

Use of ion-mobility mass spectrometry (IMS-MS) to map polyoxometalate Keplerate clusters and their supramolecular assemblies†

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We present the high-resolution (HRES-MS) and ion-mobility (IMS-MS) mass spectrometry studies of icosahedral nanoscale polyoxometalate-based $\{L_{30}\}_n(\text{Mo})\text{Mo}_5$ Keplerate clusters, and demonstrate the use of IMS-MS to resolve and map intact nanoclusters, and its potential for the discovery of new structures, in this case the first gas phase observation of 'proto-clustering' of higher order Keplerate supramolecular aggregates.

The assembly and characterisation of nanoscale structures is an exciting challenge in inorganic chemistry.¹ Polyoxometalates (POMs) are a class of inorganic nanomolecular architectures; a huge variety can be assembled through acidic condensation from MO_6 octahedra of early transition metals such as tungsten, molybdenum and vanadium, resulting in a virtually unlimited range of rigid and highly charged cluster types.² Understanding the mechanism of their aqueous self-assembly is vital to aid discovery and develop design strategies.³ Unlike coordination-based inorganic nanostructures, structural analysis of POMs is mainly limited to the solid phase (primarily XRD) and pure products. The identification and analysis in solution, and of mixtures of increasing complexity, remains a challenge.⁴

Recently, high resolution electrospray ionisation mass spectrometry (ESI-MS) has become an important tool in addressing this challenge. It has also been used to study POM self-assembly mechanisms,^{5,6} and as a discovery tool to detect new structures present in reaction mixtures prior to isolation.⁷⁻⁹ Unfortunately, deconvolution of these systems can be difficult: use of a narrow range of building blocks, often in vast numbers, yields multiple species of very similar mass-to-charge ratios (m/z), but very different sizes/structures, resulting in overlapping envelopes in the mass spectra.¹⁰ Ion mobility mass spectrometry (IMS-MS) is promising as a means to resolve this plethora of isobaric ions, separating according to their size/shape along with m/z . It has been applied extensively in the investigation of biomolecules, polymers and

carbon clusters,^{11,12} but relatively little-used in supramolecular chemistry. Its use has also been demonstrated in the separation of isomers and assemblies of hybrid organic/inorganic POMs,¹³ as well as coordination-based structures.¹⁴⁻¹⁷

Herein, we present high-resolution (HRES-MS) of a small selection of related clusters, and demonstrate that IMS-MS can be used to resolve and characterise large POM clusters. This should facilitate their continued exploration and pave the way for the discovery of new self-assembled structures – a scenario illustrated here in our first observation of 'proto' clustering of Keplerate ions in the gas phase.

We chose Keplerate clusters, a class of large POM nanostructures, as an archetypal example to explore the potential of applying IMS-MS to the self-assembly of nanocluster systems. They are spherical icosahedral, Fullerene-like assemblies consisting of twelve pentagonal $\{(\text{Mo})\text{Mo}_5\}$ building blocks that are connected by thirty linear mono- or bi-metallic linkers (see Fig. 1). Their immense size and limited solubility in many solvents makes ionisation difficult and has critically limited previous work in this area. The compounds we have investigated are detailed in Table 1.

Compounds **1**,¹⁸ **2**,¹⁹ **3**²⁰ and **4**²¹ were chosen to cover a range of linker units, sizes, and solubilities. Compounds **1-3** are water-soluble (and relatively insoluble in less polar solvents), while compound **4** is soluble in acetonitrile, by virtue of tetra(*n*-butyl)ammonium (TBA) counter-cations. Compounds **1**, **2** and **4** feature

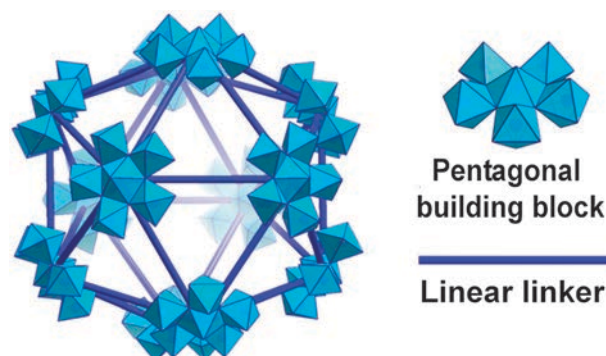


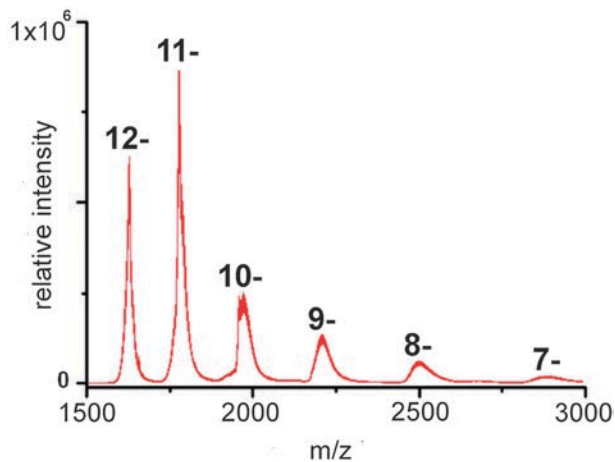
Fig. 1 Schematic representation of Keplerate cluster structure ($\{(\text{Mo})\text{Mo}_5\}$ units as light blue polyhedral, linear linkers as dark blue rods).

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† Electronic supplementary information (ESI) available: Experimental details, full HRES-MS and IMS-MS spectra, and details of corroborating MS/MS experiments. See DOI: 10.1039/c3cc38615e

Table 1 Formulation of clusters 1–4

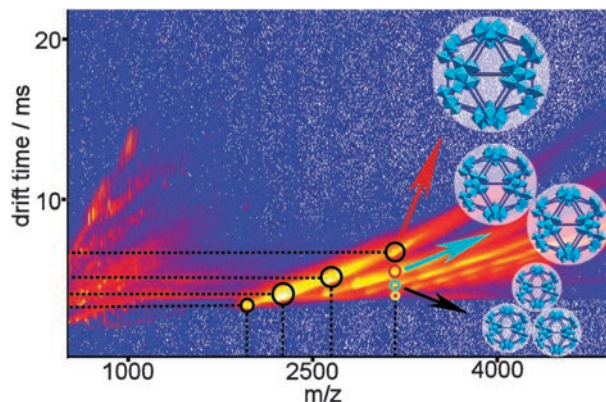
| | |
|---|--|
| 1 | $(\text{NH}_4)_{42}[\text{Mo}^{\text{VI}}_{72}\text{Mo}^{\text{V}}_{60}\text{O}_{372}(\text{CH}_3\text{COO})_{30}(\text{H}_2\text{O})_{72}]$ |
| 2 | $\text{Na}_6[\text{SiMo}^{\text{VI}}_{12}\text{O}_{40}@\text{Mo}^{\text{VI}}_{68}\text{Mo}^{\text{V}}_4\text{Fe}^{\text{III}}_{30}\text{O}_{252}(\text{CH}_3\text{COO})_{16}(\text{H}_2\text{O})_{100}]$ |
| 3 | $(\text{NH}_4)_{36}[\text{Mo}^{\text{VI}}_{72}\text{Mo}^{\text{V}}_{60}\text{O}_{372}(\text{C}_3\text{H}_5\text{COO})_{24}(\text{H}_2\text{O})_{78}]$ |
| 4 | $[\text{N}(\text{C}_4\text{H}_9)_4]_{36-n}\text{K}_n[\text{Mo}^{\text{VI}}_{72}\text{V}^{\text{IV}}_{30}\text{O}_{282}(\text{SO}_4)_{12}(\text{H}_2\text{O})_{56}]$ |

**Fig. 2** HRES-MS spectrum of compound 1 showing envelopes of ions with charges ranging from –12 to –7.

distinct linker units, while 3 has the same outer surface as 1, modified with hydrophobic butyrate ligands on its interior.

Sample preparation is crucial for ionisation in ESI-MS, especially for compounds mainly soluble in water.²² 1, 2 and 3 were dissolved in a minimum amount of water, then a portion was diluted with acetonitrile by a factor of 100 and filtered, while 4 was simply dissolved in acetonitrile. Using these solutions, we were able to acquire HRES-MS spectra of all four compounds; in each case observation of a series of highly-charged species (typical cluster mass of *ca.* 20 kDa) demonstrates that the structures remain intact in solution and in the gas-phase (corroborated by CCS data, *vide infra*). Fig. 2 shows the HRES-MS spectrum for compound 1, which yielded peak envelopes with well-resolved isotope separation in six different charge states, down to –12. Precise assignment of a formula to the broad peak envelopes is intrinsically problematic as a range of ions are formed by association/dissociation of solvent molecules and accompanying ions, however, each may be attributed to an intact Keplerate structure (see ESI† for putative assignments of each). Compounds 2, 3 and 4 are also observed with sufficient resolution to determine the charges of at least three peak envelopes in each spectrum and hence demonstrate that intact cluster ions are being detected in each case. Full spectra and samples of well-resolved isotopic separations are provided in the ESI† (S1–S4).

IMS-MS measurements were acquired for the same solutions using a commercially-available travelling wave ion-mobility spectrometer (TWIMS), where ions are separated according to their size as well as *m/z*, as the drift time is dependent on the collision cross-section (CCS).²³ For all four compounds, the HRES-MS and the IMS-MS spectra (see ESI† for IMS-MS spectra, S5–S8) exhibit similar *m/z* distribution, however since acquisition of the IMS-MS spectra was performed in “sensitivity mode”, some resolution was lost and the charge could not be observed directly. Fragmentation due to

**Fig. 3** IMS-MS of compound 2, with intensity represented by a colour-coded log scale (blue to yellow, see ESI†). Peaks circled in black can be assigned to intact monomeric clusters, the vertically displaced red, blue and yellow circles can be assigned to ‘clusters of clusters’. [nb. Keplerate schematics are for illustration and not an absolute structure]

heating within the TWIMS drift tube is a phenomenon known in delicate molecules such as proteins,²⁴ and can give misleading results. MS/MS experiments were conducted to demonstrate the clusters’ robustness in these conditions, with negligible fragmentation observed in the drift tube (see ESI† for example, Fig. S9).

Fig. 3 shows the IMS-MS spectrum of compound 2. The main feature, observed in all cases, is the intense (yellow) line of peaks which correspond to individual cluster ions (an example series is circled in black) as observed in the HRES-MS. Since an ion’s drift time is exponentially dependent on its charge, and the CCS and mass vary little between charge states, these peaks are arranged in parabolic curves. This well-defined region of the spectra may be considered similar to the areas of ‘conformational space’ previously identified in IMS-MS spectra as being characteristic for the different classes of biomolecules.²⁵ Mapping of this space represents a particularly powerful and rapid tool for cluster discovery: just as peptides and lipids occupy different regions, so the large intact nanostructures are clearly resolved from other signals, such as those corresponding to fragments (which are above or to the left in all cases; *i.e.* lower *m/z* and/or longer drift time).

The potential of this mapping approach to quickly identify large new species can be appreciated on further inspection of the IMS-MS spectrum of compound 2. Vertically displaced with respect to the hyperbola of peak envelopes corresponding to a monomeric series, a number of unexpected peaks are clearly resolved, in a space not occupied by fragmentation products (higher *m/z* and/or shorter drift time *cf.* single clusters). MS/MS experiments show that these new species dissociate to yield intact single clusters (see ESI† S10), identifying them as oligomeric ‘cluster of clusters’ assemblies, analogous to those shown in recent studies on organic POM hybrid compounds.¹³ The formation of the so-called ‘clusters of clusters’ or ‘blackberries’ of Keplerates in solution has long been known,²⁶ however we are not aware of any such species being observed in the gas-phase until now, although it is known that 2 can aggregate in the solid state.¹⁹ This observation promises to open new avenues towards the understanding of the supramolecular self-assembly of POMs over the entire range of length scales and ‘orders’, from monometallic units to building blocks, to clusters, and clusters

of clusters, a hierarchical ordering somewhat analogous to primary, secondary, tertiary and quaternary structure of protein assembly.

Unfortunately, unlike linear drift tube IMS, TWIMS instruments require calibration using ions of known CCS to relate drift time (t_D) to observed CCS.²³ While positive ion calibrants are plentiful, relatively few are known for negative ions;^{27,28} fewer still are based on published experimental data on commercially-available materials. Resolving this issue is beyond the scope of this communication; instead CCSs are estimated using calibration²³ against the only two readily available calibrants²⁷ appropriate for the analyte POMs (see ESI† for full data). Broadly, the CCSs observed for the intact monomeric clusters of each unmodified Keplerate (1–3) do not vary significantly across the observed charge states. For example the estimated CCS of **1** is near-constant across six charge states (from -12 to -7 it does not vary more than 3% from 1051 \AA^2), and not significantly different from that of **3** (the outer shell of which is identical). This further corroborates assignment of these ions as intact spherical structures, and their robustness in the gas phase, a marked contrast to proteins where large variation in charge tends to lead to adoption of alternative conformations with very different CCSs.²⁷ In **4** considerable stepwise increase in CCS is observed on reduction of charge, however this can be attributed to the addition of bulky tetrabutylammonium (TBA) cations at each of these steps, rather than instability of the spherical structure. Similarly, the observation of two differently-sized ions at the -8 charge state may be attributed to populations with different proportions of K^+ and TBA counterions.

In conclusion, we have presented the first HRES-MS and IMS-MS spectra of large inorganic POM clusters and the first observation of their higher-order assemblies in the gas phase. Furthermore, we have shown that the location of the signals in defined areas of the IMS-MS spectra is unique to intact Keplerate clusters, resolving them from fragments/building blocks even without knowledge of structure. In future, this approach will be very useful in the identification of new structures, for example from crude reaction mixtures, driving systematic efforts to isolate structures that would otherwise only be obtained serendipitously. Now that IMS-MS instruments have become commercially-available such an approach may increasingly be applied to hierarchical self-assembled systems beyond POMs, complementing current analytical techniques as they become stretched by ever-larger assemblies and more complex libraries of building blocks.

This work is limited in quantitative analysis of CCSs by the limited availability of suitable calibrants. Accurate quantitative determination of CCS would also allow further structural analysis of clusters and assemblies by comparison to suitable models (also currently lacking tested parameters suitable to study POMs) and the definition of molecular classes based on the space occupied in the IMS-MS spectrum. Based on the evidence presented here, we propose POMs as ideal candidates for calibration of negative ion TWIMS, due to their stability, high charge density, multiple accessible charge states, and structural rigidity. In future work we intend to develop POM-based

calibrants (obtaining linear drift tube IMS measurements of CCS), and continue to directly probe the self-assembly of polyoxometalate clusters from monomeric units over the entire assembly range to cluster-of-cluster assemblies.²⁶

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