related to the relatively small number of total ions utilized for determining the mass spectra.

CONCLUSION

It would be a significant advance if the mass resolution of 1500 obtained with inorganic specimens could be retained with nonvolatile and thermally labile organic specimens as well as obtaining the structurally significant fragment ion signals. This advance will require more detailed studies of substrate preparation, specimen deposition, faster lasers, and a variety of different samples.

ACKNOWLEDGMENT

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Registry No. CsI, 7789-17-5; Au, 7440-57-5; Al, 7429-90-5; sucrose, 57-50-1.

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Comparison of Relative Quasi-Molecular Ion Yields for 8-keV Ion and ^{252}Cf Fission Fragment Bombardment

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A direct comparison is made of the relative molecular ion yields for a series of peptides when the solid sample is bombarded with ^{252}Cf fission fragments and with 8-keV Cs^+ ions. The comparisons were made by using time-of-flight mass spectrometry with the same sample on the same sample foil. Remarkable similarity was observed in the mass spectra for each compound. In general the quasi-molecular ion yield was found to decrease with increasing sample molecular weight; a larger decrease was observed for low-energy bombardment than for fission fragment bombardment.

Particle-induced desorption is an important method for obtaining mass spectra of involatile organic compounds. In this technique, the compound of interest is desorbed and ionized directly from the condensed phase by particle bombardment. It has been widely applied in two distinctly different energy ranges. The bombarding particles may have energy in the ~100-MeV range or in the ~10-keV range. The principle was first demonstrated with ~100-MeV fission fragments from ^{252}Cf (*1*) and later with 2-keV Ar^+ ions (*2*); 10-keV Xe atoms or Cs^+ ions are now commonly used. In spite of the fundamentally different energy loss mechanisms involved in high- and low-energy bombardment, remarkably similar mass spectra are obtained (*3*). However, little is known about the relative yields for the two energy ranges. In general, within a class of compounds, the quasi-molecular ion yield normally decreases as the sample molecular weight increases. We have compared the dependence of quasi-molecular ion

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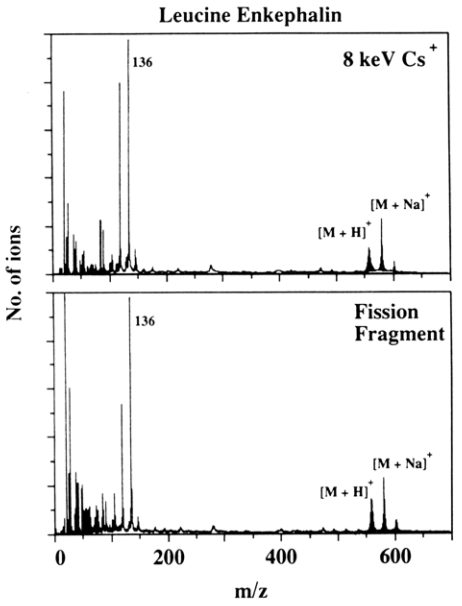

Figure 1. Leucine-enkephalin (556 u) positive ion mass spectra: upper, 8-keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment.

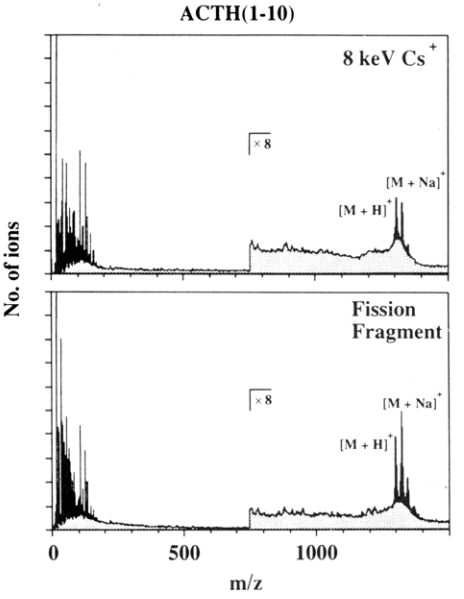

Figure 2. ACTH (1–10) (1299 u) positive ion spectra: upper, 8-keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment.

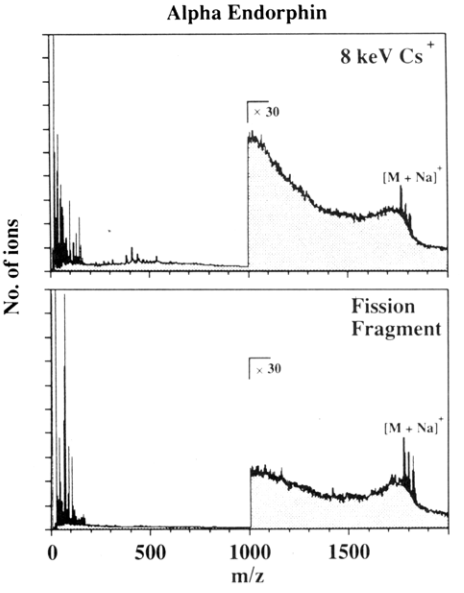

Figure 3. α-Endorphin (1746 u) positive ion spectra: upper, 8-keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment.

yield on molecular weight for a series of peptides bombarded with 8-keV Cs^+ ions and with ^{252}Cf fission fragments. In both cases, the mass spectra were taken with linear time-of-flight instruments using the same sample on the same sample foil.

EXPERIMENTAL SECTION

All the compounds examined (leucine-enkephalin (556 u), ACTH (1–10) (1300 u), substance P (1348 u), α-endorphin (1746 u), renin substrate (1760 u), dynorphin (2148 u), ACTH (1–24) (2933 u), β-endorphin (3465 u), and bovine insulin (5733 u)) were obtained from Sigma Chemical Co. They were dissolved in methanol and water (10:1) or acetic acid (insulin only) at a concentration of ~1 μg/μL and electrosprayed (*4*) onto aluminized polyester film to a thickness of ~50 $\mu g/cm^2$.

Mass spectra were first taken at Manitoba (*5*) by using 8-keV Cs^+ ions. The sample foils analyzed at Manitoba were then sent by courier to Rockefeller (*6*) where the fission fragment spectra were taken. The time between analyses was approximately 24 h, except for β-endorphin and insulin, where it was 72 h. The Cs^+ ions were incident on a 1 mm diameter spot, while the active area in the fission fragment experiment was 12 mm in diameter. For each sample, the total primary ion dose was kept below the amount at which sample damage is observed, i.e. below $\sim 10^{12}$ ions/cm^2. In all cases, secondary ions were accelerated to 10 kV across 4 mm and detected at the end of a field-free region with a chevron microchannel plate electron multiplier. The length of the flight tube was 1.5 m for primary Cs^+ ions and 3.0 m for fission fragments; an electrostatic particle guide was used for the latter. With the exception of β-endorphin and insulin the low-energy spectra were taken with no postacceleration; for the two highest molecular weight compounds it was necessary to use 9.0-kV postacceleration to observe a quasi-molecular ion peak from electrosprayed samples. The fission fragment spectra were all taken with and without 8.5-kV postacceleration.

In order to compare yields (the number of ions ejected per incident primary ion), a measure of the number of particles incident on the sample is required. Although the number of incident particles in a given fission fragment experiment is simply proportional to the spectrum accumulation time, it is more difficult to measure the incident flux directly in a pulsed ion experiment. For this reason we have used the number of secondary ions of m/z 29 ($C_2H_5^+$) as a measure of the number of incident primary particles in each run. The procedure was validated for incident fission fragments for the various targets by comparing with the spectrum accumulation time, and it seems reasonable that it is also valid for kiloelectronvolt bombardment in view of the very similar spectra in the two cases (see below).

RESULTS AND DISCUSSION

Figures 1–4 show comparisons of the spectra taken with kiloelectronvolt ion bombardment and with fission fragment bombardment for leucine-enkephalin, ACTH (1–10), α-endorphin, and bovine insulin. As in our previous comparison (*3*), we observe the pattern and shapes of the quasi-molecular ion peaks and the shape of the background continua to be remarkably similar in the spectra obtained by using the two different energy-bombarding ion species. A significant difference between the spectra obtained with the two methods is also observed. As the sample molecular weight increases, the ratio of the quasi-molecular ion yield to background decreases faster for kiloelectronvolt energy bombardment than for fission fragment bombardment. Fission fragments are observed to have a clear advantage in this respect when compared with kiloelectronvolt ion bombardment.

Figure 5 shows the relative quasi-molecular ion yields as a function of mass for incident fission fragments and 8-keV Cs^+ ions. The curves are normalized at leucine-enkephalin; only the dependence on mass is indicated, not absolute yields.

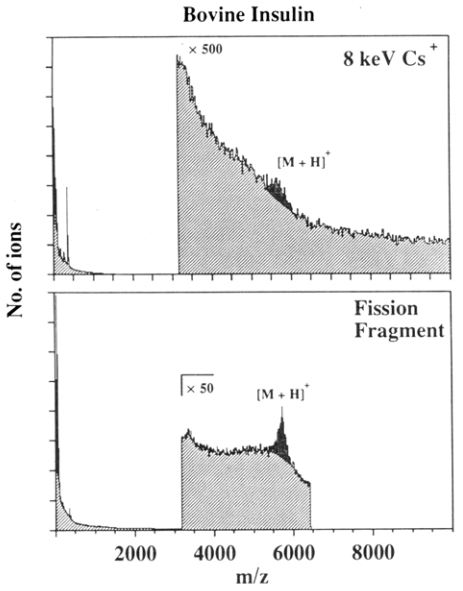

Figure 4. Bovine insulin (5733 u) positive ion spectra: upper, 8-keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment. Fission fragment data were taken up to m/z 6500.

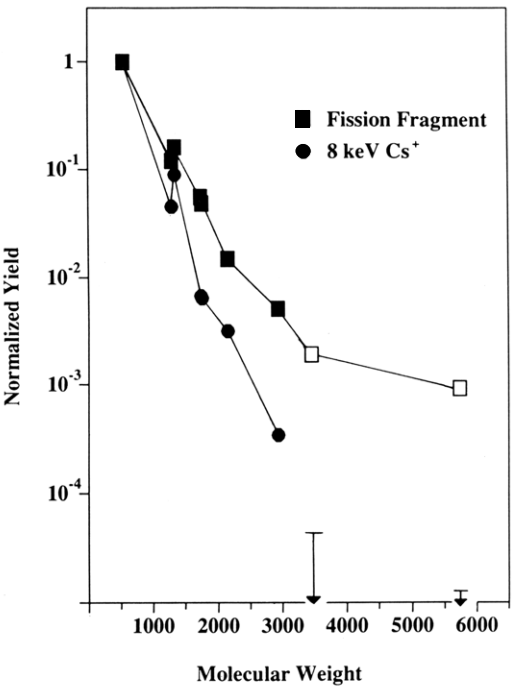

Figure 5. Dependence of the quasi-molecular ion yield (using the sharp component of the peaks) on molecular weight for a series of peptides bombarded with ^{252}Cf fission fragments (squares) and with 8-keV Cs^+ ions (circles). Data obtained on the two highest molecular weight compounds with kiloelectronvolt incident ions are given as error bars indicating the upper limits, since sharp peaks were not observed. Postacceleration was used for the data points depicted with open symbols and error bars; no postacceleration was used for the filled symbols. With the exception of the upper limits, the statistical error is smaller than the size of the symbol representing the datum. The curves are normalized to the yield for leucine enkephalin; the data give no information on absolute yields.

In general the data indicate a decrease of yield with increasing mass. The decrease is faster for kiloelectronvolt bombardment, although the specific shape of the curves depends on a number of factors. These include:

(a) Sample Preparation. The mass spectra obtained with particle-induced desorption depend strongly on sample preparation. For this reason, the same samples on the same sample foils were used in both measurements. However, the fission fragments bombard a much larger area, so some differences could be expected. We took the close similarity in the mass spectra as evidence that the surface was uniform. In cases where the spectra were considerably different, a new target was prepared and the measurements were repeated. The time between measurements might also be expected to influence the data. Since the targets were all examined with kiloelectronvolt ions immediately after their preparation, the point at issue is the target integrity in fission fragment analysis. From our experience at Rockefeller in running hundreds of electrosprayed peptide samples, we are confident that exposure to air for a period of a few days typically causes a loss of molecular ion signal of less than 30%. However, to exclude the possibility of sample degradation en route or in the Manitoba spectrometer, two targets (renin substrate (1760 u) and bovine insulin (5733 u)) were prepared at Rockefeller, analyzed there, then sent to Manitoba for analysis, and finally sent back to Rockefeller. The time between measurements at Rockefeller was 7 days, more than twice the time between any of the other analyses. For both targets, the loss of signal was <30%, small on the scale of Figure 5; the differential loss of signal is smaller still.

(b) Detection Efficiency. Instrument transmission for time-of-flight mass spectrometry is relatively high and should be independent of mass, so it should not affect the present results. Detector efficiency, however, depends on the velocity of the secondary ions striking the detector (*7*) and therefore decreases with increasing mass for sample ions of a given energy. For this reason it was necessary to use postacceleration to observe quasi-molecular ions for the two highest molecular weight compounds with low-energy bombardment; it was not applied for the other samples because of instrumental problems. Thus the shape of the curves in Figure 5 includes a possible contribution from changing detection efficiency. However, fission fragment spectra were taken with and without postacceleration for all the compounds, and up to molecular weight 2000 (dynorphin at 2148 u) no significant difference was observed. For ACTH (1–24) (2933 u) postacceleration increased the yield by about 50%, still a small effect on the scale of Figure 5. Thus it is reasonable to plot the data for the largest two peptides, for which postacceleration was used, on the same graph with data for the others, where postacceleration was not used. In any case, the same type of detector was used in both instruments, and for each compound the secondary ion velocity was the same for incident kiloelectronvolt ions and fission fragments. Therefore, while the absolute efficiencies of the two systems probably differ, the dependence on mass should be similar, so the comparison between the curves is meaningful. That is, the ratio of the yield for fission fragments and kiloelectronvolt ions should not be affected.

(c) Data Analysis. The similarity between the spectral features observed in the mass spectra obtained with the two methods is important for the comparison of yields; similar background subtractions could be performed, and the same integration limits were used. However, where the signal-to-background ratios differ strongly and the peak shapes are complex, the method of background subtraction can affect the comparison. Consider, for example, the quasi-molecular ion region of ACTH (1–24) shown in Figure 6. The sharper components (shaded black) correspond to quasi-molecular ions that remain intact during acceleration (although they may undergo fragmentation in the field-free flight tube) (*8*). The origin of the broad components (gray) is uncertain, but it probably includes contributions from a distribution of unresolved ion peaks and products of decay during acceleration. In addition a smooth continuum (hatched) is observed. At least part of this continuum results from metastable decay in the acceleration region.

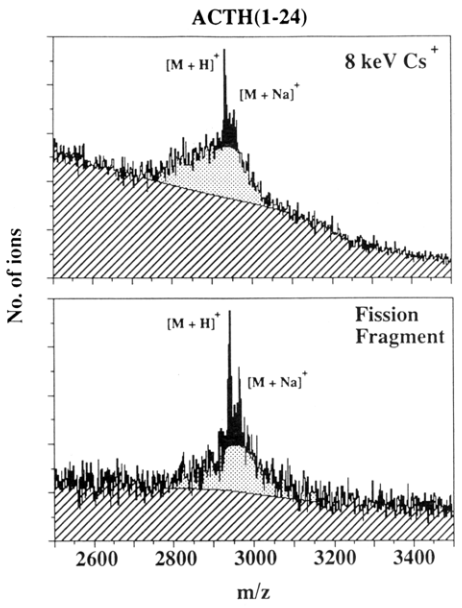

Figure 6. Quasi-molecular ion region of the positive ion spectrum for ACTH (1–24) (2933 u): upper, 8-keV Cs^+ ion bombardment; lower, ^{252}Cf fission fragment bombardment. The portion of the peak shaded black is interpreted as the quasi-molecular ion yield in Figure 5.

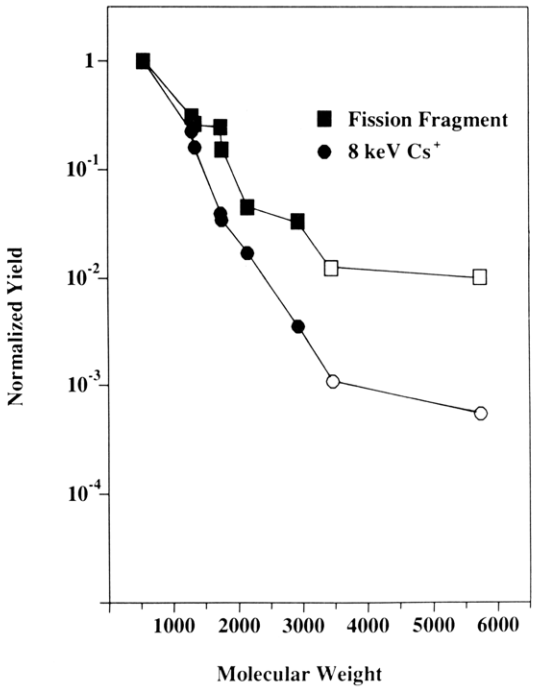

Figure 7. Relative yields as a function of molecular weight using the broad components of the quasi-molecular ion peaks for incident fission fragments (squares) and incident 8-keV Cs^+ ions (circles). (The broad component for ACTH (1–24) is shaded gray in Figure 6.) Postacceleration was used for the data points depicted with open symbols; no postacceleration was used for the filled symbols.

For most of the data shown in Figure 5 we have taken the sharper component (the black portion) as the quasi-molecular ion yield because its origin is well understood and it is the most useful portion of this part of the main spectrum; centroid determinations are usually made with this portion of the data. However, for the low-energy spectra of the two highest molecular weight compounds studied (β-endorphin and insulin), such a sharp component is not distinguishable from the broad peak (see, for example, Figure 4). These data are therefore of limited value for defining the molecular weight of these compounds but still provide some information in the form of upper limits to the yield.

The sum of the areas of the sharp components (where they exist) and the broad components of the peaks are plotted as a function of mass in Figure 7. Here a smooth extension of the continuum was estimated in the region of the peaks, as shown in Figure 4 for bovine insulin and in Figure 6 for ACTH (1–24). This background was then subtracted, and the integration was performed over the full width of the broad peak. These data represent the maximum contribution that can be identified with the molecular ion. Such broad peaks are observed for β-endorphin and insulin with kiloelectronvolt ion bombardment, so definite values (not upper limits) are shown. For both bombarding energies, the data indicate that the yield falls off more slowly for the broad component than for the sharp component. Further, when the broad component is considered, the difference between the yield curves for high- and low-energy bombardment is smaller.

With the above-mentioned qualifications in mind, we can compare the ratio of quasi-molecular ion yields from kiloelectronvolt and from megaelectronvolt ion bombardment when these yields are normalized to that of leucine enkephalin. Thus the yield of ACTH (1–24) (2933 units) *relative to leucine-enkephalin* is approximately 10 times smaller for incident 8-keV Cs^+ ions than for fission fragments; the ratio is at least 80 times smaller for bovine insulin (see Figure 5). (If the complete broad peak is taken as the yield for insulin, the relative yield is about 20 times higher for fission fragments (see Figure 7).) Absolute yields of quasi-molecular ions for kiloelectronvolt bombardment are not accurately known, but from multiplicity measurements (*9*, *10*) they are probably considerably lower than for fission fragments. Recent results from Uppsala (*11*) indicate that the absolute yield of leucine enkephalin is about an order of magnitude higher for fission fragment bombardment than for 18-keV Cs^+ bombardment. Thus, the present results indicate that the *absolute* yield of bovine insulin obtained with fission fragments is at least 800 times that obtained with kiloelectronvolt ions. On the other hand, much higher fluxes are possible with low-energy ions, so a lower yield is not necessarily a disadvantage. However, the present results indicate the yield and the signal-to-background ratio decrease faster with increasing mass for kiloelectronvolt ion bombardment than for fission fragment bombardment. This suggests a higher upper mass limit for fission fragment bombardment, at least for electrosprayed targets. Indeed, the largest quasi-molecular ions observed by mass spectrometry have been desorbed by high-energy particle bombardment (*12*).

An earlier comparison by Kamensky et al. between 3-keV Cs^+ bombardment and 54-MeV $^{63}Cu^{9+}$ bombardment (*13*) also showed that the decrease of molecular ion yield as a function of mass was faster for kiloelectronvolt bombardment. However, the divergence in the yield vs mass curves measured in that experiment for the compounds CsI (133 u), glycylglycine (132 u) ergosterol (396 u), bleomycin (1375 u), and trinucloride diphosphate (1884 u) was considerably greater than that observed in the present experiment.

As mentioned above, the mass spectra obtained by particle bombardment depend strongly on the method of sample preparation. For example, higher yields and spectra exhibiting sharper peaks and less fragmentation have been obtained with fission fragments when the sample is deposited onto nitrocellulose backing (*12*) than for electrosprayed samples. Recent experiments (*11*, *14*) show that similar effects are present at kiloelectronvolt bombarding energies and the use of nitrocellulose considerably reduces the divergence of the yield vs mass curves for kiloelectronvolt and fission fragment bombardment.

Clearly the experimental conditions strongly affect the comparison of yields for high- and low-energy bombardment, and it is important to extend the measurements to other types

of target preparation, other types of compounds, and other bombarding energies.

CONCLUSION

The desorption yield for molecular ions from peptides decreases more rapidly as a function of molecular weight for 8-keV Cs^+ ion bombardment than for ^{252}Cf fission fragment bombardment. Absolute yields were not determined, but *normalized to leucine-enkephalin* (556 u) the measured yield for ACTH (1-24) (2933 u) is about 10 times lower for kiloelectronvolt ion bombardment than for fission fragment bombardment; when normalized to leucine enkephalin, the yield for bovine insulin (5733 u) is at least 80 times lower. Also the signal-to-background ratio for the quasi-molecular ion peaks falls off more quickly with increasing mass for lower energy bombardment than for fission fragment bombardment. The results suggest a higher upper mass limit for high-energy (~100 MeV) particle bombardment than for kiloelectronvolt particle bombardment.

Note Added in Proof. Quasi-molecular ions with m/z ~34 000 have recently been observed with fission fragment bombardment (*15*). Molecular ions in the same mass range have also been observed recently from photon bombardment (*16*).

Registry No. ACTH (1-10), 2791-05-1; ACTH (1-24), 16960-16-0; leucine-enkephalin, 58822-25-6; substance P, 33507-63-0; α-endorphin, 61512-76-3; β-endorphin, 60617-12-1; renin tetradecapeptide substrate, 64315-16-8; dynorphin, 74913-18-1; bovine insulin, 11070-73-8.

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A Pyrolysis–Mass Spectrometry Investigation of Pectin Methylation

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Pyrolysis–mass spectrometry (Py–MS), in conjuction with multivariate data handling procedures, was investigated as a potential method for the rapid determination of the degree of methylation (DM) in pectin. Good discrimination between pectins of various DM was achieved. Masses m/z 85 and 96 were identified as being significantly important to the discrimination. Factor analysis and pyrolysis–gas chromatography–mass spectrometry (Py–GC–MS) were employed to study the origin of masses m/z 85 and 96. Based upon these findings pyrolysis mechanisms for galacturonic acid and methylated galacturonic subunits within pectin are proposed.

Pectins are a major group of heterogeneous polysaccharides that are of considerable importance to the food industry as gelling and thickening agents (*1*). Pectin, as illustrated in Figure 1, consists predominately of α-1,4 linked D-galacturonic acid units with varying degrees of methylation. Neutral sugars such as galactose and arabinose are associated as side chains and rhamnose units are dispersed within the polygalacturonic acid backbone (*1*, *2*).

The ability of pectin to gel depends largely on the degree of methylation (DM). Determination of DM is normally achieved by analysis (titration) of the carboxyl groups before and after hydrolysis (*1*) and/or gas chromatographic analysis of methanol released on hydrolysis (*3*, *4*). The degree of methylation has also been estimated by the ratio of carboxymethyl to carboxyl resonances using ^{13}C NMR (*5*). All these techniques require extraction/isolation of the pectin prior to analysis necessitating large quantities of sample and lengthy preparation/analysis times.

Pyrolysis is an analytical technique well suited to the analysis of nonvolatile materials such as pectins. The technique requires minimal sample and little or no sample preparation and is readily combined with separation techniques

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