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CRITICAL INSIGHT

Mass Defect from Nuclear Physics to Mass Spectral Analysis

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Abstract. Mass defect is associated with the binding energy of the nucleus. It is a fundamental property of the nucleus and the principle behind nuclear energy. Mass defect has also entered into the mass spectrometry terminology with the availability of high resolution mass spectrometry and has found application in mass spectral analysis. In this application, isobaric masses are differentiated and identified by their mass defect. What is the relationship between nuclear mass defect and mass defect used in mass spectral analysis, and are they the same?

Keywords: Mass defect, Mass excess, Delta mass, Fractional mass, Mass defect filter, Packing fraction, Nuclear binding energy, High resolution mass spectrometry

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Introduction

Mass defect and binding energy of the nucleus are two related fundamental properties of atoms. Even though they are often discussed in the context of nuclear energy, mass defect and binding energy are concepts with wider applications. Mass defect exists universally in bound systems of all sizes in which the components are bound together by force. It is applicable to small systems such as the nucleus of an atom as well as large systems such as the solar system [1]. A bound system has a lower potential energy and mass than its components in an unbound state. The difference between the mass of a bound system and its constituents in an unbound state is referred to as mass defect. Binding energy is the energy equivalent of mass defect according to Einstein's theory of mass-energy equivalence, and is more pronounced in the atomic nucleus than the solar system due to its small size and enormous amount of energy involved. Nuclear binding energy is the source of energy of the sun and nuclear power plants.

The emergence of the concepts of nuclear mass defect and binding energy goes back to the early 20th century after atomic weights were determined accurately by chemical methods and the deviation of atomic masses from whole numbers was investigated by mass spectrograph [2–8]. Early research in mass spectrometry was primarily focused on determining the accurate mass and isotopic composition of elements. However, by the 1940s this work was largely complete and mass spectrometry moved from academic laboratories into research and development facilities in the petroleum and chemical industry [9]. Resolution and accuracy of the instruments increased over the years and the accurate mass of molecules made determination of their empirical formula possible. Mass defect re-entered scientific literature this time for mass spectral analysis and applied to the identification of molecules rather than analysis of atoms [10, 11]. In order to make the following discussion clear, applications of mass defect in nuclear physics and mass spectral analysis are referred to as nuclear and chemical mass defect, respectively, even though this distinction does not exist in the literature. Chemical mass defect, defined as the difference between the monoisotopic mass and the nominal mass, became a useful criterion for sorting through a crowded mass spectrum from a complex sample in order to identify compounds of interest among many unrelated ion peaks. It was first utilized to visualize and identify different classes of compounds in petroleum samples, and later found applications in drug metabolism and pharmacokinetics studies and identification of endogenous compounds in complex biological samples [12].

Nuclear mass defect and binding energy are often discussed in connection with chemical mass defect and mass spectral analysis and are incorrectly considered to be the same [12–15]. Referring to both nuclear and chemical mass defect simply as mass defect can cause confusion, especially when discussed among a broader audience from different disciplines. For example, the nuclear mass defect for carbon-12 (^{12}C) is 0.1 mass unit (u), which when converted to energy is equal to the binding energy per nucleon (protons and neutrons in the

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nucleus) of 7.7 mega-electron volt (MeV) for a carbon atom. On the other hand, ^{12}C with the atomic mass of 12.0000 (selected as an integer value by convention to define atomic mass scale) has a chemical mass defect of zero when it comes to mass spectral analysis and this could be incorrectly interpreted as the equivalent of zero binding energy for carbon. One would only be able to differentiate the two usages of the term mass defect based on the context in which it is discussed.

The goal of this discussion is not to review different applications of mass defect in mass spectrometry, which can be found elsewhere [12], but to discuss mass defect in general. The importance of mass defect in nuclear physics is reviewed and its significance in mass spectral analysis is discussed. It is argued that nuclear and chemical mass defects are not the same and their difference is highlighted at the end, by looking at the mass defect plots of the elements in the periodic table. While nuclear mass defect reflects a physical property, chemical mass defect does not, and it is based on a convention that carbon has a mass defect of zero. It is proposed to refer to chemical mass defect as mass excess in order to eliminate confusion surrounding the usage of the term "mass defect."

Nuclear Mass Defect and Binding Energy

Mass of the nucleus is slightly less than the added masses of its constituent protons and neutrons and this mass difference is called nuclear mass defect [16]:

$$\text{Nuclear Mass Defect} = m - [(\text{Z} \times m_H) + (\text{N} \times m_n)] \quad (1)$$

where m is the atomic mass, m_H is the mass of hydrogen, m_n is the mass of neutron, Z is the number of protons, and N is the number of neutrons. The energy equivalent of the nuclear mass defect is known as the nuclear binding energy. In other words, binding energy is the energy released with the formation of a nucleus from its nucleons, or is the energy required to break a nucleus into its individual components.

The nuclear mass defect is a fundamental property of a nucleus and is a fixed value corresponding to a certain amount of binding energy for that nucleus. Mass defect and binding energy are important factors in the energy involved in nuclear reactions. Looking at how mass defect and binding energy change from one element to another will make the relationship between mass defect, binding energy, and nuclear energy more apparent. A plot of nuclear mass defect versus mass number for different elements is shown in Figure 1.

The nuclear mass defect changes with mass number from zero for hydrogen to a value close to –2 for uranium. The nuclear mass defect per nucleon, which is mass defect divided by mass number, is a more useful value. It provides a more meaningful way of comparison between different elements and is plotted against mass number in Figure 2a. The corresponding energy, the binding energy per nucleon, is the amount of energy that is released per nucleon upon the formation of a nucleus and is an indication of its stability (Figure 2b).

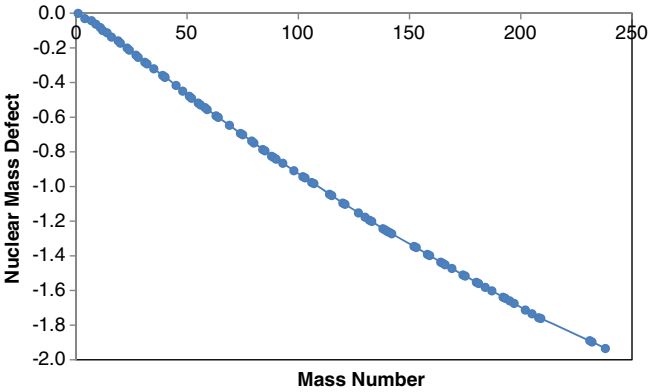

Figure 1. Nuclear mass defect versus mass number for the most abundant isotopes. Masses are based on the ^{12}C mass scale

If we start from hydrogen and move to heavier atoms on the curve in Figure 2b, we find that binding energy per nucleon increases and reaches a maximum around 56, the mass number for iron. If we continue past iron, the binding energy per nucleon decreases gradually. Therefore, the medium mass nuclei are the most stable. Iron has the highest binding energy per nucleon and is the most stable nucleus (^{62}Ni is more stable than ^{56}Fe but it is not the most abundant isotope of nickel). The difference in the binding energy between elements provides an opportunity for producing energy if elements with lower

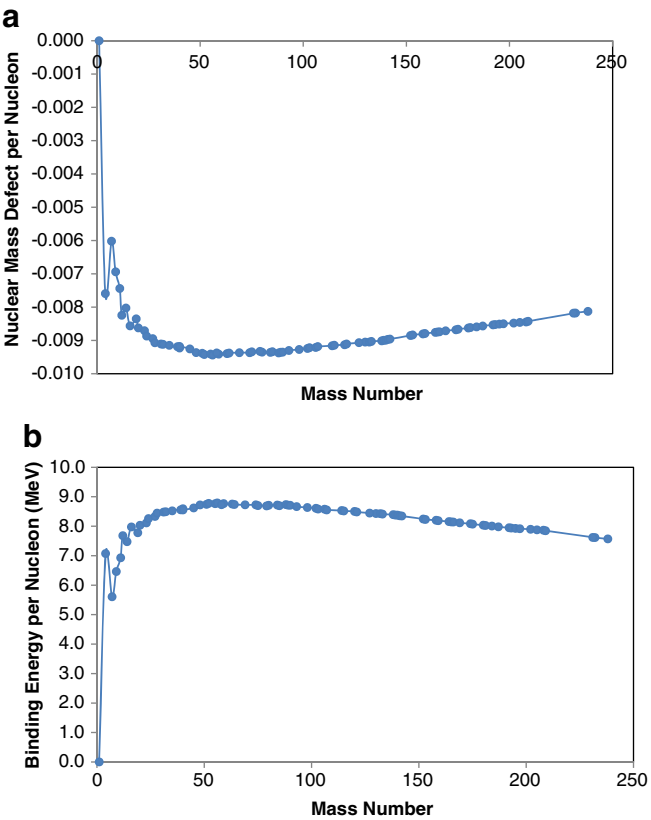

Figure 2. **(a)** Nuclear mass defect per nucleon based on the ^{12}C mass scale, and **(b)** binding energy per nucleon versus mass number for the most abundant isotopes

binding energy per nucleon are converted into more stable elements with higher binding energy per nucleon. Figure 2b shows that heavy nuclei gain stability and therefore give off energy if they are fragmented into two mid-sized nuclei. The process of splitting a nucleus into smaller nuclei is known as fission. The breakdown of the uranium nucleus into more stable nuclei as a result of collisions with neutrons releases energy and is an example of a fission reaction [17]:

$$^{1}_{0}n + {}^{235}_{92}U \rightarrow {}^{142}_{56}Ba + {}^{91}_{36}Kr + 3{}^{1}_{0}n \quad (2)$$

where the subscript shows atomic number Z, superscript shows mass number A, and n represents neutrons. This is the process by which nuclear energy is produced in nuclear power plants. Energy is also released if light nuclei are combined or fused together to form more massive nuclei with greater binding energy per nucleon than that of reacting species. This too is a change towards a greater stability. The process that is called fusion is exothermic only for the nuclei of mass number below 56. The reaction of deuterium and tritium to form helium is an example of a fusion reaction [17]:

$$^{2}_{1}H + {}^{3}_{1}H \rightarrow {}^{4}_{2}He + {}^{1}_{0}n \quad (3)$$

Nuclear fusion is the source of energy of the sun and other stars. Combination of hydrogen nuclei to form more complex nuclei was first proposed as the mechanism of production of stellar energy in 1920 after the publication of masses of isotopes by Aston [18]. The difference in the binding energy per nucleon between hydrogen and helium is much more than between uranium and a mid-mass element such as iron and as a result hydrogen fusion can produce more energy, kilogram for kilogram, than the nuclear fission of uranium.

Mass Excess and Q-Value

The released energy as a result of the formation of nuclei can be compared to the heat of formation of molecules. The heat of formation of a molecule is the energy released with the formation of a molecule from its elements and it is a measure of the stability of the molecule. A large heat of formation is an indication of a stable molecule since a large amount of energy is required to decompose the molecule into its constituent atoms. The energy released with the formation of a nucleus from its constituent protons and neutrons is a measure of the stability of the nucleus in a similar way. The heat of formation of molecules is considerably less than the energy released with the formation of nuclei. The standard heat of formation of CO_2 from carbon and oxygen is 394 KJ/mole, which is the amount of heat released under standard conditions per mole of CO_2. (For comparison, the energy released as a result of the formation of a carbon nucleus from protons and neutrons is 8.9×10^9 KJ/mole). This corresponds to a mass loss of 4.4×10^{-9} g for each mole of CO_2 formed. Unlike nuclear mass defect, the mass loss is too minute to be measured and is largely ignored.

The energy change in chemical reactions is calculated from the heat of formation of the reactants and products. Similarly, we can calculate the amount of energy released or consumed in a nuclear reaction. The Q-value is the energy involved in a nuclear reaction and is defined as:

$$Q\text{-}value = \sum_{initial} mc^2 - \sum_{final} mc^2 \quad (4)$$

where m is the atomic mass and c is the speed of light. The Q-values are usually reported in units of MeV (c^2 = 931.5 MeV/u). A positive Q-value is an indication of an energetically favored reaction. The Q-value for the reaction 3, for example, is calculated using atomic masses of reactants and products:

$$\begin{aligned} Q\text{-value} &= [(2.014102 + 3.016049) - (4.002603 + 1.008665)] \\ &\quad \times 931.5 \\ &= 17.6\,\text{MeV} \end{aligned}$$

The neutral isotopic mass is not always given in isotope tables, but the mass excess is listed instead in units of mass or energy (MeV). It is defined as the difference between the measured atomic mass (m) and the mass number (A):

$$\Delta_A = m - A \quad (5)$$

where Δ_A is the mass excess. Since the sum of the mass numbers on either side of a nuclear reaction is the same, A is conserved. If $(A + \Delta_A)$ is substituted for m in Equation 4, A cancels out because the total number of protons and neutrons between reactants and products are unchanged (charge and mass conservation). We are left with an equation for the Q-value that depends only on the mass excess. For the above example, one can use the mass excess in mass unit (u) to find the Q-value:

$$\begin{aligned} Q\text{-value} &= [(0.014102 + 0.016049) - (0.002603 + 0.008665)] \\ &\quad \times 931.5 \\ &= 17.6\,\text{MeV} \end{aligned}$$

However, it is easier to use the energy equivalent of the mass excess, which is commonly listed in tables [19]:

$$\begin{aligned} Q\text{-value} &= (13.136 + 14.950) - (2.425 + 8.071) \\ &= 17.6\,\text{MeV} \end{aligned}$$

The value that is reported in the tables of nuclear properties is the mass excess (in units of energy) rather than the mass. The above example shows that the use of mass excess makes prediction of the Q-values straightforward and simplifies the calculation of energy involved in nuclear reactions. Definition of mass defect in general chemistry and physics textbooks is

consistent with Equation 1 and what has been discussed so far [17, 20]. The definition used in mass spectrometry is discussed next.

Chemical Mass Defect

Application of mass spectrometry was extended from mainly atomic to molecular analysis as commercial instruments became available to meet the demands of the chemical and petroleum industry. Resolution of molecular ions with the same nominal mass but arising from different combination of elements became possible with the increasing resolution of the instruments. Petroleum samples contain many compounds with the same nominal mass but with different elemental compositions that could be identified by high resolution instruments. A goal of petroleum analysis was to identify compounds based on their class, type, and the degree of alkylation. Compound class is collectively defined as all of elemental compositions with the same heteroatom content. Compounds with the same heteroatom but with various numbers of hydrogen in their empirical formula belong to different compound types. Compound type arises from different number of double bonds or rings in the molecule. Within the same class and type, there are many compounds with varying degrees of alkylation, which differ only in the numbers of methylene (CH_2) groups in their formula. They are commonly known as homologous series. Presence of a large number of different molecules made the interpretation of the mass spectra of petroleum samples a difficult task. The high-resolution spectra of such samples with many resolved peaks at the same nominal mass demanded a new approach for data interpretation. A data interpretation strategy was developed based on the chemical mass defect. Mass defect was defined as the difference between the accurate mass of the ion in question and a reference hydrocarbon ion with the same nominal mass [10]. This approach was used to identify several new compound classes and types not reported before.

Chemical mass defect was later defined as the difference between the nominal mass and the measured monoisotopic mass by Kendrick and was utilized to facilitate analysis of petroleum samples [11]:

$$\text{Chemical Mass Defect} = A - m \quad (6)$$

where A is the nominal mass and m is the monoisotopic mass. Nominal mass is the mass calculated using the integer mass of the most abundant isotopes of each element. It is equivalent to mass number expressed in mass unit [21] and they are both represented by the same symbol, A, here. Therefore, chemical mass defect is the difference between the monoisotopic mass and a whole number mass, which may not be the closest integer mass. Nominal mass is the closest integer mass to the monoisotopic mass for low molecular weight compounds but this is not necessarily true at higher masses where the difference between monoisotopic and nominal masses can be quite large [22]. For example, polystyrene, $C_4H_9(C_8H_8)_{100}H$, has a nominal mass of 10458 u and monoisotopic mass of 10464.338 u.

Chemical mass defect as defined in Equation 6 is what is currently used in mass spectral analysis. Because homologous series constitute a large portion of the compounds found in petroleum samples, Kendrick introduced a new mass scale based on $CH_2 = 14.0000$ (^{12}C scale based masses are multiplied by 14.0000/14.01565 in order to be converted to the Kendrick mass scale). On this mass scale, the repeating mass of methylene does not change the mass defect and all of the compounds in a homologous series that belong to the same class and type will have the same chemical mass defect. Plotting chemical mass defect versus nominal mass will help visualize all of the compounds present in the spectrum in a way that would not be possible by just viewing the spectrum. Figure 3 shows an example of such a plot for peaks from the high-resolution mass spectrum of a crude oil sample [23]. Compounds belonging to the same class and type but with different number of CH_2 groups will fall on a horizontal line on this plot. Similarly, compounds of the same class but different type differ by two hydrogens and will fall on horizontal lines separated by the mass defect of H_2. Compounds belonging to different classes

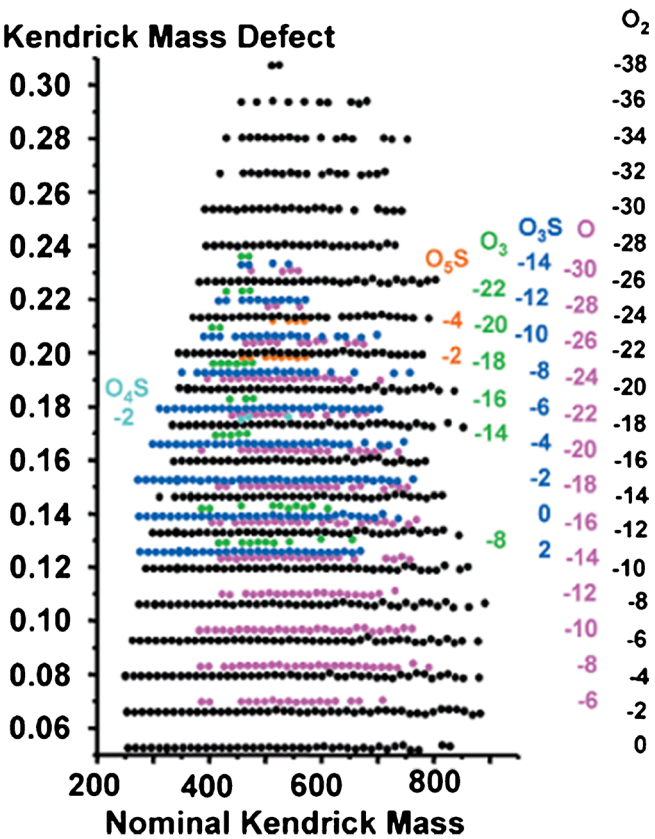

Figure 3. Kendrick mass defect versus nominal Kendrick mass for about 1000 odd-mass ion peaks from an ESI FT-ICR mass spectrum of a crude oil sample. Six different classes of compounds are identified. Different compound types are listed under each class. Compound type is reported as the value of Z in the general elemental composition formula of $C_cH_{2c+z}N_nO_oS_sP_p$. Points on horizontal lines differ in the number of CH_2 groups and belong to homologous series. Reprinted from reference 23. Copyright 2001 American Chemical Society

are now readily identified because their chemical mass defect will be displaced vertically from each other. Visualization of a complex mass spectrum is simplified by using a simple two-dimensional graphical display of the data based on chemical mass defect. Patterns are recognizable on the plot, and the outlier data are easily identified. Identification of a few compounds on the plot, at least one from each class, is the key to identifying the majority of the compounds. Such a plot has been used for analyzing data from a single high resolution mass spectrum of a crude oil sample containing several thousand ion peaks [24]. Class and type assignment for so many compounds in the sample was accomplished by taking advantage of their chemical mass defect, a task that would be difficult to achieve in the absence of such a powerful data interpretation strategy. A CH_2 based mass scale is historically the first one used for the analysis of crude oil samples by mass defect. Other mass scales (^{16}O- and H_2-based for example) have also been used to plot data on two-dimensional plots similar to the one shown in Figure 3, and are useful for environmental samples [25]. The use of more than two mass scales for graphical visualization of data on higher-order plots makes data interpretation easier and increases the number of assigned chemical formulas [26].

Applications of Chemical Mass Defect in Mass Spectrometry

Applications of chemical mass defect in mass spectrometry have been the subject of a recent review [12] and can be divided into two general categories. In both cases, chemical mass defect is used to facilitate the identification of compounds of interest in a complex sample in the presence of many other mass spectral peaks.

In one category, the existing chemical mass defect in the compound of interest is used to identify or track it. The characterization of crude oil samples [23, 24] or identification of metabolites [27–30], proteins [31], lipids [32], and natural organic matter [25] falls in this category. Mass defect filters have become common in metabolite identification in the pharmaceutical industry [33]. The central structure of drugs does not change drastically after metabolism. Therefore, the chemical mass defect of the parent drug and its metabolites usually remain similar and fall into a narrow range. In mass defect filtering, a limited chemical mass defect range is defined and ions with chemical mass defects outside of this range are removed from the spectrum through post-acquisition data processing. The result is a clean mass spectrum with less background noise or signals unrelated to the compounds of interest.

In the second category, a chemical mass defect tag is introduced into the compound of interest, which will help its differentiation from chemical noise or other compounds. In a typical complex mass spectrum such as the spectrum of a whole cell protein digest, ion signals are clustered around certain spots and there are gaps in the mass spectrum where no signal is detected because no peptides have masses at those values [34]. Mass tags contain atoms with large chemical mass defects per nucleon. The presence of a mass tag in a compound will shift its ion signal from the crowded areas of the spectrum to the gaps where it can be easily detected and identified [13]. Tagging proteins, for example, has been used to improve protein sequencing and identification [35]. Fluorinated compounds have long been popular for mass calibration and as internal standards because they have chemical mass defects that are different from naturally occurring compounds and less likely to interfere with the analysis [36].

Comparison between Nuclear and Chemical Mass Defect

Chemical mass defect as defined by Equation 6 was originally developed for the mass spectral analysis of molecules in petroleum samples. Because chemical mass defect in a molecule is due to contribution from its constituent atoms, Equation 6 can also be used to find the chemical mass defect of atoms. This provides an equal platform for a comparison between the nuclear and chemical mass defects. In applications other than Kendrick mass defect, it is common to calculate chemical mass defect such that elements with a lower mass than carbon, such as hydrogen, have a positive mass defect (mass defect = $m - A$). This definition is used for plots in Figures 4 and 5. Figure 4 shows a plot of chemical mass defect versus mass number for different elements in the periodic table. One of the curves in Figure 4 is based on the ^{12}C mass scale, whereas the other is based on the ^{16}O mass scale. Apart from the obvious differences between the nuclear and chemical mass defect plots in Figures 1 and 4, a comparison between the two reveals a few points:

1. Nuclear mass defect in Figure 1 changes to increasingly negative values with increasing mass number. This means that the binding energy increases with mass number and is higher for heavier atoms. The last element on the plot in Figure 1, uranium, has the highest total binding energy. Such a relationship does not exist for the chemical mass defect in Figure 4.

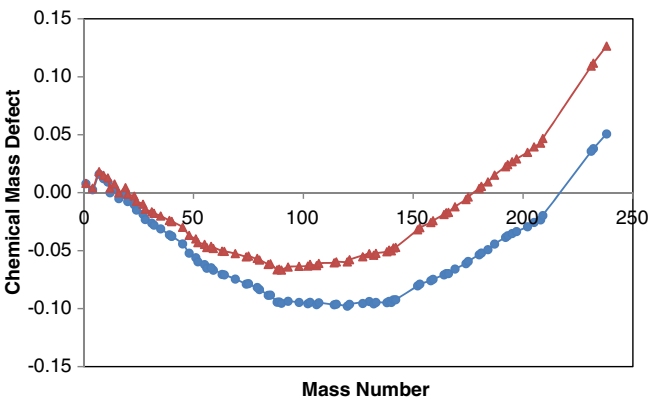

Figure 4. Chemical mass defect versus mass number for the most abundant isotopes based on the ^{12}C (●) and ^{16}O (▲) mass scales

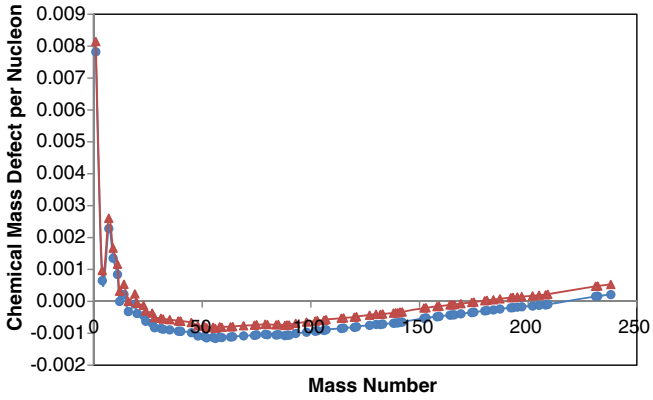

Figure 5. Chemical mass defect per nucleon versus mass number for the most abundant isotopes based on the ^{12}C (•) and ^{16}O (▲) mass scales

2. Nuclear mass defect is a negative value and has the same sign for all elements and therefore binding energies as the energy that keeps the nucleus together will all have the same sign as expected. On the other hand, chemical mass defect is positive for some and negative for others.
3. The chemical mass defect curve in Figure 4 changes by changing the mass scale from ^{12}C to ^{16}O, which shows that chemical mass defect is dependent on the mass scale. This is in contrast to the mass scale dependency of the nuclear mass defect. The nuclear mass defect values in Figure 1 (which is based on the ^{12}C mass scale) will be 0.03% higher when calculated based on the ^{16}O mass scale, a change that will not be noticeable on the graph. This dependency is small and can be ignored compared with the mass scale dependency of the chemical mass defect. Even though the nuclear mass defect for a certain nucleus is slightly higher on the ^{16}O mass scale, the corresponding binding energy is the same whether it is calculated based on the ^{16}O or ^{12}C mass scale. The conversion factors from the nuclear mass defect to the binding energy are different for the ^{16}O and ^{12}C mass scales such that the binding energies calculated based on different mass scales will have the same value. The nuclear mass defect and binding energy are intrinsic properties and represent physical values in absolute units of mass or energy (grams or joules). Chemical mass defect is defined relative to the atomic mass unit scale, which is a matter of convention.

In addition, nuclear mass defect by definition is only concerned with the nucleus and does not include electrons, wheras chemical mass defect includes electrons. Mass defect per nucleon is another value that one can compare between the nuclear and chemical mass defects. This is the same concept reported by Aston as the packing fraction [4]. Chemical mass defects are divided by the corresponding mass numbers and plotted as a function of mass number in Figure 5. One of the curves in Figure 5 is based on the ^{12}C mass scale, wheras the other one is based on ^{16}O. A comparison between Figure 5 and the nuclear mass defect per nucleon in Figure 2a shows that the position of the curve along the vertical axis is changed. This is because chemical mass defect is a relative value, with its reference zero point depending on the mass scale. The position of the curve along the vertical axis changes so that in the case of ^{12}C mass scale, carbon-12 has a chemical mass defect of zero. On the ^{16}O mass scale, the curve shifts along the *y*-axis so that oxygen-16 has a zero chemical mass defect. Consequently, both nuclear and chemical mass defects per nucleon show similar trends moving on the curves from hydrogen to uranium, but a certain element will have two different values for the nuclear and chemical mass defects. On a mass scale based on hydrogen (H = 1.0000), the nuclear and chemical mass defects will have values that are close to each other.

It is interesting to see that despite the differences between the nuclear and chemical mass defect plots in Figures 1 and 4, the nuclear and chemical mass defect per nucleon curves in Figures 2a and 5 have similar trends. This is because the underlying phenomenon behind them, the strong nuclear force, is the same. The strong nuclear force is one of the four basic forces in nature (along with the weak nuclear force, electromagnetic force and gravity), that holds the subatomic particles of the nucleus together. It pulls the protons and neutrons in the nucleus tightly together, slightly more or less tight in different nuclei. It is useful to compare an atom in the presence and absence of the nuclear force to understand its effect. In the absence of the nuclear force, an atom can be considered as a collection of protons, neutrons, and electrons. The mass of such a hypothetical atom can be calculated from the mass and number of electrons, protons, and neutrons present in the atom. This calculated mass divided by mass number for different elements, called mass per nucleon, is plotted against mass number in the top section of the plot in Figure 6 (CV-1). The mass per nucleon in the presence of the nuclear force is calculated by dividing ^{12}C-based masses by mass numbers and is plotted against mass number in the bottom section of the plot in Figure 6 (CV-2). The effect of the nuclear force on atomic masses is clear on the plot. The almost straight line trend (CV-

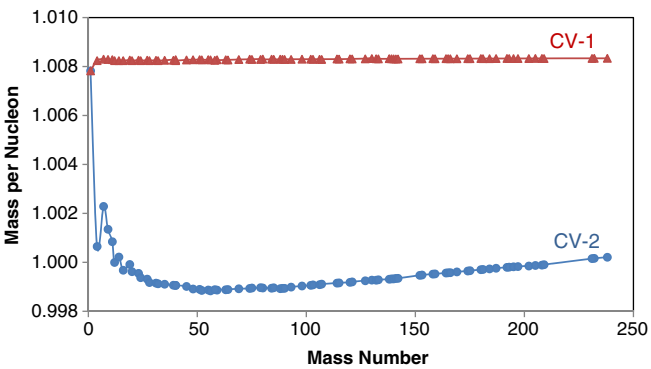

Figure 6. Mass per nucleon versus mass number for the most abundant isotopes in the absence (▲) and presence (•) of the strong nuclear force. Data points on the top curve are calculated according to $[(Z \times m_H) + (N \times m_n)]/A$ (for the definition of the symbols in the formula, see Equation 1). The points in the bottom curve are calculated by dividing ^{12}C bases masses by corresponding mass numbers (m/A)

1), in the presence of the nuclear force, is converted to the familiar trend seen in the mass defect curves (CV-2). Therefore, the trend seen in Figures 2a and 5 is the result of the nuclear force acting inside the nucleus. We can get a hint of the strength of the nuclear force in different nuclei by measuring nuclear mass defect, a higher nuclear mass defect per nucleon indicating a stronger force per nucleon. The nuclear mass defect is the difference between the mass of a nucleus in the presence and absence of the nuclear force. Therefore, it can be calculated by subtracting the two curves (CV-1 and CV-2) in Figure 6 (electrons are present in both curves and will cancel out after subtraction). Subtracting a straight line from a function such as a sine wave will not change its trend and will produce another sine wave. Similarly, because CV-1 is close to a straight line, the resulting nuclear mass defect curve from the subtraction of CV-1 and CV-2 will have a trend similar to CV-2. Figure 6 is in fact a graphical representation of Equation 1 divided by mass number. CV-2 is a plot of m/A versus mass number and CV-1 is the second part of Equation 1 divided by mass number ($[(Z \times m_H) + (N \times m_n)]/A$) plotted against mass number. CV-2 has the exact same trend as the chemical mass defect plot in Figure 5. However, there is a slight difference between CV-2 and the nuclear mass defect curve in Figure 2a. Because CV-1 in Figure 6 is not a straight line (it has slight variations from one element to the next), its deviation from a straight line will cause the nuclear mass defect curve resulting from the subtraction of CV-1 and CV-2 to have a slightly different trend than CV-2. Therefore, nuclear and chemical mass defect will have slightly different trends. Superimposing the nuclear and chemical mass defect curves will reveal their differences and show that despite their similar trends, they do not follow the exact same track.

Mass Defect or Mass Excess?

The discussion so far concludes that nuclear and chemical mass defects are different. On the other hand, chemical mass defect has the same definition as mass excess, a concept introduced to simplify the calculations of energy change involved in nuclear reactions. They are both defined as the difference between the measured monoisotopic mass and nominal mass. It is therefore logical to restrict the use of mass defect to discussions of binding energy and nuclear science and use mass excess in mass spectral analysis. Looking back at the example discussed in the Introduction, ^{12}C has a mass defect of 0.1 u and binding energy per nucleon of 7.7 MeV, but as far as mass spectral analysis is concerned, it has a mass excess of zero. However, this is against the recommendation of the International Union of Pure and Applied Chemistry (IUPAC), which supports the use of mass defect and defines it as the difference between the nominal mass and the monoisotopic mass of an atom, molecule, or ion [21]. In applications where the decimal value of a reported mass is of interest [37], the use of fractional mass is more appropriate (polystyrene with the monoisotopic mass of 10464.338 u has a mass defect of 6.338 and a fractional mass of 0.338).

Conclusion

Why are atomic masses not whole numbers? The quest to answer this question and to evaluate the divergence of atomic masses from whole numbers led to the concepts of nuclear mass defect and binding energy. Later on, chemical mass defect played an important role in mass spectral analysis. Nuclear and chemical mass defects are both caused by the strong nuclear force. However, despite their common origin and close relationship, they are different concepts. Nuclear mass defect is an absolute parameter whereas chemical mass defect is a relative value. Nuclear mass defect and binding energy are intrinsic properties and are fixed values for a certain atom. On the other hand, chemical mass defect is not a fixed value and depends on the mass scale. Nuclear mass defect reflects a physical property whereas chemical mass defect is not a physical property and is based on a convention that carbon has a mass defect of zero. Considering the differences between the nuclear and chemical mass defects, it is proposed to refer to the latter as mass excess. This will eliminate confusion surrounding the use of the term mass defect especially among different disciplines and will harmonize the terminologies used in nuclear physics and mass spectrometry.

References

1. Miki, H.: Binding energy and mass defect. Phys. Educ. **25**, 322–324 (1990)
2. Harkins, W.D., Wilson, E.D.: The changes of mass and weight involved in the formation of complex atoms. J. Am. Chem. Soc. **37**, 1367–1383 (1915)
3. Harkins, W.D., Wilson, E.D.: Energy relations involved in the formation of complex atoms. Philos. Mag. **30**, 723–734 (1915)
4. Aston, F.W.: A new mass spectrograph and the whole number rule. Proc. Royal Soc. Lond. A **115**, 487–514 (1927)
5. Gamow, G.: Mass defect curve and nuclear constitution. Proc. Royal Soc. Lond. A **126**, 632–644 (1930)
6. Aston, F.W., Maxwell, C.: The mass-spectra of chemical elements. Philos. Mag. **39**, 611–625 (1920)
7. Budzikiewics, H., Grigsby, R.D.: Mass spectrometry and isotopes: a century of research and discussion. Mass Spectrom. Rev. **25**, 146–157 (2006)
8. Bauer, S.H.: Mass spectrometry in the mid-1930s: Were chemists intrigued? J. Am. Soc. Mass Spectrom. **12**, 975–988 (2001)
9. Grayson, M.A.: Measuring mass from positive rays to proteins. CHF Press, Philadelphia, PA (2002)
10. Carlson, E.G., Paulissen, G.T., Hunt, R.H., O'Neal, M.J.: High resolution mass spectrometry: interpretation of spectra of petroleum fractions. Anal. Chem. **32**, 1489–1494 (1960)
11. Kendrick, E.: A mass scale based on CH_2 = 14.0000 for high resolution mass spectrometry of organic compounds. Anal. Chem. **35**, 2146–2154 (1963)
12. Sleno, L.: The use of mass defect in modern mass spectrometry. J. Mass Spectrom. **47**, 226–236 (2012)
13. Hall, M.P., Ashrafi, S., Obegi, I., Petesch, R., Peterson, J.N., Schneider, L.V.: 'Mass defect' tags for biomolecular mass spectrometry. J. Mass Spectrom. **38**, 809–816 (2003)

14. Bruce, C., Shifman, M.A., Miller, P., Gulcicek, E.E.: Probabilistic enrichment of phosphopeptides by their mass defect. Anal. Chem. **78**, 4374–4382 (2006)
15. Liu, H., Yang, L., Khainovski, N., Dong, M., Hall, S.C., Fisher, S.J., Biggin, M.D., Jin, J., Witkowska, H.E.: Automated iterative MS/MS acquisition: a tool for improving efficiency of protein identification using a LC-MALDI MS workflow. Anal. Chem. **83**, 6286–6293 (2011)
16. Choppin, G.R., Liljenzin, J.-O., Rydberg, J.: Radiochemistry and nuclear chemistry, 3rd ed. Butterworth-Heinemann, Woburn (2002)
17. Brown, T.L., LeMay, H.E., Bursten, B.E.: Chemistry the central science, 8th edn. Prentice-Hall, Upper Saddle River (2000)
18. Eddington, A.S.: The internal constitution of the stars. Nature **106**, 14–20 (1920)
19. Audi, G., Bersillon, O., Blachot, J., Wapstra, A.H.: The NUBASE evaluation of nuclear and decay properties. Nucl. Phys. A **729**, 3–128 (2003)
20. Young, H.D., Freedman, R.A., Ford, A.L.: University Physics, 13th ed. Pearson, San Francisco (2012)
21. Murray, K.K., Boyd, R.K., Eberlin, M.N., Langley, G.J., Li, L., Naito, Y.: Definitions of terms relating to mass spectrometry (IUPAC recommendations 2013). Pure Appl. Chem. **85**, 1515–1609 (2013)
22. Yergey, J., Heller, D., Hansen, G., Cotter, R.J., Fenselau, C.: Isotopic distributions in mass spectra of large molecules. Anal. Chem. **55**, 353–356 (1983)
23. Hughey, C.A., Hendrickson, C.L., Rodgers, R.P., Marshall, A.G., Qian, K.: Kendrick mass defect spectrum: a compact visual analysis for ultrahigh-resolution broadband spectra. Anal. Chem. **73**, 4676–4681 (2001)
24. Hughey, C.A., Rodgers, R.P., Marshall, A.G.: Resolution of 11,000 compositionally distinct components in a single electrospray ionization Fourier transform ion cyclotron resonance mass spectrum of crude oil. Anal. Chem. **74**, 4145–4149 (2002)
25. Reemtsma, T.: Determination of molecular formulas of natural organic matter molecules by (ultra-) high-resolution mass spectrometry status and needs. J. Chromatogr. A **1216**, 3687–3701 (2009)
26. Roach, P.J., Laskin, J., Laskin, A.: Higher-order mass defect analysis for mass spectra of complex organic mixtures. Anal. Chem. **83**, 4924–4929 (2011)
27. Barbara, J.E., Castro-Perez, J.M.: High-resolution chromatography/time-of-flight MS^E with in silico data mining is an information rich approach to reactive metabolite screening. Rapid Commun. Mass Spectrom. **25**, 3029–3040 (2011)
28. Shahidi-Latham, S.K., Dutta, S.M., Prieto Conaway, M.C., Rudewicz, P.J.: Evaluation of an accurate mass approach for the simultaneous detection of drug and metabolite distributions via whole-body mass spectrometric imaging. Anal. Chem. **84**, 7158–7165 (2012)
29. Ekanayaka, E.A.P., Celiz, M.D., Jones, A.D.: Relative mass defect filtering of mass spectra: a path to discovery of plant specialized metabolites. Plant Physiol. **167**, 1221–1232 (2015)
30. Zhu, M., Ma, L., Zhang, D., Ray, K., Zhao, W., Humphreys, W.G., Skiles, G., Sanders, M., Zhang, H.: Detection and characterization of metabolites in biological matrices using mass defect filtering of liquid chromatography/high resolution mass spectrometry data. Drug Metab. Dispos. **34**, 1722–1733 (2006)
31. Lehmann, W.D., Bohne, A., von der Lieth, C.-W.: The information encrypted in accurate peptide masses – improved protein identification and assistance in glycopeptide identification and characterization. J. Mass Spectrom. **35**, 1335–1341 (2000)
32. Jones, J.J., Stump, M.J., Fleming, R.C., Lay, J.O., Wilkins, C.L.: Strategies and data analysis techniques for liquid and phospholipid chemistry elucidation by intact cell MALDI-FTMS. J. Am. Soc. Mass Spectrom. **15**, 1665–1674 (2004)
33. Zhang, H., Zhang, D., Ray, K., Zhu, M.: Mass defect filter technique and its applications to drug metabolite identification by high-resolution mass spectrometry. J. Mass Spectrom. **44**, 999–1016 (2009)
34. Frahm, J.L., Howard, B.E., Heber, S., Muddiman, D.C.: Accessible proteomics space and its implications for peak capacity for zero-, one-, and two-dimensional separations coupled with FT-ICR and TOF mass spectrometry. J. Mass Spectrom. **41**, 281–288 (2006)
35. Hernandez, H., Niehauser, S., Boltz, S.A., Gawandi, V., Phillips, R.S., Amster, I.J.: Mass defect labeling of cysteine for improving peptide assignment in shotgun proteomic analyses. Anal. Chem. **78**, 3417–3423 (2006)
36. Moini, M.: Ultramark 1621 as a calibration/reference compound for mass spectrometry. II. Positive- and negative-ion electrospray ionization. Rapid Commun. Mass Spectrom. **8**, 711–714 (1994)
37. Pourshahian, S., Limbach, P.A.: Application of fractional mass for the identification of peptide-oligonucleotide cross-links by mass spectrometry. J. Mass Spectrom. **43**, 1081–1088 (2008)