Investigation of Structural Isomers of Monosaccharides by Electrospray Ionization-Ion mobility-Time of Flight Mass Spectrometry (ESI-IM-TOFMS)

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OVERVIEW

Purpose: **In this study we investigated the separation of structural isomers for monosaccharides by ion mobility mass spectrometry.**

Method: **Ambient pressure ion mobility time-of-flight mass spectrometry (AP-IM-TOFMS) was used to evaluate sixteen D-methyl glycoside isomers and four N-acetylhexosamine isomers in positive mode as $[M+Na]^+$.**

Results: **Six anomer and fourteen epimer pairs were separated by AP-IM-TOFMS.**

INTRODUCTION

- **Glycomics is a comprehensive study of carbohydrates. Isomeric variety of carbohydrate structures is an important aspect in the field of glycomics. Figure 1 shows the isomeric variation of sixteen D-methyl pyranosides and four methyl N-acetylhexosamines.**
- **Detailed information about the anomeric configuration and specific stereochemical variants of monosaccharides in the gas phase are not obtained by mass spectrometry (MS) alone because these compounds would have identical mass spectra. Examples of mass spectral data for sixteen D-methyl pyranosides and four methyl N-acetylhexosamines are shown in Figure 2 and 3.**
- **Ambient pressure IMS is a gas phase separation technique based on ion's charge, size, and shape characteristics. Chemical structures with configuration variations can be potentially resolved by IMS. The reduced mobility K_0 is used to compare isomeric compounds by the following equation:** $$Ko = \frac{L^2}{t_d \times V} \times \frac{P}{760} \times \frac{273}{T}$$

L: drift tube length in cm; t_d: drift time in seconds; V: voltage across drift tube in V; P: pressure in torr; T: temperature in K.

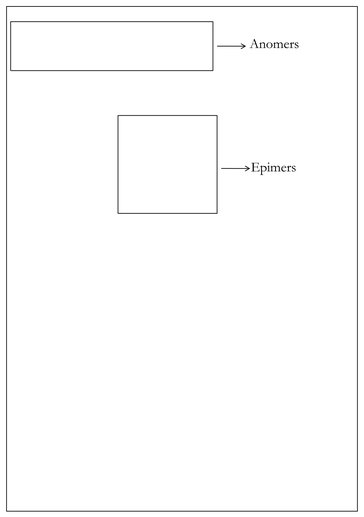

Figure 1: Structures and nomenclatures of 16 D-methyl pyranosides and 4 methyl N-acetylhexosamines

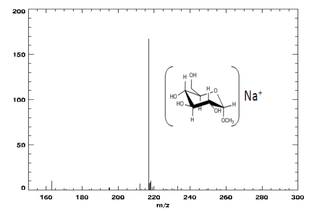

Figure 2: Mass Spectrum of α-Me-glucopyranoside as $[M+Na]^+$ at m/z 217. The other 15 D-methyl-pyranosides have the same mass spectra as shown above .

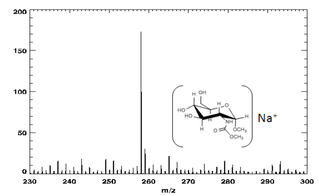

Figure 3: Mass spectrum of α-Me-N-acetyl glucosamine as $[M+Na]^+$ at m/z 258. The other 3 N-acetylhexosamines have the same mass spectra as shown above.

EXPERIMENTAL

Table 1: Summary of the electrospray and ion mobility operating parameters

Parameter	Setting
ESI voltage	14.5 KV
ESI solvent	50:50 CH_3OH/H_2O
First ring voltage	11 KV
Gate voltage	9007 V
Last ring voltage	773 V
Gate pulse width	0.2ms for individuals 0.1ms for mixtures
Electric field	412V/cm
Temperature	92°C
Sample conc.	10μM
Drift gas	Nitrogen
Gas flow rate	1.5L/min

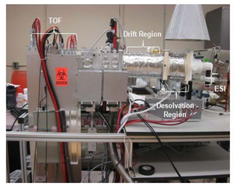

Figure 4: Instrument image of AP-IM-TOFMS

- New design: compact resistive glass tube, easy to construct.
- Mass spectrometer maximum resolution: 7000
- High transmission interface, vacuum interface efficiency is increased by addition of a series of lenses and two segmented quadrupole ion guides.
- High sensitivity: 1 μM to 10 μM
- High resolving power: 40 to 90

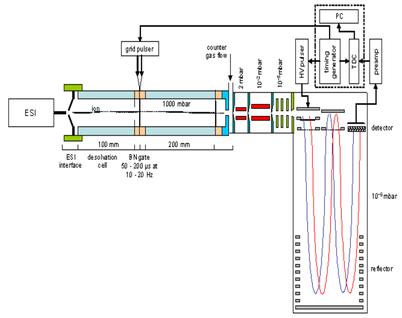

Figure5: Schematic diagram of resistive glass tube ion mobility spectrometer interfaced to a time-of-flight mass spectrometer via a 300 μm i.d. pinhole leak followed by two segmented quadrupole ion guides and a set of focusing ion lenses.

RESULTS

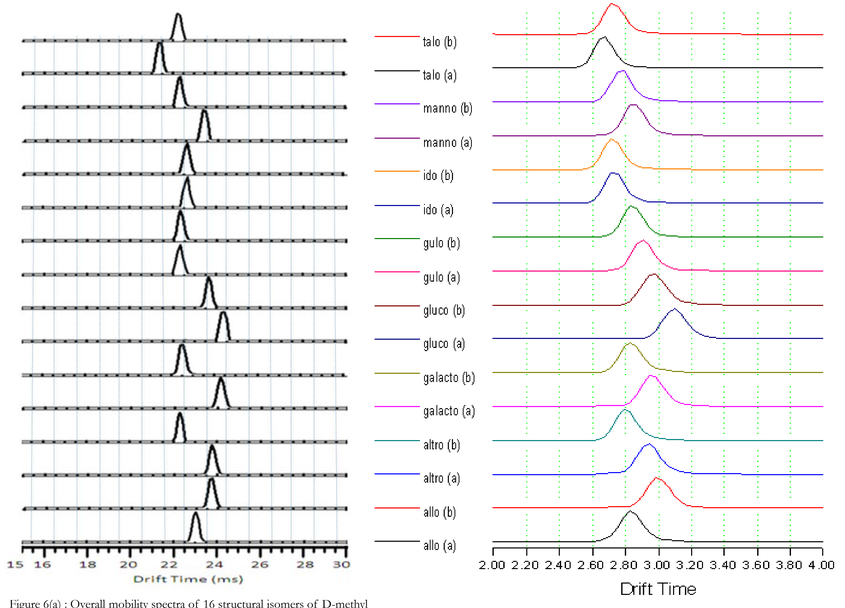

Figure 6(a) : Overall mobility spectra of 16 structural isomers of D-methyl pyranosides as $[M+Na]^+$ on AP-IM-TOFMS. Mobility extracted spectra for m/z 217 collected in 10 s was used to generate the mobility peaks. However, α&β-Me-altrosides and α&β-Me-Gulosides were collected in 50 s.

Figure 6(b) : Overall mobility spectra of 16 structural isomers of D-methyl pyranosides as $[M+Na]^+$ using WATERS Synapt G2 . Each data was acquired for two minutes with at a flow rate of 5 μl/min. The sample concentration was10 μM in 1:1 $MeOH/H_2O$. The IMS cell was operated at nominally 3mb N_2 with a 40V, 900 m/s T-Wave. This data set was provided by the courtesy of Dr. Kevin Giles of WATERS MS technologies center, Manchester, UK

RESULTS

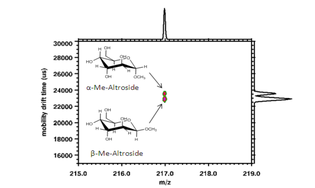

Figure 7: 2D IM-TOFMS spectrum illustrating the separation of anomers of α-Me-altroside and β-Me-altroside as sodiated adducts at m/z 217

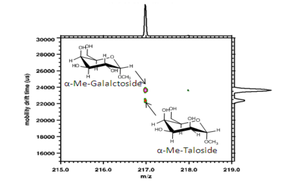

Figure 8: 2D IM-TOFMS spectrum illustrating the separation of epimers of α-Me-Taloside and α-Me-Galactoside as sodiated adducts at m/z 217

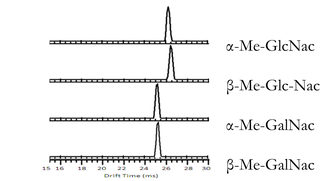

Figure 9: Overall Mobility peaks for α&β-Methyl-N-acetylglucosamine and α&β-Methyl-N-acetylgalactosamine

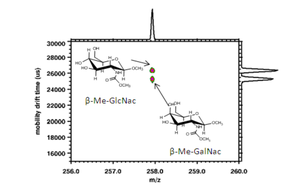

Figure 10: 2D IM-TOFMS spectrum illustrating the separation of epimers of β-Me-GlcNac and β-Me-GalNac as sodiated adducts at m/z 258

Table 2: Reduced mobilities, drift times and resolving powers for all 16 structural isomers of D-Me-Pyranosides and 4 structural isomers of Methyl N-acetylhexosamines

Compound	T_d(ms)	K_o	R_p	Compound	T_d (ms)	K_o	R_p
β-Me-Taloside	22.28	1.45	66	β-Me-Galactoside	22.53	1.47	66
α-Me-Taloside	21.44	1.53	73	α-Me-Galactoside	24.15	1.39	70
β-Me-mannoside	22.26	1.48	70	β-Me-Altroside	22.31	1.48	77
α-Me-Mannoside	23.21	1.43	73	α-Me-Altroside	23.89	1.40	72
β-Me-Idoside	22.64	1.48	71	β-Me-Alloside	23.70	1.40	68
α-Me-Idoside	22.56	1.47	71	α-Me-Alloside	23.03	1.44	72
β-Me-Guloside	22.30	1.49	69	α-Me-GlcNac	26.34	1.26	82
α-Me-Guloside	22.31	1.48	66	β-Me-GlcNac	25.96	1.26	81
β-Me-Glucoside	24.23	1.40	74	α-Me-GalNac	24.91	1.32	81
α-Me-Glucosie	22.90	1.37	75	β-Me-GalNac	24.93	1.32	84

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CONCLUSIONS

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- **WATERS Synapt G2 can separate monosaccharides isomers even though in low resolving power (15); Higher resolving power and sensitivity are observed in the AP-IM-TOFMS compared to the Waters G2.**
- **In general, many drift time patterns match between the two instruments.**
- **Monosaccharides structural isomers exhibited different mobility drift times depending on anomeric and stereochemical configurations.**
- **IMS is valuable in providing ion structural information, resolving subtle conformational variations and then serves as a complementary tool for MS analysis of structural isomers.**

ACKNOWLEDGEMENT

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