

## Mass Spectrometry Forum

# Masses in Mass Spectrometry: Balancing the Analytical Scales

Mass spectrometrists have been measuring masses in the ultrasmall world for the past 50 years. Ions routinely traverse our instruments and reveal their structural secrets through a number of well-known experiments. However, a sense of our own accomplishments does not derive from the same perspective linking ultrasmall masses to the normal-mass world.

**Kenneth L. Busch**

A science news release in early 2004 (1) described how scientists “weighed” a single bacterium suspended on the end of a cantilevered atomic force microscopy (AFM) probe. The writer of the article took care to explain the scales of weights in the ultrasmall world, making sure readers could handle the trillionths and billionths, and masses counted in the pico- and femtogram scales. We appreciate the process of weighing the bacterium because we know how we weigh ourselves, and the difference in scales encourages our interest.

Readers of this column know, of course, that mass spectrometrists have been measuring masses in the ultrasmall world for the past 50 years. Ions, starting with atomic ions and growing into large ionic complexes containing thousands of atoms, routinely traverse our instruments, and reveal their structural secrets in a multitude of well-known experiments. However, a sense of our own accomplishments does not derive from the same perspective linking ultrasmall masses to the normal-mass world, as in the example of the bacterium. Instead, mass spectrometrists operate stealthily, and technical achievements seem subsumed within images of a robotic laboratory filled with blinking beige boxes, spitting out reams of data arrayed in neat charts and tables. Mass spectrometers are seen (briefly) on the news when the dopant screening test of an Olympic competitor is revealed to be positive. Public recog-

niton of mass spectrometry (MS) seems to be derived not from our outstanding technical feats, but instead (citing a few examples) for revelations of wrongdoing, from feats of forensic analysis, or from discovery of environmental degradation. In a quest for a different recognition for our accomplishments, should we relabel our mass scales from “kDa” or “ $m/z$ ” to yoctopounds (yocto denotes  $10^{-24}$ ), so that we can connect with a broader scientific population? Such a strategy reveals some inherent inability to grasp the great breadth of scales within which we work. To increase our appreciation, this column returns to the topic of mass scales and analytical scopes.

### Back to Basics

The writer of the bacterium article mentioned above connected the scale of the mass for a single bacterium (about 665 fg) to masses of the everyday world (kilograms and pounds). Within the even smaller world of masses in our mass spectra, we seem to forget the scales of the mass units with which we work everyday. Nothing brings this point to mind as explicitly as teaching the first few sections of an introductory course on MS (2). We gloss over the connection between daltons (Da) and grams, and between the world of isolated ions (where we measure masses from  $m/z$  1 of hydrogen to ions/particles with masses of several million daltons, and intriguing structural subtleties), and the world of still larger particles such as bacteria. We need to extend our small atomic and molecular world into the higher reaches of the still ultrasmall masses of particles and conglomerates. We already are extending our analytical reach into that domain. Study of noncovalent interactions in complex ions is now common practice. It even has been suggested that viral particles that pass as ions through a mass spectrometer retain their viability once collected on a surface and reconstituted. In our appreciation of an analytical scale encompassing the 1 Da mass of a proton through to gigadalton masses of prebiotic ions, we must think with fresh perspective about basics: what mass is, how we measure mass with our instrumentation, and what mass-changing reactions are at the core of MS.

**Kenneth L. Busch** wages an unceasing, and so far unsuccessful, battle to replace the common term “molecular weight” with “molecular mass,” as well as a personal battle against a steadily increasing, age-related body mass. One of the first mass spectrometers used by F.W. Aston to refine understanding of mass and isotopes is in the British Science Museum, though not displayed with anywhere near sufficient prominence. However, the collection archives of the British Museum are now open for tours. The views in this article are those of the author and not those of the National Science Foundation. The author can be contacted at [wvverners@yahoo.com](mailto:wvverners@yahoo.com).



This series of four consecutive articles in “Mass Spectrometry Forum” therefore visits and revisits various aspects of reality, subtlety, and new perspectives in the ultrasmall-mass world, including basic definitions, common misconceptions, approaches to calibrations, and mass informatics. This first column deals primarily with standard definitions and uses. The second column includes a discussion of calibration in classical organic MS, emphasizing topics such as perfluorinated mass calibration compounds, and the realities of internal versus external mass calibration. The third column expands the issue of mass calibration into the higher masses of biomolecular or polymeric ions, and special challenges intrinsic in dealing with higher mass and often highly charged ions in terms of both analysis and detection. The fourth and final installment in the series collects more specialized and perhaps new perspectives relevant to mass in MS — the sizes and shapes of ionic particles, locations of charges on megamolecules, and the evanescence of charge. We first provided an overview of the basic topic of mass 10 years ago in “Mass-scale Calibration and the Case of the Mass Defect,” published in this column in June 1994. The basics have not changed, but our uses and users of MS have. We are overdue for a revisit to the fundamentals, and build here upon the content of the previous article.

### Linking Masses

We begin with the working definition of mass appropriate for molecular MS. Then we will proceed methodically both back to the standards of definition, and to an example of modern practice in analysis. The molecular ion mass derived from a molecule *M* is defined as the summation of the masses of its atomic components, including the change in mass required to produce a charge (loss of an electron or addition of a proton, usually). For a singly charged positive ion ( $M^+$ ), add the atomic masses of all the

component atoms and then subtract the mass of the electron. Similarly, for  $M^-$ , add the atomic masses and then add the mass of an electron. For each such summation then, we must know the accurate values of the masses of the atomic components. Underlying atomic masses are the masses of the subatomic particles, and so we start there. Scientists have labored for many years to measure these values, and establish accuracy and precision for them. For example, we can specify with a known degree of accuracy and precision the value of the exact mass of a proton, neutron, or electron (see Table I). The masses in the table are given in kilograms and daltons, and, in keeping with this column’s focus on a linkage of scales, the value of the dalton is converted into units of pounds.

We establish quickly, however, that simple summation of the masses of these subatomic particles does not equal the masses of the atoms, which also are measured with great accuracy and precision. The difference in summed masses and actual masses corresponds to the nuclear binding energy between the subatomic particles. Aston, who won the Nobel Prize in Chemistry for his work in MS, played a central role in the development of the concept of the binding energy, and the mass defect that results. Such values as the exact masses given in Table II occupy our attention in the practice of modern MS. In previous “Mass Spectrometry Forum” columns, we presented the carbon-12 mass standard and its use as the anchor point for the masses of all the other atoms. Note that the masses in Table II are given in daltons; conversions into kilograms or pounds can be accomplished with the values in Table I. Knowing that a mole of carbon-12 atoms has a mass of exactly 12 g, the calculation of Avogadro’s number is left as an exercise for the reader. Master the connection between the tables, and the link between masses in MS, the bacterium, and the bathroom scale that reads in pounds is concomitantly forged. Remember that the

**Table I. Exact masses of fundamental subatomic particles and the dalton**

Proton	$1.67262171 \times 10^{-27}$ kg	1.00727646688 Da
Neutron	$1.67492728 \times 10^{-27}$ kg	1.00866491560 Da
Electron	$0.91093826 \times 10^{-30}$ kg	$5.4857990945 \times 10^{-4}$ Da
Dalton	$1.66053873 \times 10^{-27}$ kg	$3.660861212 \times 10^{-27}$ lb

Data is taken from the CODATA NIST site: <http://physics.nist.gov/cgi-bin/cuu/Constants/index.html>

**Table II. Exact masses of selected isotopes of atoms (in Da)**

<sup>1</sup> H	1.007825
<sup>12</sup> C	12.000000
<sup>13</sup> C	13.993355
<sup>14</sup> N	14.003074
<sup>15</sup> N	15.000108
<sup>16</sup> O	15.994915
<sup>17</sup> O	16.999133
<sup>18</sup> O	17.999160

masses listed in Table II suffice for use within the velocity regime of most MS ( $10^6$  m/s or slower); we are not concerned here with relativistic corrections.

Compilations of the exact masses of the atoms can be found in multiple reference sources, and they should be posted above the desk of every mass spectrometrists.

### A "Clear" Example

As an example of the determination and calculation of the exact mass of an ion, we choose an ion from a compound of some interest for those who followed the 2004 summer Olympic games, and the associated dopant controversies. The news stories continue even now about the analytical discovery of an "undetectable" dopant called "clear." The (then) anonymous receipt by a testing laboratory of a syringe containing traces of the dopant led to the structural elucidation of "clear" and the subsequent inclusion of this compound in the steroid testing protocol. "Clear" ultimately was identified as tetrahydrogestrinone (THG), with the molecular formula of  $C_{21}H_{28}O_2$ . The analytical detective story of its identification followed a standard approach, including measurement of an exact mass, which we describe briefly here. The full story is presented by Catlin et al. (3).

A first analysis of the underivatized sample from the rinse of the syringe used a gas chromatography (GC) separation followed by electron ionization and mass spectral measurement at a resolution of 10,000. The predominant component of the residue was evident in an isolated GC peak characterized by a mass spectrum that suggested a molecular ion  $M^{+}$  at  $m/z$  312. The high-resolution mass spectrometer (properly calibrated) produced an exact mass measurement of 312.2080 Da for the putative molecular ion of the compound eluted in that GC peak.

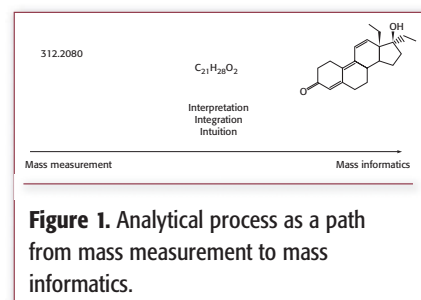
Only certain ion empirical formulas

for the putative molecular ion of "clear" will be in concordance with the measured value, and the errors known to be associated with that measurement. Previewing the answer, calculate the exact mass of the formula, using the values given in Table II. Calculation provides the answer of 312.2089 ( $[21 \times 12] + [28 \times 1.007825] + [2 \times 15.994915]$ ), within the range of measurement error. This is the exact mass of the molecular ion of THG consisting of <sup>12</sup>C, <sup>1</sup>H, and <sup>16</sup>O, and the exact masses of these isotopes were used in the summation. To prove that the mass spectrometer measures the mass of that ion correctly, one must prove that the instrument measures the mass of standards to the requisite accuracy. The standard compound used to calibrate the mass scale for instrument operation and to demonstrate proper instrument operation for mass accuracy is termed the mass calibrant, and we deal with that issue in the second column of this article series.

That first exact mass measurement of the compound in the syringe provided immediate clues about possible structures. Other experiments followed, and the identification of "clear" as THG followed more or less routine unknown identification procedures. In addition to identification, quantification is important. Catlin et al. established a limit of detection of 0.5 ng/mL for THG in urine, using GC coupled with MS. How much is this, actually? Scales and units come into play again. That limit of detection is about half a part per million in urine. Half a nanogram of THG is about 1 picopound of THG, and about  $10^{12}$  molecules of THG. Each individual molecule of THG weighs 1.14 yoctopounds. We seem far removed from the weight of even a single bacterium, but read on.

### Detective Work

Figure 1 illustrates in a very general sense the progression from a measured value to an empirical formula through to a molecular structural formula, and the transition from mass measurement to mass informatics through processes of spectral interpretation, integration of other chemical information, and analytical intuition. Such extraordinary detective stories, which mass spectrometrists accomplish on an everyday basis, are ex-



**Figure 1.** Analytical process as a path from mass measurement to mass informatics.

emplified in the story of THG. In determining the structure of an unknown such as THG, analysts use the basic measurement strategy of determining an exact mass, providing clues as to the empirical formula of the compound, and then interpretation of the mass spectral fragmentation pattern, structural analogies to known compounds, and a knowledge of common synthetic methods. Publicity (and a Web search will confirm for you that there was indeed a great deal of publicity) derived from the findings, and not from the process. Eventually, the elegant AFM experiment by which the single bacterium was weighed will fade from direct public view, to simply become part of the scientific armamentarium along with MS. We are ahead of this game because millions of mass spectra are recorded every day, weighing individual ions and quantifying them in an ultrasmall world. Our ultrasmall world is getting bigger as mass spectrometrists develop proteomics protocols, expand polymer and dendrimer analysis, and experiment with prebiotic particle manipulation and examination. Some balance bacteria on the end of an AFM tip; mass spectrometrists already seek to identify bacterial strains through clever experiments in mass informatics (4).

### References

1. <http://news.bbc.co.uk/2/hi/science/nature/3607993.stm>
2. Short Course "Introduction to Mass Spectrometry" taught by the author at PittCon every year since 1995.
3. D.H. Catlin, M.H. Sekera, B.D. Ahrens, B. Starcevic, Y.-C. Chang, and C.K. Hatton, *Rapid Commun. Mass Spectrom.* **18**, 1245–1249 (2004).
4. V. Ruelle, B. El Moulaj, W. Zorzi, P. Ledent, and E. De Pauw, *Rapid Commun. Mass Spectrom.* **18**, 2013–2019 (2004). ■