

Structural analysis (CH-314)

Week 2

Problems and Solutions

Problem 1. Consider the isotopic distribution of a protonated peptide shown below. Estimate the charge state (z) of this peptide, its monoisotopic and average masses (of the neutral species), and the number of carbon atoms in it.

Solution:

The spacing between adjacent peaks is about 0.5 Th, thus the charge state is +2 (the peptide is a cation due to protonation), or in more details:

$$\left(\begin{matrix} 0.5018 \text{ Th} + 0.5002 \text{ Th} + 0.5013 \text{ Th} + \\ 0.5021 \text{ Th} + 0.5037 \text{ Th} \end{matrix} \right) / 5$$

$$= 0.50182 \text{ Th}$$

$$1.00335 \text{ Da} / 0.50182 \text{ Th} = 1.999e$$

To calculate the monoisotopic mass of the neutral peptide, one just multiplies the m/z value of the first peak (in this case, its intensity is high enough to be sure the first peak does correspond to monoisotopic ion) by the previously estimated charge and then subtract the mass of the added protons, that is:

$$2e \cdot 957.01 \text{ Th} - 2 \cdot 1.007276 \text{ Da} \approx 1912 \text{ Da}$$

To evaluate the average mass, one sums up the m/z values of the peaks multiplied by the respective abundances (or normalized peak intensities as in the equation below), multiplies the result by the previously estimated charge, and then subtract the mass of the added protons:

$$\left(\begin{matrix} 93 \cdot 957.01 \text{ Th} + 100 \cdot 957.5118 \text{ Th} + 77 \cdot 958.012 \text{ Th} + \\ 43 \cdot 958.5133 \text{ Th} + 26 \cdot 959.0154 \text{ Th} + 5 \cdot 959.5191 \text{ Th} \end{matrix} \right) / \left(\begin{matrix} 93 + 100 + 77 + \\ 43 + 26 + 5 \end{matrix} \right) = 957.756 \text{ Th}$$

$$957.756 \text{ Th} \cdot 2e - 2 \cdot 1.007276 \text{ Da} = 1913.5 \text{ Da}$$

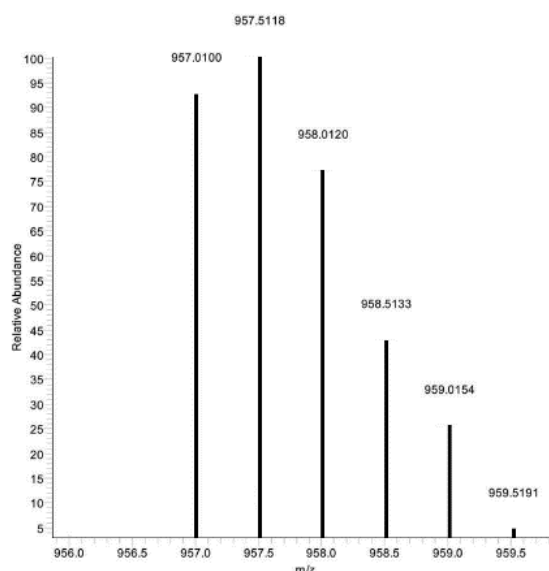
To estimate the number of carbon atoms (n), one may use the intensities of the two consecutive peaks and the following formula:

$$\frac{I_{A+m+1}}{I_{A+m}} = \frac{n-m}{m+1} \cdot \frac{1-\alpha}{\alpha}$$

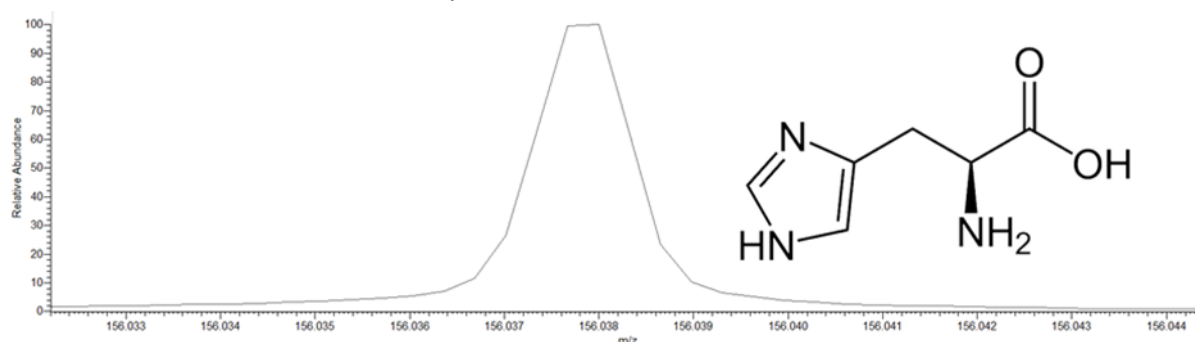
where m is the number of ^{13}C isotopes corresponding to $A+m$ peak and α is the abundance of ^{12}C isotope. Then, the number of carbon atoms can be evaluated as:

$$n = (m+1) \cdot \frac{\alpha}{1-\alpha} \cdot \frac{I_{A+m+1}}{I_{A+m}} + m$$

If one takes the first two peaks ($m = 0$), then $n = \frac{\alpha}{1-\alpha} \cdot \frac{I_{A+1}}{I_A} = \frac{0.9889}{0.0111} \cdot \frac{100}{93} \approx 96$. For $m = 1, 2, 3, 4$ one gets $n \approx 138, 151, 218, 90$, respectively. As the final estimate, one may take either one of these five numbers, or their average ($n \approx 139$), or the range from 90 to 219.



Problem 2. Shown below is the monoisotopic peak of a singly protonated amino acid, histidine. Estimate mass measurement accuracy and mass resolution.



Solution:

The molecular formula of histidine is $C_6H_9N_3O_2$. Thus, its monoisotopic mass is:

$$m = 6 \cdot 12 \text{ Da} + 9 \cdot 1.007825 \text{ Da} + 3 \cdot 14.003074 \text{ Da} + 2 \cdot 15.994915 \text{ Da} = 155.069477 \text{ Da}$$

The theoretical m/z of singly protonated histidine is:

$$m/z = \frac{155.069477 \text{ Da} + 1.007276 \text{ Da}}{1e} = 156.076753 \text{ Th}$$

The experimentally measured value is ca. 156.0378 Th (the center of the peak). The mass measurement accuracy is then

$$\delta m/m = \frac{156.076753 \text{ Th} - 156.0378 \text{ Th}}{156.076753 \text{ Th}} \approx 2.5 \cdot 10^{-4} = 250 \text{ ppm}$$

To estimate mass resolution, one has to find the m/z values that correspond to the half of the peak maximum (156.0372 and 156.0384 Th), calculate the difference between them ($FWHM = 0.0012 \text{ Th}$), and then divide the m/z value of the peak maximum by this difference:

$$R = \frac{156.0378 \text{ Th}}{0.0012 \text{ Th}} \approx 1.3 \cdot 10^5.$$

Problem 3. Which resolution is required to distinguish monoisotopic peaks of deprotonated aspirin ($C_9H_8O_4$) and deprotonated glucose ($C_6H_{12}O_6$)? And to resolve isotopic distribution (due to ^{13}C) of a 24 kDa protein?

Solution:

First, one has to calculate the mass-to-charge ratios of the two ions:

$$m/z([C_9H_8O_4 - H]^-) = (9 \cdot 12 + 8 \cdot 1.007825 + 4 \cdot 15.994915 - 1.007276) \text{ Th} = 179.034984 \text{ Th}$$

$$m/z([C_6H_{12}O_6 - H]^-) = (6 \cdot 12 + 12 \cdot 1.007825 + 6 \cdot 15.994915 - 1.007276) \text{ Th} = 179.056114 \text{ Th}$$

Then, the required resolution is estimated as follows:

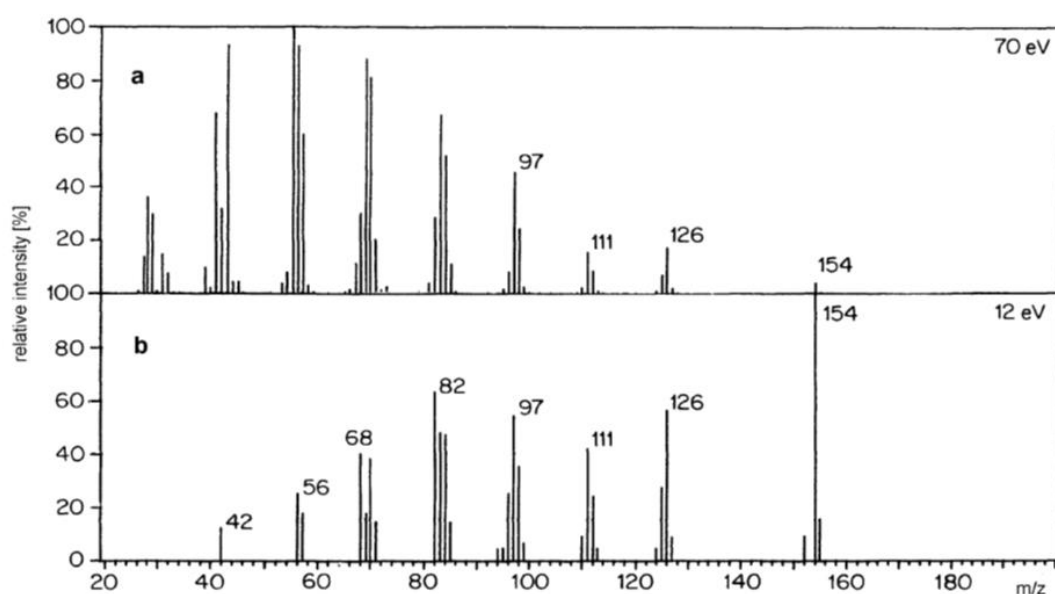
$$R = 1.8 \cdot \frac{\frac{1}{2} \cdot (m/z([C_9H_8O_4 - H]^-) + m/z([C_6H_{12}O_6 - H]^-))}{m/z([C_6H_{12}O_6 - H]^-) - m/z([C_9H_8O_4 - H]^-)} = 1.8 \cdot \frac{179.045549}{0.02113} \approx 15000$$

Two consecutive peaks in the isotopic distribution of the protein are separated by the mass difference between ^{12}C and ^{13}C . The required resolution does not depend on the protein charge state. Indeed, denote the protein's charge as Z , the mass of the lower m/z peak as M , and the mass difference between ^{12}C and ^{13}C as ΔM . Then, to resolve peaks at M/Z and $(M + \Delta M)/Z$, one needs the resolution to be:

$$R = 1.8 \cdot \frac{\frac{1}{2} \cdot \left(\frac{M + \Delta M}{Z} + \frac{M}{Z} \right)}{\frac{M + \Delta M}{Z} - \frac{M}{Z}} = 1.8 \cdot \frac{\frac{M}{Z} + \frac{\Delta M}{2Z}}{\frac{\Delta M}{Z}} = 1.8 \cdot \frac{M}{\Delta M} + 0.9$$

For a 24 kDa protein, $R \approx 1.8 \cdot 24 \text{ kDa} / 1.00335 \text{ Da} \approx 43000$.

Problem 4. Electron ionization mass spectra of undecan-1-ol ($\text{C}_{11}\text{H}_{23}\text{OH}$) recorded at two different electron energies are shown below. Which of them, do you think, corresponds to higher electron energy? Explain why.



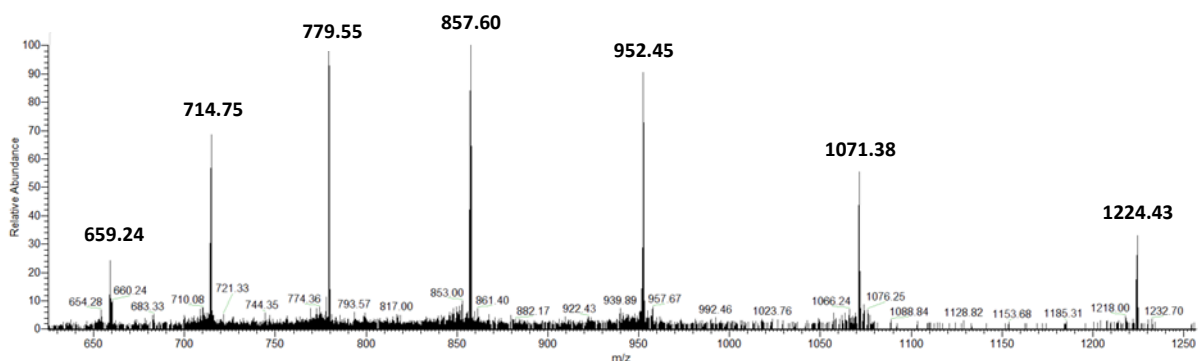
Solution:

Higher electron energy results in more extensive fragmentation. In these two cases the electron energy is so high that the molecular ion ($m/z = 172$ Th) is not observed. Panel **a** exhibits extensive fragmentation with the most abundant fragments at low m/z . The distribution of fragment ions in Panel **b** is shifted to higher m/z (the most abundant fragment at $m/z = 154$ Th is due to a neutral water loss), which means less fragmentation. Therefore, Panel **a** corresponds to higher electron energy (70 eV) and Panel **b** – to lower electron energy (12 eV).

Problem 5. Shown below is the mass spectrum of a small protein recorded using positive mode electrospray ionization (ESI). Estimate the molecular weight of the protein?

Solution:

Let's denote the molecular weight of the protein as M and the leftmost and rightmost peaks as i and j , respectively: $(m/z)_i = 659.24$ Th and $(m/z)_j = 1224.43$ Th.



Then, $z_i \cdot (m/z)_i = M + z_i \cdot m(H^+)$ and $z_j \cdot (m/z)_j = M + z_j \cdot m(H^+)$. Because peaks in the spectrum correspond to a series of charge states with decreasing charge, $z_j = z_i - 6$ and the second equation transforms to:

$$(z_i - 6) \cdot (m/z)_j = M + (z_i - 6) \cdot m(H^+)$$

After subtracting the first equation, one gets:

$$z_i \cdot ((m/z)_j - (m/z)_i) - 6 \cdot (m/z)_j = -6 \cdot m(H^+)$$

$$z_i = \frac{6 \cdot ((m/z)_j - m(H^+))}{(m/z)_j - (m/z)_i} = \frac{6 \cdot (1224.43 - 1.007276)}{1224.43 - 659.24} = 12.988 \approx 13$$

Therefore, the charge states of the protonated protein are:

m/z (Th)	z	M (Da)
659.24	+13	8557.0
714.75	+12	8564.9
779.55	+11	8564.0
857.60	+10	8565.9
952.45	+9	8563.0
1071.38	+8	8563.0
1224.43	+7	8564.0

where the protein's molecular weight for each charge state is calculated as:

$$M = z_i \cdot (m/z)_i - z_i \cdot m(H^+)$$

Finally, to get a better estimate, one may average the evaluated values:

$$\langle M \rangle = \frac{8557.0 + 8564.9 + 8564.0 + 8565.9 + 8563.0 + 8563.0 + 8564.0}{7} \text{ Da} = 8563.1 \text{ Da.}$$

Problem 6. Shown below is the mass spectrum of a mixture of proteins. Which ionization technique, do you think, was used? Why?

Solution:

Proteins are quite large non-volatile molecules (e.g., the molecular weight of Ubiquitin is 8.5kDa). Therefore, either ESI or MALDI was used in this case. Also, proteins have multiple protonation sites

(e.g., there are 13 protonation sites in Ubiquitin). However, the recorded mass spectrum demonstrates peaks that mainly correspond to singly charged ions (except for [Cytochrome + 2H]⁺) and no charge state distribution. This suggests that the ions were produced using positive mode MALDI.

