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Observing the Mass Spectra of atoms and small isotopic molecules

**Introduction**

Mass spectrometers can work by ionizing atoms into positive ions and passing them through a magnetic field. Particles with a larger charge/mass ratio are deflected less than those with a smaller charge/mass ratio. A mass analyzer and detector separate the ions and measure the quantity of each ion. The degree to which the particles are deflected corresponds to isotope or atom.

The Lorentz force the magnetic field exerts upon the particle is given by:

Using Newton’s second law and some algebraic manipulation:

Another method by which mass spectrometers can work is called Matrix assisted laser desorption ionization (MALDI), which aims a laser pulse at a matrix of molecules. The mass enters the gas phase and the Time of Flight analyzer (TOF) separates masses by the time at which they arrive at the detector. Molecules arriving first have a higher acceleration. This experiment will use simulations of these two methods in order to determine the abundance of mercury, chlorine, methylene chloride, and hemoglobin.

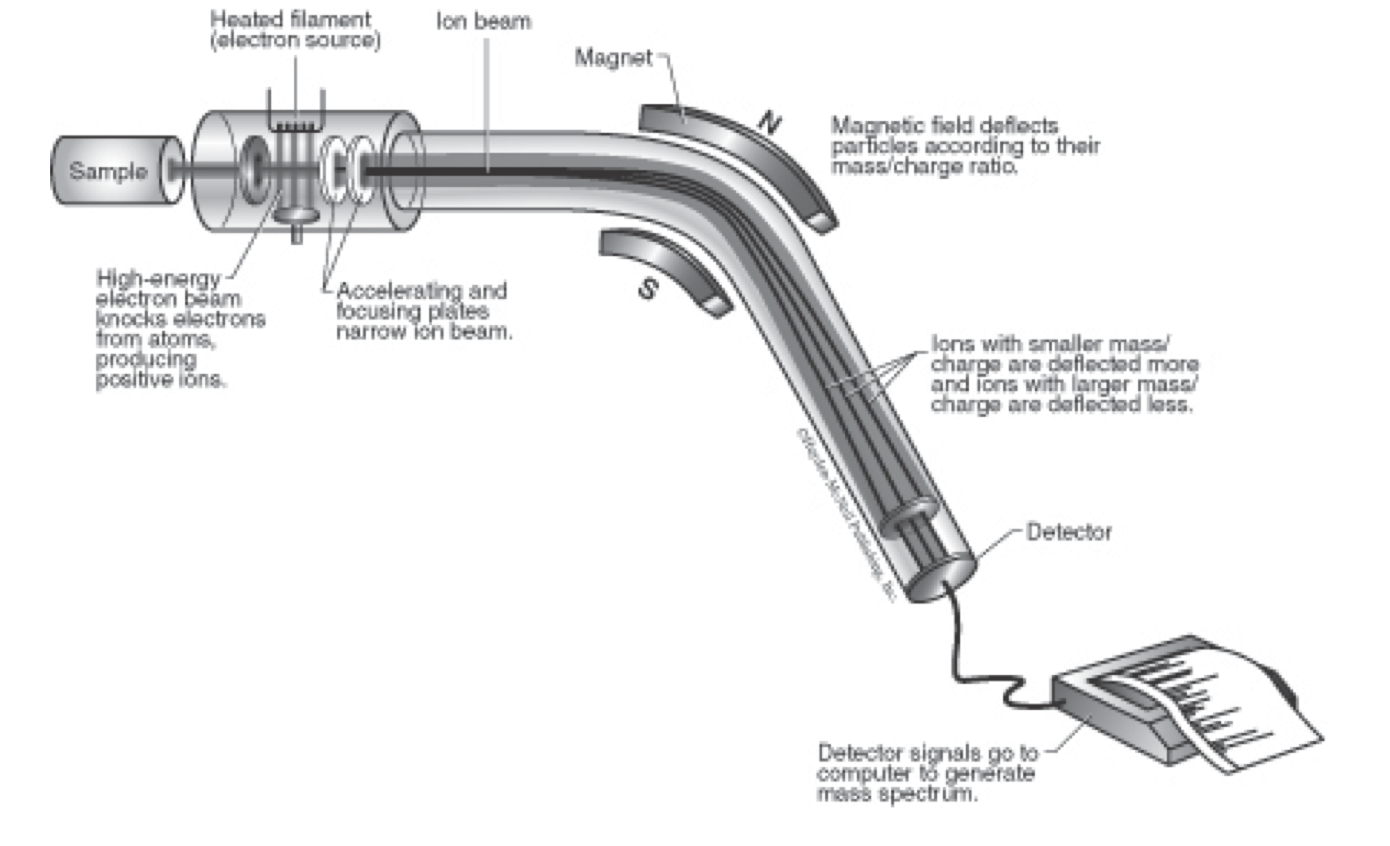


Fig 1: The apparatus for mass spectrometry is shown with a bent tube and detector at the end to detect the different particles. (“Mass Spectrometry”)

**Results/Discussion**

First, the isotopic abundance of mercury was measured. The relative observed abundance percentages for masses of 196, 198, 199, 200, 201, 202, 204 amu were measured to be .174%, 10.161, 17.066, 23.486, 13.210, 29.330, and 6.573, respectively by dividing the absolute intensity for each mass by the total absolute intensity. By taking these percentages, multiplying them by the corresponding measured masses, and adding them up, the observed average atomic mass was found to be 200.64 amu. From Table 2 of exact masses and fractional abundances (“Mass Spectrometry”), the same technique was used to calculate the theoretical average atomic mass 200.58 amu. The percent difference was found by subtracting the observed from theoretical atomic mass and divinding the difference by the theoretical atomic mass. The percent difference was .03074%. The reasonable guess of why there was a peak at 207.07 amu was that the molecule underwent radioactive absorbtion to increase its atomic mass.

Second, the mass spectrum for Cl2 was given to determine that isotopes Cl-35 and Cl-37 were observed. For the peaks at 35, 37, 70, 72, and 74 amu, the ions that might have contributed were Cl-35, C;-37, Cl-35, Cl-35 & Cl-37, and Cl-37 (2x), respectively. The relative intensity of the most abundant isotope, Cl-35, was found to be 75.76%; the relative intensity for the least abundant isotope, Cl-37, was found to be 24.24%. For Cl-35 and Cl-37, the abundances calculated are lower than the actual abundances.

Third, the mass of methylene chloride was determined. It was decided that H-2 could be ignored due to its small abundance and size. For the peak at 87.96 amu, the ions might have been Cl-37 (2x), C-13, and H-1. Cl-37 is moderately low and C-13 is very low in abundance, so it makes sense that the peak is very small. For the peak at 86.96, the ions might have been Cl-37 (2x), C-12, and H-1. Cl-37 is moderately low in abundance, so it makes sense that the peak is small. For 85.96, the ions might have been Cl-37, Cl-35, C-12, and H-1 (2x). Cl-37 is moderately low in abundance, but C-12 and H-1 are very high, so it makes sense that the peak is very high. For 84.96, the ions might have been Cl-37, Cl-35, C-12, and H-1 or Cl-35 (2x), C-13, and H-1 (2x). Cl-37 is moderately low and C-13 is very low in abundance, so it makes sense that the peak is very small. For 83.96, the ions might have been Cl-35 (2x), C-13, and H-1. All these atoms are very high in abundance, so it makes sense that the peak is very high. For 82.95, the ions might have been Cl-35 (2x) and C-13. C-13 is low in abundance, so it makes sense that the peak is very low. Also, the molecule is unstable since there carbon atom wouldn’t have four groups. For 81.94, the ions might have been Cl-35 (2x) and C-12. The molecule is very unstable since there carbon atom wouldn’t have four groups. For 69.94, the ions might have been Cl-35 (2x). This is unlikely to be stable because there are not enough protons to attract the electrons.

Fourth, the sickle cell hemoglobin’s MALDI-TOF analysis was observed to determine which amino acid substitution would account for the mass difference between the normal chain and sickle cell chain. Valine was guessed, and, after checking online (Wing), it was discovered that glutamic acid (GAG) was substituted for valine (GTG).

For the final part of the lab, MS-Fit (mass spectrometry that fits the data) tryptic digest from UCSF was used with given data about the hemoglobin protein sequence to identify similar proteins/sequences in other organisms and species. After entering the data, the top 10 best matches were shown. The top 5 results, from highest to lowest, were of human subunit beta in *Homo sapiens*, bonobo, chimp, whitetoad gibbon, and lowland gorilla. The six, seventh, and eighth results were subunit alpha of human, bonobo, and chimp, respectively. The ninth and tenth results were subunit beta of ring-tailed coati and brown wooly monkey, respectively. Next, the % coverage map was determined for Beta chain human hemoglobin by dividing the number of amino acids found from the MS-Fit in the amino acid sequence by the total number of amino acids in the sequence. 112 were found out of 150, giving 74.66%. The same was done for the Alpha chain human hemoglobin, yielding 85 amino acids found over 141 for 60.3%. The coverage may not be 100% because not all the protein peptide mapping data is available. The amino acid sequence of human beta hemoglobin was compared to the beta hemoglobin of gorilla and ring-tailed coati, giving one amino acid difference between human and gorilla, and one between human and ring-tailed coati. For the difference between human and gorilla, arginine was replaced with Lysine, giving little difference. For human and ring-tailed coati, there is a greater difference, but still not very significant.

**Conclusion**

The experiment successfully used two different techniques, ionization and MALDI-TOF, to determine the relative abundances of different molecules with simulations. Analysis of the data generated by the MALDI-TOF can determine genetic and evolutionary trends across different organisms.

Works Cited

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Wing, Michael K., PhD. "Sickle Cell Anemia." *The Medical Biochemistry Page*. Themedicalbiochemistrypage, n.d. Web. 10 Sept. 2013.