

# Isotopic Distributions in Mass Spectra of Large Molecules

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**Molecular ion distributions are calculated and presented for three sets of homologues with different elemental compositions and hence different mass defects and isotopic distributions in the 1000, 10 000, and 100 000 amu mass ranges. The usefulness is examined of different values characteristic of molecular weights: nominal mass, monoisotopic mass, most abundant mass, and average mass. Experimental problems are described which affect the determination of average molecular weights in the mass range 3000-4000 amu.**

In recent years, there has been a steady growth in the number and kinds of compounds which can be studied with mass spectrometry. In large part this has paralleled the introduction of a number of new ionization techniques, beginning perhaps with chemical ionization (1).

Most recently, it has been the desorption techniques which have revolutionized the field. Field desorption (2), thermal desorption (3, 4), plasma desorption (5), laser desorption (6, 7), secondary ion mass spectrometry (SIMS) (8), and fast atom bombardment (FAB) (9, 10) have almost reversed the order in which organic compounds are amenable to mass spectral analysis, desorbing organic salts and highly polar compounds more readily than less polar ones (11). With these techniques at the mass spectroscopist's disposal, the prognosis for analysis of heavier compounds is good. To date, oligomeric mixtures of industrial polymers such as polystyrene and poly(ethylene glycol) have been quantitatively analyzed in the mass range 1000-6000 using field desorption (12-14) and electrohydrodynamic ionization (15). Biopolymers such as glucagon (16), insulin (17, 18), and endorphin (19) have been weighed in the mass range 3000-6000 by use of fast atom bombardment and plasma desorption. Molecules of insulin and endorphin were analyzed with a time-of-flight analyzer, with considerably less than unit mass resolution at that mass.

There has been some interest in preserving unit mass resolution at high mass and, if at all possible, in achieving accurate mass measurements for elemental composition. At this point in time, there are really two concerns. The first is, through what mass range analyzers will keep pace with the ionization techniques to provide at least unit mass resolution. The second question, which is the concern of this particular paper, assumes that unit resolution will continue to be possible, but asks whether unit mass resolution at very high masses will provide the same information as it does below 2000 amu.

In this paper, we present calculated molecular ion distributions of three sets of compounds with different elemental compositions and hence different mass defects and isotopic distributions in the 1000, 10 000, and 100 000 amu ranges.

The usefulness is examined of different values characteristic of molecular weights: nominal mass, monoisotopic mass, most abundant mass, and average mass. Empirical assessments are made of problems affecting the accuracy of determination of average molecular weights in the mass range 3000-4000.

## EXPERIMENTAL SECTION

Three compounds, all of which have been studied experimentally in our laboratory, were used as models for high mass

molecular ion distributions (12, 16). Polystyrene oligomers, with the formula  $C_4H_9(C_6H_5)_nH$ , have been analyzed by using field desorption to masses beyond 5000 amu, retaining unit resolution. Values of  $n = 10, 100$ , and  $1000$  can be used to generate the distributions in the three mass ranges under study here. The polypeptide glucagon,  $C_{153}H_{224}H_{42}O_{50}S$ , contains almost two heteroatoms for every three carbon atoms, while phosphazine 2121,  $C_{42}H_{18}N_3O_6P_3F_{72}$ , has a negative mass defect. For these compounds, high mass molecular ion clusters were generated by using multiples of their formulas.

The computer program used to generate the theoretical mass spectra shown in the following figures calculates the exact mass and relative abundance of each of the isotopic permutations of the input molecular formula and outputs a listing of these data along with the nominal, monoisotopic, and average masses and a plot of the theoretical distribution. The masses of the ions are plotted to within 0.1 amu. The distribution of the exact masses of the isobars which comprise each unit mass peak is also generated.

A Kratos MS-50 mass spectrometer with a 23 kG magnet was used for the experimental measurements, with EI, FD, and FAB sources provided by Kratos. Spectra were compiled from 5 to 20 scans by multichannel analyzer or with the Kratos DS-55 data system. Programs were written in Fortran IV for a Nova 3 computer.

## RESULTS AND DISCUSSION

In the figures which follow are the following:

**NOMINAL MASS** of the molecular ion, calculated by using the most abundant isotope, without regard for the mass defect, i.e.,  $H = 1, C = 12, N = 14$ , etc.

**MONOISOTOPIC MASS**, which again refers to the molecular ion peak composed from the most abundant isotopes of the elements, but includes the mass defect, i.e.,  $H = 1.007825, C = 12.000000$ , and  $N = 14.00307$ .

**MOST ABUNDANT MASS** of the molecular ion peak group.

**AVERAGE MASS**, calculated from the average masses of the elements, weighted for abundance, i.e., the "centroid" of the distribution.

**A. The 1000 to 3000 Mass Range.** This range is currently accessible in unit mass resolution on most instruments and in high resolution on some. Many of the features of the molecular ion regions of compounds in this range are well-known.

The molecular ion distribution of polystyrene,  $N = 10$ , is shown in Figure 1. In this mass range, the effect of the mass defect is observed, so that while the fictitious NOMINAL mass is 1098, the exact MONOISOTOPIC mass is 1098.704 and is still the MOST ABUNDANT mass. The AVERAGE mass is, of course, somewhat higher, and the shape of the group peaks is nonsymmetrical, as is generally expected at low mass.

In Figure 2 the monoisotopic mass of phosphazine shows a slight negative mass defect from the nominal mass of 2121. This mass defect slowly becomes more negative as the mass of the perfluorinated compound increases. The singularity of the atomic weight of fluorine simplifies this molecular ion array relative to those of polystyrene or glucagon in the same mass range.

With a NOMINAL mass of 3480 (Figure 3), glucagon's monoisotopic mass shows a smaller positive mass defect than polystyrene of comparable weight. This reflects the presence

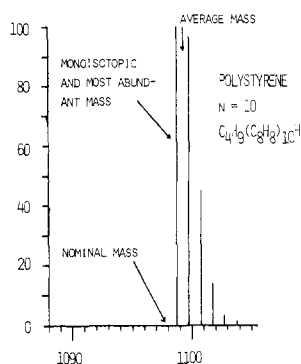


Figure 1. Theoretical molecular ion distribution of polystyrene  $n = 10$ .

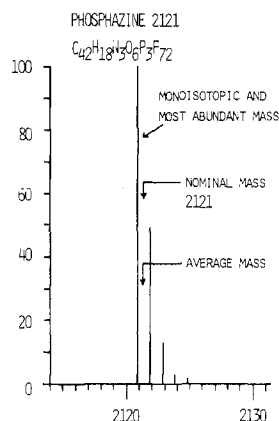


Figure 2. Theoretical molecular ion distribution of phosphazine 2121.

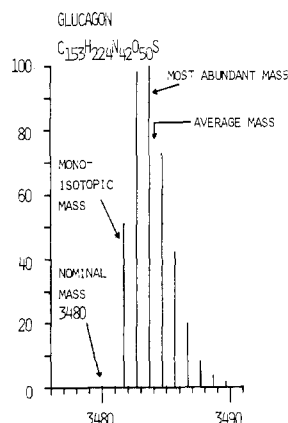


Figure 3. Theoretical molecular ion distribution of glucagon.

of one oxygen or nitrogen atom for every 2.5 hydrogen atoms. The most abundant isotopic species here is not the monoisotopic mass, but appears two atomic mass units higher, reflecting the  $^{13}\text{C}$  contribution from 150 carbon atoms as well as contributions from  $^{15}\text{N}$  and  $^{18}\text{O}$ . The monoisotopic mass is still of significant abundance and the peak group is closer to being symmetrical than those in Figures 1 and 2.

**B. 10 000 Amu.** In this mass range the cumulative mass defect is so large for polystyrene (Figure 4) that the monoisotopic mass at 10 464.34 is six mass units above the nominal mass of 10 458. The monoisotopic mass is no longer the most abundant mass and may not, in fact, be visible in the spectrum because of its low probability of occurrence, 0.09% relative to the most abundant ions at 10 473.37. The most abundant isotopic species weigh 15 mass units more than the nominal mass and 9 more than the monoisotopic mass. Moreover, the adjacent peak at 10 472.37 is 99.6% as abundant and flanking peaks at 10 471.36 and 10 474.37 have relative intensities of 88% and 90%. Thus there is no individual peak in this

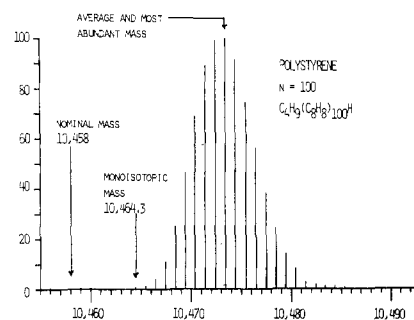


Figure 4. Theoretical molecular ion distribution of polystyrene  $n = 100$ .

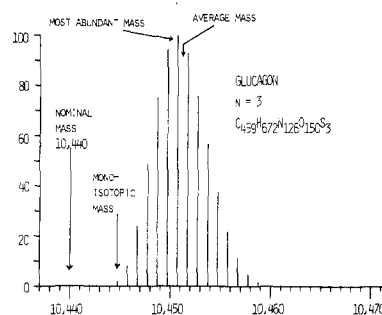


Figure 5. Theoretical molecular ion distribution of glucagon  $n = 3$ .

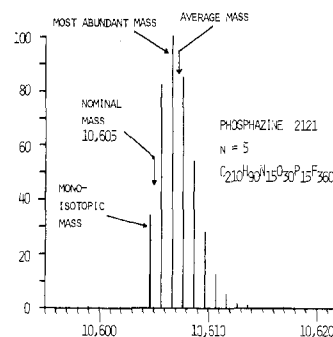


Figure 6. Theoretical molecular ion distribution of phosphazine 2121  $n = 5$ .

molecular ion peak group which is distinctive because of its intensity. The group is close to symmetrical and is perhaps best characterized by its center of gravity or average mass. As another feature of this molecular ion distribution, peaks with intensities  $>1\%$  occur through 22 mass units between 10 464 and 10 486.

The distribution is shown in Figure 5 of molecular species of a trimeric multiple of glucagon. The distribution in this envelope is close to symmetrical. The mass defect (the difference between the nominal and monoisotopic ions) is smaller than that of polystyrene, reflecting the high proportion of heteroatoms. The full width at half maximum of the envelope is essentially the same as that of the polystyrene envelope in Figure 4.

In contrast, the range of molecular ions for phosphazine molecules of the formula given in Figure 6 is relatively narrow, with peaks  $>1\%$  relative intensity falling between 10 604 and 10 614. The monoisotopic mass still carries a high proportion of the total ion current and lies 0.4 amu below the nominal mass. The most abundant mass is only two units above the nominal mass. The most distinctive features of the envelope are its narrowness and its asymmetry. In this mass range perfluorinated compounds may continue to be useful as mass standards, because ion current is distributed among a small number of peaks (sensitivity is not substantially degraded) and one peak in the group can be readily distinguished by its dominant intensity.

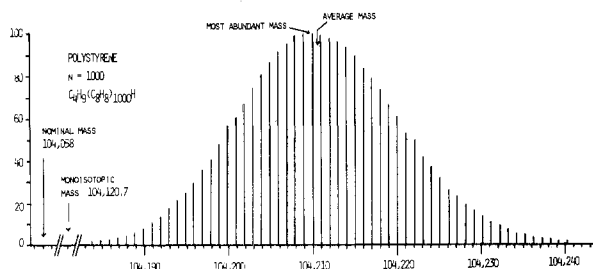


Figure 7. Theoretical molecular ion distribution of polystyrene  $n = 1000$ .

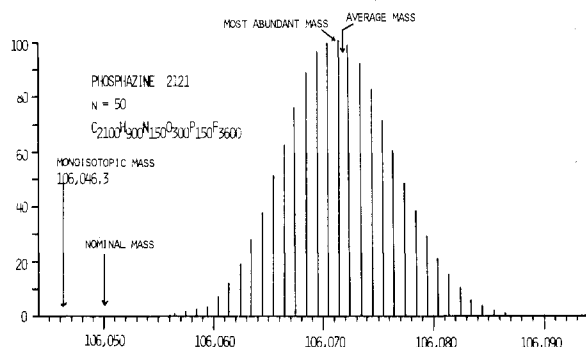


Figure 8. Theoretical molecular ion distribution of phosphazene 2121  $n = 50$ .

**C. 100 000 Amu.** Polystyrene  $n = 1000$  has a nominal mass of 104 058 and a monoisotopic mass of 104 120.7. The most abundant peak in the distribution corresponds to a mass of 104 210.0, or 151 mass units above the nominal mass (Figure 7). Looking only at peaks with abundances  $>1\%$  relative to that of the most abundant peak, the first "visible" mass would be 104 184.7 or 64 mass units above the monoisotopic mass. There are 57 molecular ion peaks with relative abundances  $1\%$  or greater.

The phosphazene type compound shows a less severe situation in this mass range (Figure 8), since the cumulative mass defect is negative (and moreover, small) and the number of carbon-13 possibilities is fewer. In this case 30 peaks with relative abundance  $>1\%$  of the most abundant peak make up the symmetrical ion distribution.

**D. Molecular Weight Assignments.** Historically the molecular ion in a mass spectrum has provided information on the molecular weight of a compound. Examination of relative abundance of isotopic peaks has permitted deductive assessment of the elemental composition, while accurate mass measurements have provided more direct evidence. An important prerequisite to using either approach has been the identification of the peak corresponding to the monoisotopic ion. The low relative intensity of the monoisotopic mass for elemental compositions like those of polystyrene and glucagon in the 10 000 mass range will make its observation difficult in many situations. Even the most abundant mass in a molecular ion envelope is not always distinguishable (Figures 4, 7, and 8), and only in well-defined situations, e.g., the sample is known to be a polymer of styrene, can the relationship be extrapolated between most abundant mass and an unobservable monoisotopic mass.

It is also interesting that ions of these nominal heavy masses are not homogeneous. For example, in the case of polystyrene ( $n = 100$ ) the most abundant peak contains contributions from three isobaric ions which have the same mass to 1 part in  $10^6$ , i.e., 10 473.37 amu. These ions,  $^{12}\text{C}_{897}^{13}\text{C}_7^1\text{H}_{808}^2\text{H}_2$ ,  $^{12}\text{C}_{896}^{13}\text{C}_8^1\text{H}_{808}^2\text{H}_1$ , and  $^{12}\text{C}_{895}^{13}\text{C}_9\text{H}_{810}$ , have relative abundances of 0.67%, 12.2%, and 100%, respectively. Ions in the 10 000 amu region with the carbon to heteroatom ratio of glucagon are also interesting. The many heteroatoms present generate

Table I. Composition of the Most Abundant Peak in the Molecular Ion Cluster of Glucagon ( $n = 3$ )  
 $\text{C}_{459}\text{H}_{672}\text{N}_{126}\text{O}_{150}\text{S}_3$

formula <sup>a</sup>	mass	% composition of the peak at $m/z = 10\ 450$
$^{13}\text{C}_3^{15}\text{N}_1^{34}\text{S}_1$	10 450.80	5.61
$^{13}\text{C}_2^{18}\text{O}_1^{34}\text{S}_1$	10 450.80	2.18
$^{13}\text{C}_3^{15}\text{N}_3$	10 450.80	1.47
$^{13}\text{C}_4^{34}\text{S}_1$	10 450.80	15.45
$^{13}\text{C}_2^{15}\text{N}_1^{33}\text{S}_1$	10 450.80	1.28
$^{13}\text{C}_2^{15}\text{N}_2^{18}\text{O}_1$	10 450.81	1.74
$^{13}\text{C}_4^{15}\text{N}_2$	10 450.81	12.34
$^{13}\text{C}_3^{15}\text{N}_1^{18}\text{O}_1$	10 450.81	12.90
$^{13}\text{C}_2^2\text{H}_1^{34}\text{S}_1$	10 450.81	1.21
$^{13}\text{C}_4^{15}\text{N}_1^{17}\text{O}_1$	10 450.81	3.02
$^{13}\text{C}_3^{33}\text{S}_1$	10 450.81	2.83
$^{13}\text{C}_5^{15}\text{N}_1$	10 450.81	54.69
$^{13}\text{C}_2^{18}\text{O}_2$	10 450.82	2.49
$^{13}\text{C}_2^{18}\text{O}_1^{15}\text{N}_1$	10 450.82	35.53
$^{13}\text{C}_4^2\text{H}_1^{15}\text{N}_1$	10 450.82	5.38
$^{13}\text{C}_3^{17}\text{O}_1^{18}\text{O}_1$	10 450.82	1.56
$^{13}\text{C}_5^{17}\text{O}_1$	10 450.82	6.64
$^{13}\text{C}_6$	10 450.82	100.00
$^{13}\text{C}_3^2\text{H}_1^{18}\text{O}_1$	10 450.82	2.79
$^{13}\text{C}_5^2\text{H}_1$	10 450.82	11.84

<sup>a</sup> Remainder of the molecular formula is made up of the most abundant isotope of each element,  $^{12}\text{C}$ ,  $^1\text{H}$ ,  $^{14}\text{N}$ ,  $^{16}\text{O}$ ,  $^{32}\text{S}$ .

many isobaric ions, and the most abundant peak in this envelope represents 22 isobars of relative intensity greater than  $1\%$ , as listed in Table I. These 22 isotopic isobars from a single chemical compound span a range of only 0.02 mass units with asymmetrically distributed abundances. Resolution of  $10^7$  would be required to permit any of the isobars to be accurately measured free of interference from others.

The multiplicity of isotopic isobars increases with mass. In the case of polystyrene,  $n = 1000$ , the most abundantly populated mass ( $m/z$  104 210.0) is contributed mainly by the six isotopic isobars containing between 84 and 89 atoms of  $^{13}\text{C}$ . Thus unit resolution of molecular ion species of polystyrene  $n = 1000$  requires resolving power of  $10^5$  and separation of these isobars would require  $10^7$ .

Perhaps the best approach to characterizing molecular weights from molecular ion distributions of heavier molecules will be to determine the average mass and to consider the shape of the entire envelope. Average masses have, of course, been used already at low and high masses in situations where unit resolution was not achievable (18, 20, 21). For different formulas the mass range varies in which values other than average mass become difficult to distinguish or meaningless to report. In contrast to glucagon and polystyrene, phosphazene may still be characterized by its monoisotopic mass at 10 000. At 100 000 the average mass is probably the least ambiguous value for any organic compound. However, even in the 100 000 mass range the full width at half maximum of the phosphazene envelope is distinctly narrower (Figure 8) than those of glucagon or polystyrene.

Working in the mass range 1000–4000 we have found that useful evidence supporting or disproving compound identification can be obtained by comparing theoretical molecular ion distributions with observed ones (16, 22).

Table II contains average masses for several compounds in the 3000–4000 mass range. These average masses have been calculated theoretically for each compound, and they have been calculated from molecular or fragment ion distributions experimentally determined at unit resolution using three different ionization techniques. Phosphazene ( $\text{M} - \text{F}$ )<sup>+</sup> ions were obtained by electron impact and are a good match with

Table II. Comparison of Theoretical and Experimentally Determined Average Masses

compound	theoretical	experimental	error, ppm
phosphazene $C_{72}H_{24}N_4P_4O_8F_{127}$	3609.70	3609.75	13
polystyrene ( $n = 34$ ) $C_{276}H_{382}$	3599.32	3599.39	19
glucagon $C_{153}H_{224}N_{42}O_{50}S_1$	3483.81	3483.43	109

the theoretical distribution. It can be seen in Table II that the average mass calculated from this experimentally determined envelope matches well the theoretical average mass. The molecular ion group of polystyrene  $n = 34$  generated with unit resolution by field desorption has been published (22). Comparison with the theoretical distribution indicates that a mixture of  $M^+$  and  $(M + H)^+$  species have been recorded. This kind of phenomenon is typical of field desorption. The theoretical average mass differs from that calculated from this overlapping set of  $M^+$  and  $(M + H)^+$  ions by 19 ppm (Table II). The molecular ion region of glucagon ionized by fast atom bombardment is presented in ref 16. Comparison with the theoretical distribution indicates that the  $(M + H)^+$  ions formed by FAB are accompanied by some weak downfield fragment ions,  $M - H$  and/or  $M - 2H$  ions, characteristic of many FAB spectra. In this case the theoretical average molecular weight differs by 109 ppm from that calculated from the experimentally determined distribution which includes the fragment ions.

### CONCLUSION

As advances in analyzers and ionization allow us to work readily in unit resolution above 3000 amu, new approaches are needed to interpret such spectra: abundances of monoisotopic ions become vanishingly low; a multiplicity of isotopic isobars contributes to a single mass number; no individual peak will always be distinctively the most intense in a molecular ion distribution. We suggest that average masses

be calculated with the best accuracy possible and that the width and symmetry of molecular ion envelopes be exploited to assign and confirm molecular weights.

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## Hadamard Transform Alternating Current Polarography

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**The method combines the advantages of its Fourier transform cousin with greater operational speed and simplicity, making it ideally suited for microprocessor applications. Harmonic distortion can easily be avoided by using sufficiently small excitation signal amplitudes.**

In a previous paper (1) we have shown that Hadamard transformation can be used as an efficient alternative to Fourier transformation in characterizing the electrical transfer function of an electrochemical cell. Specifically, we demonstrated the use of this technique for the determination of the double layer capacitance, emphasized its relation to synchronous rectification, and stressed the inherent speed and

simplicity of the approach which makes it ideally suited for microprocessor application. We also pointed out the potential problem associated with the use of excitation signals which differ from their neighbors in the frequency spectrum by exactly one octave. The present study was undertaken in order to evaluate whether this latter aspect presents a practical obstacle to the general applicability of Hadamard transformation to ac polarography. The conclusion is that it is not.

### EXPERIMENTAL SECTION

The equipment used has been described before (1). Solutions were made from reagent-grade chemicals and water which had been pyrodistilled (2) and, subsequently, distilled twice in quartz. The usual polarographic precautions of thermostating and of deaeration with water-saturated nitrogen were taken: temperature, 25 °C; drop time 5.5 s.