# Readers' Tribune

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## Molecular weights under discussion?

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The molecular weight ($M_r$) of a compound is a fundamental value, which describes an important aspect of a molecule in a quantitative way. In the European Pharmacopoeia (Ph. Eur.), it is given in monographs for information only; in other words, it is not part of the mandatory quality specifications but the value given should be used, if needed, in the practical analytical tests. The value given, $M_r$, is the isotopic-weighted average: it is calculated from the molecular formula and the atomic weights of the constituting atoms, which are dependent on the atomic masses of the isotopes of each element and the corresponding mole fractions of these isotopes. However, contrary to general belief, the atomic weight ($A_r$) is not a fixed error-free theoretical value: conceptually, it will not only vary with place (deep-sea versus mountain) and time (on a geological time scale), but the method used for this $A_r$ determination will also influence the uncertainty accounting for both systematic and random errors. Using different normal terrestrial samples and analytical techniques, different results are thus obtained by different groups for the $A_r$ values. In all cases, the reference value used is $A_r$ ($^{12}C$) equals 12. The International Union of Pure and Applied Chemistry (IUPAC) biennially publishes an updated Table of Standard Atomic Weights, based upon careful evaluation of results obtained and an overall statistical evaluation. This evaluation also explicitly includes the uncertainty, which is reflected in the number of significant figures. Hence, in its most recent Table of Standard Atomic Weights [1], interval values [*a* ; *b*] are given for some elements rather than a single value corresponding to the best individual estimate [2]. The lowest value *a* is obtained from the lowest experimental value found with a representative terrestrial sample, lowered by its uncertainty and rounded to the last certain digit. In the case of carbon, the fifth digit after the decimal point was uncertain, hence, its $A_r$ value is reduced to four figures after the decimal point. The IUPAC commission may still deviate from the accepted rounding rules and widen the limits in the rounding step to ensure that normal terrestrial materials all safely fall within the interval. The interval is thus obtained by a so-called type B uncertainty evaluation, and it should not be considered as some Gaussian-type of confidence interval; hence, the mid-value of the interval cannot be taken as a best single estimate. As it is impractical to work with interval values in a pharmacopoeial context, even though it is more correct, we have moved to abridging the lower *a* and upper *b* bounds to fewer figures until *a* equals *b*. These abridged atomic weights are given in the last column of Table 1.

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Table 1 - *Atomic weights*

| Element name | Symbol | Atomic number | Atomic weight[1] | Atomic weight interval [a ; b][2] | Abridged atomic weight |
|---|---|---|---|---|---|
| Carbon | C | 6 | 12.0107 (8) | [12.0096 ; 12.0116] | 12.01 |
| Hydrogen | H | 1 | 1.00794 (7) | [1.00784 ; 1.00811] | 1.008 |
| Oxygen | O | 8 | 15.9994 (3) | [15.99903 ; 15.99977] | 16.00 |
| Nitrogen | N | 7 | 14.0067 (2) | [14.00643 ; 14.00728] | 14.01 |

[1] Standard values as from IUPAC (2007) [2]. The uncertainty for given atomic weights is given in parentheses following the last significant figure to which it is attributed.

[2] Standard values as from IUPAC (2009) [1].

While for low molecular weight compounds, this will hardly make a difference, this is by no means so for higher molecular weight compounds such as peptides, proteins, oligo/polynucleotides and oligo/polysaccharides. The number of these oligo/polycompounds is expected to increase in the pharmaceutical field as they are steadily replacing small molecules which, even though they currently predominate, are being rapidly phased out. Moreover, the importance of clearly agreeing on $A_r$ values and their definition will also increase due to technical advances, where mass spectrometry (MS) is progressively becoming a routine analytical technique in the pharmaceutical quality environment due to its increased analytical performance and its decreased costs and operator complexity. Since isotopic distribution and monoisotopic masses are commonly used in MS, for current pharmacopoeial monographs, at least an agreed consensus on $A_r$ and hence $M_r$ values should be reached and formally communicated. Within the analytical MS community, software is often used which will also give the user "an average" molecular mass. In general, these types of software are consistent in their results. For example, polymyxin $B_1$ has the molecular formula $C_{56}H_{98}N_{16}O_{13}$ and the different values obtained for its $M_r$ are given in Table 2. We have included all figures and did not perform any rounding.

Table 2 - *Polymyxin $B_1$ $M_r$ values*

| Source | Molecular weight | Rounded molecular weight |
|---|---|---|
| Ph. Eur. [3] | 1204 | 1204 |
| MolE [4] | 1203.49416 | 1203 |
| Lenntech [5] | 1203.47 | 1203 |
| Environmental chemistry [6] | 1203.494 | 1203 |
| BMRB [7] | 1203.479587 | 1203 |
| IUPAC (2009) a [1] | $1203.3961_9$ | 1203 |
| IUPAC (2009) b [1] | $1203.5578_7$ | 1204 |
| IUPAC (2009) abridged | $1203.50_4$ | 1204 |
| IUPAC (2007) [2] and Exploring chemical analysis [8] | $1203.4767_2$ | 1203 |

It is thus observed that different values are obtained: if we round them to the unit (amu), we thus obtain 1204 or 1203. Both values reflect in some way the uncertainty interval, and in this case the IUPAC abridged value gives indeed the Ph. Eur. value of 1204. Taking the correct full IUPAC approach, an interval of [1203.3962 ; 1203.5579] is defined.

In conclusion, as the Ph. Eur. represents state-of-the-art science, it is recommended that atomic weights, and derived molecular weights, are aligned with the most recent IUPAC data and that they are explicitly clarified. This will also prepare the Ph. Eur. for more detailed MS information which will be required in the near future.

## References

[1] Wieser ME, Coplen TB. Atomic weights of the elements 2009 (IUPAC Technical report). *Pure Appl. Chem*. 2011;**83**(2):359-96.

[2] Wieser ME, Berglund M. Atomic weights of the elements 2007 (IUPAC technical report). *Pure Appl. Chem*. 2009;**81**(11):2131-56.

[3] Polymyxin B sulfate, monograph 0203. Ph. Eur. 7th Edition. Strasbourg, France: Council of Europe; 2010 (vol 2).

[4] Rozenski, J. MolE – Molecular mass calculator v2.02 [website] [available at: http://library.med.utah.edu/masspec/mole.htm, accessed 2011 Oct 5].

[5] Lenntech molecular weight calculator [website] [available at: http://www.lenntech.com/calculators/molecular/molecular-weight-calculator.htm, accessed 2011 Oct 5].

[6] Barbalace, RC. Molar mass calculations and molecular weight calculator [website] [available at: http://environmentalchemistry.com/yogi/reference/molar.html, accessed 2011 Oct 5].

[7] Biological Magnetic Resonance Data Bank. Molecular mass calculator [website] [available at: http://www.bmrb.wisc.edu/metabolomics/mol_mass.php, accessed 2011 Oct 5).

[8] Harris, DC. Exploring chemical analysis. 3rd Edition. New York, USA: W.H. Freeman; 2005.