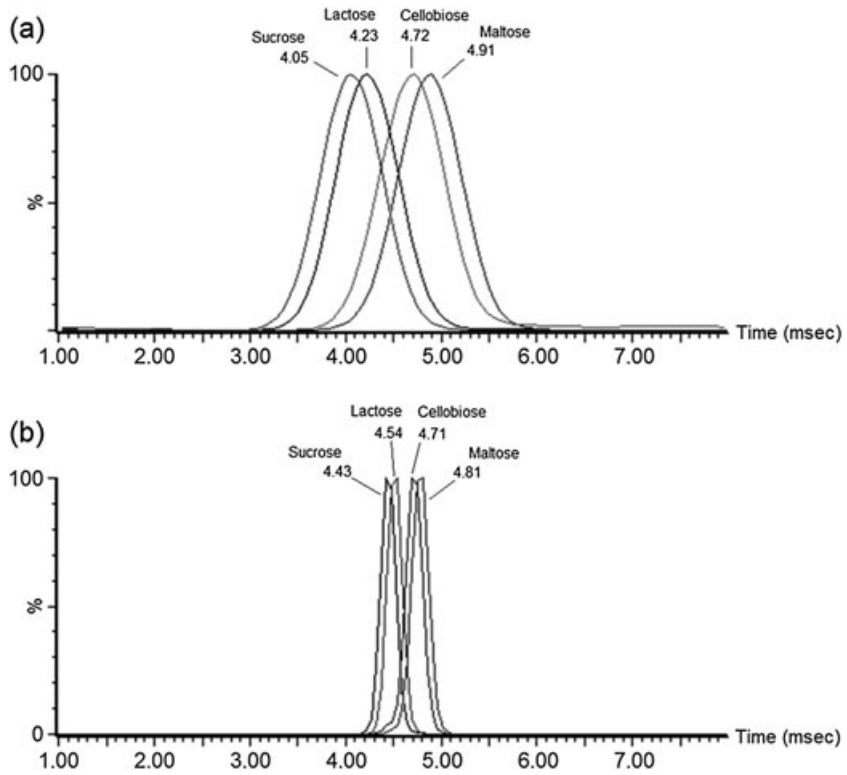

Figure 1. (a) Overlaid drift time plots using CO_2 as drift gas for sucrose, lactose, cellobiose and maltose as the $[M + Na]^+$ with Synapt G1 (2.00 mbar, wave velocity of 100 m·s^{-1}, wave height of 30 V) and (b) Synapt G2 (2.00 mbar, wave velocity of 550 m·s^{-1}, wave height of 40 V).

Table 2. Resolving power (R_p) for each isomer with Synapt G1 and Synapt G2

	R_p	
Isomer	G1	G2
Lactose	5.4	22.3
Maltose	6.4	21.9
Cellobiose	5.8	22.5
Sucrose	5.0	23.7
Average	5.6	22.4

Table 3. Separation factor (α) and peak-to-peak resolution ($R_{p\text{-}p}$) calculated for each pair of isomers with the G1 and G2 cells

	G1		G2	
Isomeric pairs	α	$R_{p\text{-}p}$	α	$R_{p\text{-}p}$
Cellobiose/sucrose	1.17	0.47	1.06	0.76
Maltose/cellobiose	1.04	0.13	1.02	0.30
Cellobiose/lactose	1.12	0.34	1.04	0.45
Maltose/sucrose	1.21	0.58	1.09	1.04
Lactose/sucrose	1.04	0.14	1.02	0.32
Maltose/lactose	1.16	0.46	1.06	0.74

the same for both instruments; hence, better $R_{p\text{-}p}$ are obtained in the G2 cell because of sharper peaks, that is, approximately four times higher R_p (Table 2).[16]

To illustrate the increase in $R_{p\text{-}p}$ offered by CO_2 as a massive and polarizable drift gas for the separation of isomeric disaccharides, separation of a lactose/maltose mixture was attempted in both the G1 and G2 cells using either N_2 and CO_2 (Fig. 2). Note the drastic effect; peaks are as expected much sharper using the G2 cell, but G2 $R_{p\text{-}p}$ using N_2 [Fig. 2(a)] is nearly as poor as the G1 $R_{p\text{-}p}$ [Fig. 2(b)] with nearly overlapping peaks. When CO_2 is used as the drift gas in the G1 cell [Fig. 2(c)], $R_{p\text{-}p}$ increases, but it is still unacceptable for the resolution of mixtures with variable proportions. But when CO_2 is used in the G2 cell [Fig. 2(d)], two well-defined peaks with nearly baseline $R_{p\text{-}p}$ are obtained for lactose and maltose. This result illustrates that the R_p of TWIM-MS cells can indeed be improved by more sophisticated designs, but the proper selection of the drift gas may be crucial for satisfactory $R_{p\text{-}p}$. The drift-time plots shown in Fig. 2 are representative examples, but different mobility conditions were tested such as varying drift gas pressure and wave parameters with no substantial changes in $R_{p\text{-}p}$.

The polarizability of the drift gas is an important parameter. CO_2 increases the resolution in ion mobility experiments likely when it establishes stronger ion–dipole interactions with the ion with longer drift time. Therefore, even if two analytes have very close cross-section values but display different interactions with the drift gas, they can migrate during different times through the drift cell, thus becoming spatially separated.

Conclusions

The ability of TWIM cells to separate disaccharides isomers has been evaluated using the G1 and G2 cells and N_2 or CO_2 as drift

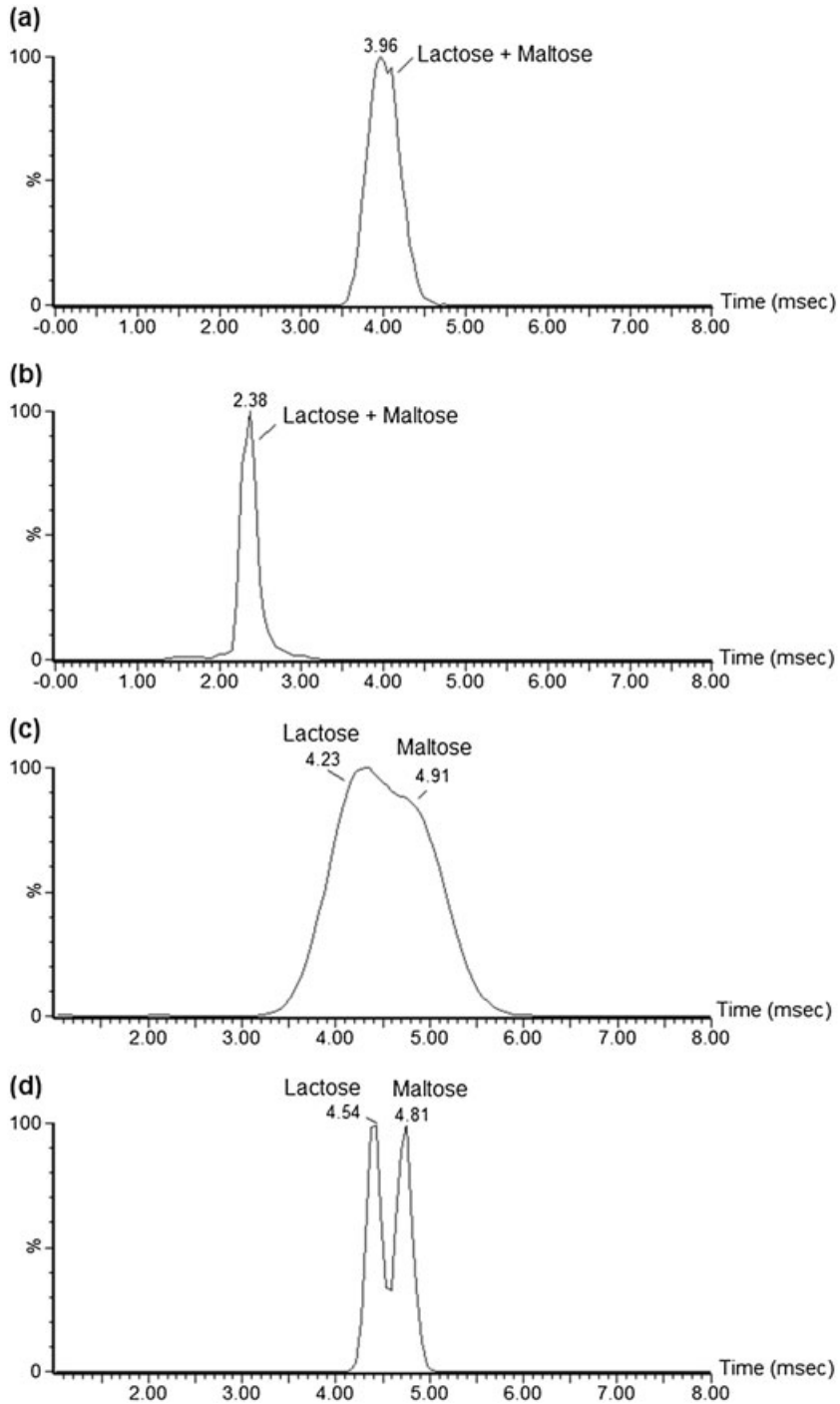

Figure 2. Overlaid drift time plots for lactose and maltose in $[M + Na]^+$ forms in the (a) G1 cell with N_2 (2.00 mbar, wave velocity of 250 m·s^{-1}, wave height of 30 V); (b) G2 cell with N_2 (2.00 mbar, wave velocity of 550 m·s^{-1}, wave height of 40 V); (c) G1 cell with CO_2 (2.00 mbar, wave velocity of 100 m·s^{-1}, wave height of 30 V) and (d) G2 cell with CO_2 (2.00 mbar, wave velocity of 550 m·s^{-1}, wave height of 40 V).

gases. Despite the higher R_p of the G2 cell, proper $R_{p\text{-}p}$ resolution of the isomers was not attained using N_2 as the drift gas. The more polarizable CO_2 was shown to greatly improve $R_{p\text{-}p}$, and near or baseline $R_{p\text{-}p}$ was achieved in the G2 cell for three pairs of disaccharide isomers, that is, of cellobiose and sucrose (0.76), maltose and sucrose (1.04), and maltose and lactose (0.74). TWIM(CO_2)-MS is found therefore to offer a fast and efficient technique for the separation of several disaccharide pairs with potential analytical applications such as for food quality control.[18] Disaccharides are often the products of enzymatic activity; hence, TWIM(CO_2)-MS may also be applied in this field. The results show also that the proper resolution of the most challenging sucrose/lactose and cellobiose/maltose isomeric pairs is still not attained via TWIM(CO_2) in the G2 cell but is feasible and would require IM cells with just a twofold to threefold increase in $R_{p\text{-}p}$.

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