Suspended Trapping Procedure for Alleviation of Space Charge Effects in Gas Chromatography/Fourier Transform Mass Spectrometry

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A suspended trapping pulse sequence is implemented in Fourier transform mass spectrometry detection of capillary gas chromatography effluent as a means to alleviate space charge effects in the trapped ion cell. The combination of intense ionization conditions and a suspended trapping delay extends the working range of gas chromatography/Fourier transform mass spectrometry (GC/FTMS) for which high-performance spectra are generated to 5 orders of magnitude, from detection limits of 10–100 pg to the limit of gas chromatography (GC) column capacity. This corresponds to a factor of 10^3 improvement compared to conventional trapping methods. Shifts in cyclotron frequency over the eluting GC peak profile are also reduced from as much as 210 Hz to less than 3 Hz over the same range of neutral analyte concentrations, which indicates accurate mass calibration can be achieved independent of initial ion population in the trapped ion cell. This capability is demonstrated as frequency assignments with low part-per-million error are obtained by GC/FTMS for mixture components of varying concentration from a single suspended trapping calibration table.

The application of Fourier transform mass spectrometry (FTMS) as a detector for capillary gas chromatography (GC) is recommended by the simultaneous ion detection, high mass resolution, and low part-per-million (ppm) error mass measurement capabilities of the spectrometer. The source/analyzer pressure conflict (*1*) that plagued early GC/FTMS work (*2*, *3*) has been reconciled through the development of first the pulsed valve interface (*4*) and then differentially pumped external source (*5*, *6*) and dual cell (*7*, *8*) instrument configurations that now extend detection limits well below 1 ng without compromising the FTMS performance achieved at lower analyzer pressures. A continuing impediment to routine implementation of GC/FTMS, however, is the modest working range of the detector before space charge perturbs ion motion in the trapped ion cell (*9*, *10*). The actual range of neutral analyte concentration for which acceptable spectra are generated for constant ionization parameters before Coulombic line broadening (*11*) and peak shape distortion arise, depends upon trapped ion cell capacity (*12*) but is only about 2 orders of magnitude in the 2 in. cubic cell. The working range for low ppm error mass measurement is even more restricted, typically less than an order of magnitude. Given that the minimum acceptable range for GC/MS performance should extend from the low picogram detection limits achieved with quadrupole and sector mass analyzers to the capacity of capillary GC columns which is typically tens of micrograms, it is necessary that present GC/FTMS performance be extended by 2 to 3 orders of magnitude to be competitive with other GC/MS instruments.

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The restrictions to dynamic range imposed by Coulombic repulsive effects in the FTMS trapped ion cell are endemic to all ion trapping devices and similar problems were encountered with the ion trap detector (ITD) developed by Finnigan for GC/MS (*13*). The automatic gain control (AGC) solution arrived at for the GC/ITD utilizes a real-time interactive adjustment of electron ionization beam time to match ionization conditions with the expected neutral analyte population. The linear dynamic range for GC/ITD was found to improve 1000-fold with AGC as a 10^6 change in neutral concentration could be detected before the onset of space charge effects. An approach analogous to AGC for extending GC/FTMS performance is conceivable, but modifications to the trapped ion cell and detection circuitry to facilitate ion current monitoring and adjustment would be difficult and to date have not been implemented.

In a recent paper we described an alternative method for regulation of the ion population detected by FTMS that is based upon the suspension of trap plate potentials after the ionization event and prior to detection to permit the efflux of excess ions that contribute to space charge (*14*). The insertion of a suspended trapping delay of a few milliseconds was found to establish an ion population for detection that was independent of initial ionization conditions and neutral populations and yielded FTMS spectra with nearly identical relative mass intensities, mass resolution, and effective cyclotron frequencies. Application to detection of chromatographic effluent and to desorption experiments, each of which generate transient or fluctuating neutral analyte population, was suggested as a means to extend the working range for FTMS and to simplify mass calibration. In this paper the merits of suspended trapping for FTMS detection of GC effluent are evaluated.

EXPERIMENTAL SECTION

The FTMS instrument used for all experiments is described elsewhere (*14*). Briefly, the system includes a 3-T superconducting magnet, a Nicolet analytical instruments dual cell assembly with 2 in. cubic cells, differential pumping with 700 L/s UHV diffusion pumps, and Nicolet FTMS-2000 data system and analog electronics. System base pressures are maintained in the mid 10^{-9} Torr range as measured by Bayard–Alpert gauges mounted above the diffusion pumps outside the strong magnetic field. Electron ionization is accomplished with the standard Nicolet filament assembly mounted in the analyzer chamber. During the beam event an electron beam is gated to traverse the dual cell and strike a probe mounted collector placed 37 cm behind the source trap plate in the source chamber. Measured electron beam currents through the cell are 20% of software-requested values.

A Hewlett-Packard 5890 gas chromatograph was interfaced to the FTMS to perform the GC/FTMS measurements. A capillary split injector with 10:1 split ratio was used for sample injection onto a wide bore, short length capillary column with zero-grade helium as the carrier gas. This column was selected to increase sample capacity and to permit rapid repetitive separation of the simple mixtures used to evaluate suspended trapping performance. Methylnaphthalene separations from methylene chloride solvent

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were performed isothermally at 50 °C. The three-component mixtures dissolved in methylene chloride were separated with a temperature program from 50 to 100 °C.

The interface to the mass spectrometer consisted of an open split measured at 100:1 at the column exit that is achieved by inserting a 100 μm i.d. × 10 cm length of fused silica a distance of 3 cm into the GC column. The 100 μm i.d. restrictor was connected with a zero-volume union to a 250 μm i.d. × 2 m length of deactivated fused silica transfer line that terminated in the vacuum chamber a distance of 2 cm from the source side trapped ion cell. Source and analyzer pressures during GC experiments were 1.1×10^{-6} and 1.1×10^{-8} Torr, respectively. The transfer line to the FTMS was maintained at 100 °C with resistive heating and the FTMS vacuum system temperature was 165 °C.

FTMS data acquisition parameters were controlled with Nicolet FTMS-2000 software version 5.1R2. The suspended trapping pulse sequence used was identical with the conventional single resonance swept excitation pulse sequence (SGL) except for a variable delay event inserted after the beam event during which collector plate, source trap, conductance limit, and analyzer trap were set to 0.0 V. For all experiments a −21.5-V electron beam was used to minimize helium ion formation. Various combinations of electron beam currents, ranging from 5 to 40 μA, and beam times, ranging from 5 to 30 ms, generated desired initial ion populations. Ion equilibration between source and analyzer during the beam event with 1.3-V trap plate potentials and 0.0 V at the conductance limit yielded transfer efficiencies between 35% and 40% of the initial source population. Trap potentials for analyzer cell excitation and detection events were 1.3 V. Common to all experiments was 2.66-MHz broad-band excitation at a 3200 Hz/μs sweep rate. An 800-kHz bandwidth with 32K data points was used for GC/FTMS broad-band detection of the three-component mixture experiments. In experiments that examined frequency shifts in the molecular ion region of methylnaphthalene, narrow band detection of 16K data points over a 25.8-kHz window from masses 137 to 145 was used. Data processing of transients included base-line correction, addition of an equivalent number of zeroes, sine bell apodization, and a magnitude Fourier transform.

For the GC software subroutine employed, the minimum GC/FTMS scan time of 1.9 s was limited by the software overhead for background storage of transients to the storage module. It was found that a total of ten transients could be coadded and stored every 1.9 s for the broad-band experiments and four coadded transients could be stored in 1.9 s for mixer mode experiments. With typical peak widths of 10 to 20 s at the base line, the chromatographic profile was adequately defined. No threshold for data storage was used.

Chemical information extracted from spectra during postrun processing with Nicolet subroutines included relative peak height, signal to noise, peak centroid frequency, and mass resolution. In particular, the algorithm to calculate the peak centroid frequency extracts the peak maximum from the fit of an inverted parabola to the top of a sampled peak (*15*). Mass calibration tables were generated from spectra of perfluorotributylamine (PFTBA) that was leaked through a volatile inlet. Subsequent chromatography experiments were performed in the absence of calibrant. The software supplied calibration equation used to assign calibrant ion frequencies (*16*) accounts for the magnetic and trapping electric fields but does not include an ion density dependent electric field term.

RESULTS AND DISCUSSION

Selection of a Suspended Trapping Delay. Application of suspended trapping to GC/FTMS measurements first requires the determination of an appropriate delay time during which excess ions exit the trapped ion cell. In previous work optimum delays of 1 to 2 ms were indicated by the suspended trapping ion flight profiles (*14*). However it was also noted that because the ion population remaining in the cell at extended delays was a function of cell cleanliness, the profile was not constant and occasional reevaluation of an optimum delay time would be necessary. In Figure 1a the flight of naphthalene molecular ions from the dual cell, obtained at the time of these GC/FTMS experiments, is presented. The large initial ion population at zero delay (which corresponds

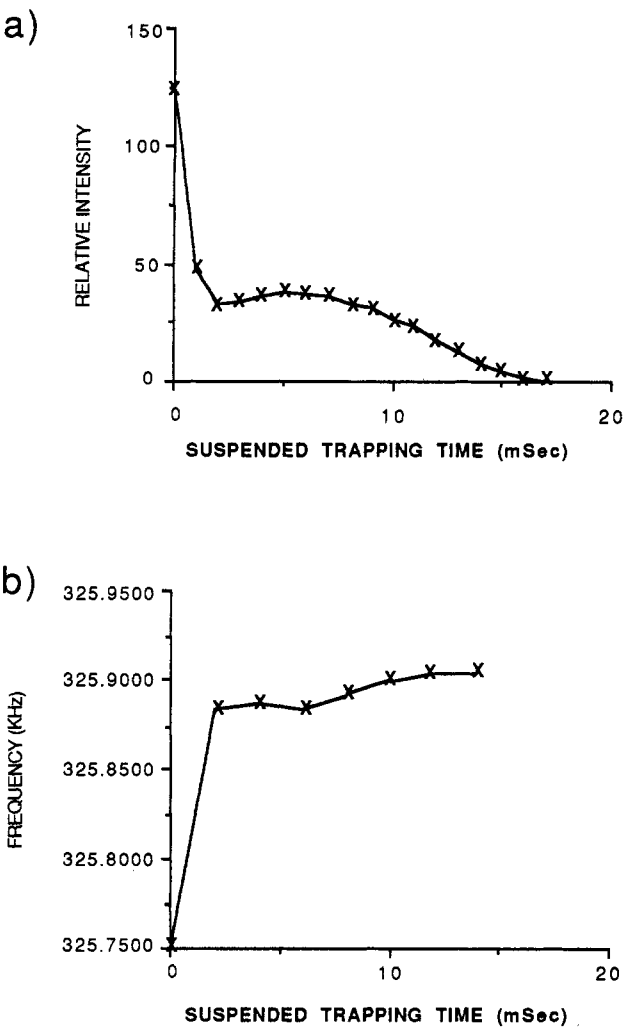

Figure 1. Profiles of suspended trapping ion intensity (a) and effective cyclotron frequency (b) at increasing suspended trapping delay times. Data are for the naphthalene molecular ion. Sample was leaked into the source at 5×10^{-8} Torr and ionized with a 10-ms, 10-μA beam.

to the conventional ion trapping population) decays rapidly in the first several hundred microseconds due to strong local ion Coulombic forces. The sustained signal that follows is attributed both to the influx of ions during suspended trapping from the external well between the collector plate and source trap and to the low-energy ions retained in the cell due to surface charging on contaminated trap plates (*17*). Because the trapped ion cell had not been cleaned for several weeks, cell contamination was significant and the plateau in the ion profile extended for nearly 8 ms before the onset of a more pronounced decay.

Selection of an appropriate suspended trapping delay time is complicated in this work by the desire to generate spectra with high mass measurement accuracy. Sensitivity benefits achieved by reducing the selected delay time conflict with the expected reduction in mass measurement errors associated with smaller ion populations at longer trapping delays. As indicated in Figure 1b, the leveling of effective cyclotron frequency with increased suspended trapping delays is nearly complete at 12 ms. As a compromise between sensitivity and mass calibration requirements, a 10-ms suspended trapping time was selected for all subsequent GC/FTMS measurements. At the beginning of each work period the ion flight profile was reevaluated to ensure that suspended trapping conditions were not altered.

Dynamic Range Enhancement. The primary benefit of suspended trapping to GC/FTMS applications should be extension of the working range over which spectral quality

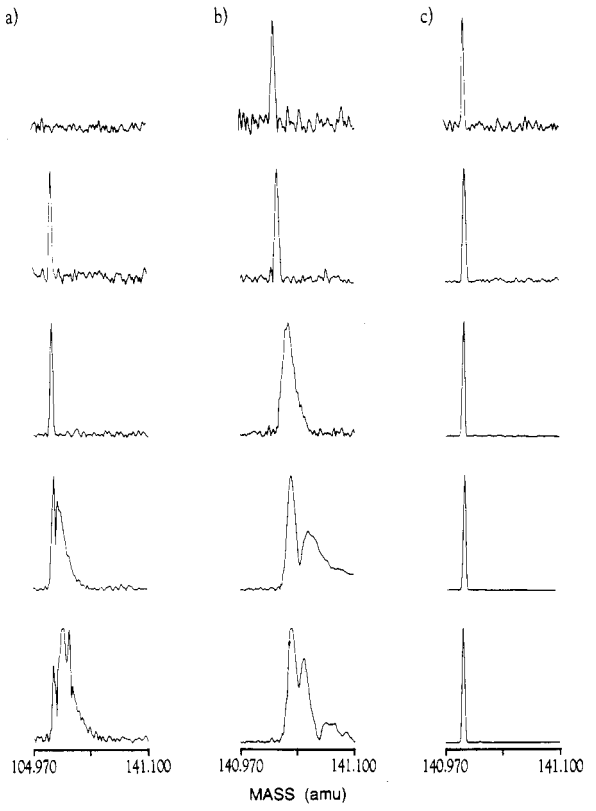

Figure 2. GC/FTMS spectra of methylnaphthalene in the region around mass 141 for increasing concentrations of the analyte injected on the GC column. The estimated amounts of analyte introduced to the source are, in descending order in the figure, 10–100 pg, 100 pg–1 ng, 1–10 ng, 10–100 ng, and 50–500 ng. These absolute amounts are obtained from estimates of pre- and postcolumn split ratios, but relative ratios are correct. The maximum intensity spectrum from each GC profile is presented for three sets of ionization parameters: (a) 10-μA electron current, 10-ms beam time with conventional trapping; (b) 40-μA current, 30-ms beam with conventional trapping; (c) 40-μA current, 30-ms beam with a 10-ms suspended trapping delay.

does not deteriorate due to space charge. Justification for suspended trapping detection when large neutral analyte populations elute from the GC column is apparent. However, the question arises whether this benefit is negated by a corresponding decrease in ion population for the case of small neutral populations. We argue that the signal detected for these smaller neutral populations with suspended trapping will not decrease but will actually improve, for two reasons. First, the rate of ion flight from the cell early in the suspended trapping period is reduced for smaller ion populations because ion Coulombic repulsive effects are smaller. Second, by use of stronger ionization conditions than space charge considerations would deem practical for conventional trapping experiments, the initial ion population generated would be larger.

Presented in Figure 2 are results from experiments designed to compare the working range of suspended trapping GC/FTMS with conventional trapping. The FTMS spectra presented are from GC separations in which increasing amounts of methylnaphthalene were injected on column. For each injection the maximum intensity spectrum from the GC profile expanded in the region around mass 141 is shown. Injections were made at 11 increasing concentrations of methylnaphthalene in solvent over a 6 order of magnitude range from below the FTMS detection limits up to the limit of GC column capacity. An examination of spectral peak shape was found to provide the best measure of the onset of space charge distortion. The data set in Figure 2a is for the ionization conditions typical of those commonly used in GC/FTMS, a 10-ms beam and a 10-μA requested current with conventional trapping. The first signal detected in a spectrum by spectrum search was for the GC injection that corresponded to between 100 pg and 1 ng being introduced to the source. Acceptable peak shapes were then observed for subsequent injections over a 2 order of magnitude range before obvious peak distortion in a maximum intensity file occurred. The spectra in Figure 2b are for a 30-ms beam and 40-μA current with conventional trapping. As would be expected with more intense ionization conditions, detection limits were lowered, in this case by an order of magnitude. The ion capacity of the cell is constant, however, and again a 2 order of magnitude working range was observed before the onset of space charge. These spectra provide a clear indication of the limited accommodation of the FTMS trapped ion cell for any experiment in which the neutral population cannot be controlled.

Figure 2c presents suspended trapping spectra acquired for the identical experimental conditions that yielded the spectra in Figure 2b, with the single modification that a 10-ms suspended trapping delay time was inserted after the beam event. The most obvious feature of the suspended trapping data, in marked contrast with the data sets in parts a and b of Figure 2, is retention of peak shape quality and mass resolution over a nearly 5 orders of magnitude change in initial neutral population. The detection limit of 10–100 pg was similar to the limit found for conventional trapping. Surprisingly, spectral quality is superior for the suspended trapping case even though the number of analyte ions must be smaller. This reproducible effect may be due to a mass-dependent explusion of lower mass background ions from air, water, and carrier gas during the suspended trapping event.

It is apparent from the data in Figure 2 that suspended trapping provides an effective approach to extending the range of FTMS detection of GC effluent. The magnitude of the improvement is similar to that observed with AGC for ion trap detectors. Suspended trapping has the advantage of being much simpler to implement because the detected ion population is self-regulating. The most important disadvantage of suspended trapping is the apparent loss of quantitative information usually available from integration of chromatographic peak areas. It can be argued however that given the already modest working range of conventional trapping FTMS measurements, this lost information is a minor inconvenience. In fact, reconstructed chromatograms from suspended trapping experiments are quite similar to those obtained by conventional trapping because if initial ion populations are within the space charge limit of the cell, then suspended trapping ion loss due to Coulombic repulsion is small. It is only for initial ion populations that exceed the space charge limit that the rate of ion loss due to Coulombic repulsive effects becomes large and quantitative information is lost. At these ion densities, however, conventional spectra also lose quantitative significance. There are, however, several approaches to regaining lost quantitative results including the use of auxiliary detectors like the flame ionization detector, implementing ion collection electrodes that monitor ion current exiting the cell during suspended trapping, or combining alternate suspended trapping and conventional pulse sequences during the GC experiment.

Simplified Mass Calibration. FTMS mass measurement accuracy at the low- and sub-ppm error level has been shown in numerous applications (*15*, *18–20*), including GC/FTMS measurements in which low-ppm errors over a wide mass range in the absence of calibrant ions were demonstrated for simple synthetic mixtures (*21–23*). Routine low-ppm errors are possible, however, only when the analyte ion population is well controlled. Under these circumstances the important contributions to measured cyclotron frequency are a homogeneous magnetic field and the static electric trapping field. Several

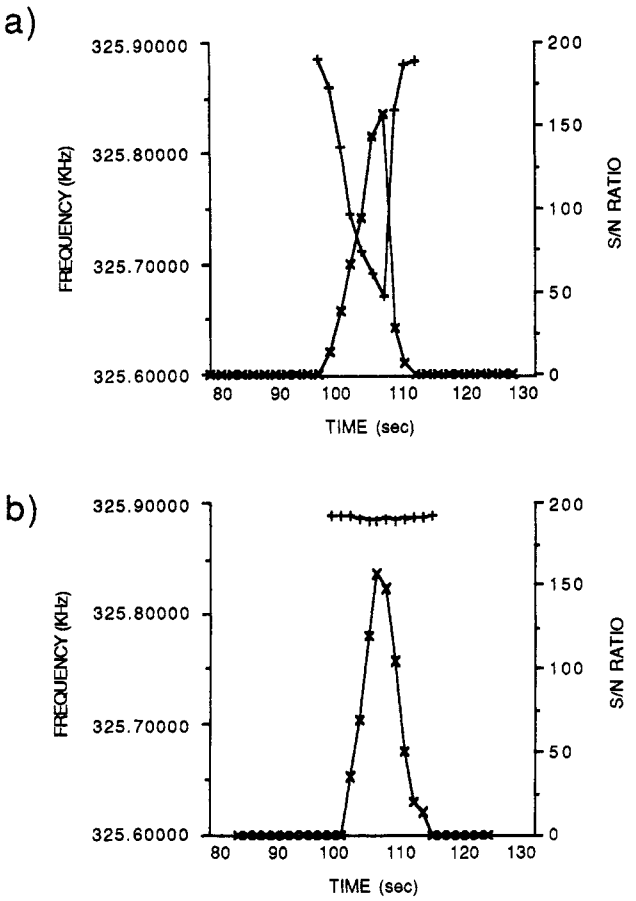

Figure 3. Chromatographic profile recreated for injection of 100 ng of methylnaphthalene into the GC with FTMS detection. Parameters extracted from individual FTMS spectra to yield the profiles are as follows: ×, S/N of the ion at nominal mass 141; +, effective cyclotron frequency. Part a is for FTMS detection with 10-μA beam and 10-ms beam time with conventional trapping, and part b is for 30-ms beam time and 40-μA current with 10-ms suspended trapping time.

calibration equations that account for these two factors have been demonstrated (*16*). Unfortunately, as Gross and coworkers first showed, the local ion Coulombic electric field in the trapped ion cell also contributes with the trapping electric field to decrease the measured cyclotron frequency (*15*). Frequency shifts can be several hundred hertz for large ion populations, which renders calibration tables based solely on magnetic and trapping fields useless for other than nominal mass assignment. Calibration equations developed to account for the ion density dependent electric field (*24*) are effective only if the ion population is known or can be measured. This is rarely possible in routine analytical work and instead calibration equations are constructed either from ion populations similar to those expected for the analyte or at low ion densities so that the added ion Coulombic electric field term is negligible. Obviously for chromatography experiments in which neutral populations vary over several orders of magnitude, neither option is feasible. Successful accurate mass measurement studies reported in previous GC/FTMS work were obtained for mixtures with components of approximately equal concentration (*21*).

Suspended trapping is effective in simplifying mass calibration because the detected ion population is reduced to levels at which the ion Coulombic electric field term is negligible. It would be expected then that the measured frequency should be constant over the course of an eluting GC profile that yields spectra for a wide range of analytical concentrations. To demonstrate this, GC profiles that track the elution of a 100-ng injection of methylnaphthalene are presented in Figure 3. In Figure 3a, the signal to noise ratio (S/N) and

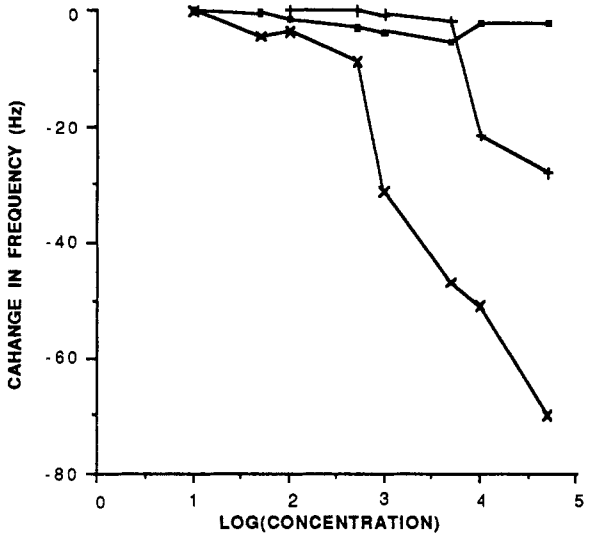

Figure 4. Comparison of effective cyclotron frequencies for the ion of methylnaphthalene at mass 141 for increasing concentrations injected on column. Frequencies are from spectra shown in Figure 2: ×, 10 ms, 10 μA with conventional trapping; +, 30-ms beam, 40-μA current with conventional trapping; ■, 30-ms beam, 40-μA current with 10-ms suspended trapping delay.

cyclotron frequency of the mass 141 ion are extracted and plotted as a function of time to obtain chromatograms for a 10-μA, 10-ms beam experiment with conventional trapping; both parameters are observed to provide a useful measure of neutral analyte population. Unfortunately, the 210-Hz change in frequency corresponds at mass 141 for a 3-T magnet to a minimum fluctuation of about 650 ppm over the eluting GC peak if accurate mass measurement of the ion is attempted. The actual mass assignment error could be even greater if the calibration table generated for the calibrant ion population is outside the concentration range of the methylnaphthalene profile. In contrast with the results in Figure 3a, the S/N profile for suspended trapping also mimics the conventional trapping result but the frequency profile varies by less than 3 Hz, which suggests the possibility of low ppm error in mass measurement for fluctuating neutral populations.

To better assess the effective range of suspended trapping for improved mass calibration, frequency values from the spectra in Figure 2 were extracted and are plotted in Figure 4. As expected, large frequency shifts for conventional trapping spectra both precede and then accompany Coulombic broadening and peak distortion. The use of a 30-ms beam and 40-μA current with conventional trapping apparently creates such a large trapped ion cell population (including both analyte and background ions) that mass calibration would be difficult over any concentration range. Better results would be expected with a 10-ms beam and 10-μA current as the frequency shift of less than 10 ppm over a 10- to 100-fold concentration change suggests. Clearly, however, the coupling of suspended trapping with intense initial ionization affords the best performance as frequencies corresponding to less than a 10 ppm change are exhibited over the entire 5 order of magnitude range accessible to GC/FTMS.

Application of suspended trapping FTMS mass calibration to fluctuating GC populations is now demonstrated. The accurate mass data presented in Table I are from GC/FTMS analyses of two mixtures of *p*-xylene, methylnaphthalene and bromonaphthalene, one with components in equal concentration by weight and the other with concentrations varying by 2 orders of magnitude. Frequencies were assigned to the electron ionization fragments by interpolation of a PFTBA calibration table constructed just prior to the GC/FTMS experiments with the same ionization conditions and sus-

Table I. Accurate Mass Data for Suspended Trapping GC/FTMS Measurements of a Three-Component Mixture

	calculated mass, amu	measured mass, amu	error, ppm
mixture 1[a]			
bromonaphthalene	207.970 516	207.972 122	−7.7
	205.972 562	205.973 117	−2.7
	127.054 226	127.055 73	−11.9
methylnaphthalene	142.077 701	142.078 650	−6.7
	141.069 876	141.070 110	−1.7
	115.054 226	115.055 040	−7.1
p-xylene	106.077 701	106.078 098	−3.7
	91.054 226	91.054 633	−4.8
	77.038 576	77.040 307	−22.5
mixture 2[b]			
bromonaphthalene	207.970 516	207.967 230	+15.8
	205.972 562	205.972 790	−1.1
	127.054 226	127.055 172	−7.4
methylnaphthalene	142.077 701	142.078 440	−5.2
	141.069 876	141.069 900	−0.2
	115.054 226	115.054 850	−5.7
p-xylene	106.077 001	106.077 820	−1.1
	91.054 226	91.054 530	−2.9
	77.038 576	77.038 920	−4.5

[a] Equal amounts by weight of each compound were injected with approximately 10 ng of each entering the source. [b] Mixture components in the ratio of 100 ng of *p*-xylene, 10 ng of methylnaphthalene, and 1 ng of bromonaphthalene entering the source.

pended trapping time. Errors for the two mixtures averaged 7.6 and 7.3 ppm for nine fragment ions and are consistent with previous wide-band accurate mass GC/FTMS measurements for specific acquisition parameters used (*21*). The primary source of error for the data in Table I is associated not with the suspended trapping event but rather with the inadequate number of data points used to define the bandwidth. This is reflected by the increase in error for higher mass ions. Errors on the order of 2 to 3 ppm could be achieved out to mass 300, but this would require a reduction in bandwidth or increase in the number of data points acquired, which is not always feasible (*21*). The advantage of suspended trapping demonstrated here is not a reduction in absolute error of the measurement compared to static system measurements but rather the opportunity to obtain low ppm errors from mixture components differing by orders of magnitude in concentration from a single calibration table. With little concern given to either analyte or calibrant neutral populations, the leveling effect of suspended trapping should permit assignment of elemental composition for the relatively low mass ions encountered in GC/FTMS.

One possible limitation of suspended trapping that has not been addressed is the skewing of relative abundances compared to conventional spectra. The reason for this mass discrimination effect is that if all ions possess similar kinetic energies independent of mass, then during the suspended trapping event lower mass ions with higher velocities would preferentially exit the cell. Although this effect is observed, the skewing of relative peak intensities does not follow that expected from simple time-of-flight considerations. For example, the comparison electron ionization spectra in Figure 5 are from the suspended trapping GC/FTMS separation of the three-component mixture used to produce the data in Table I and from conventional trapping measurements of pure samples introduced through a volatile inlet. Two explanations are given for the nearly identical relative intensities. First, ions created in the external reservoir enter the trapped ion cell during suspended trapping thereby forming a more uniform distribution of the ion population to be detected. Second, the population of low-energy ions continuously retained in the cell by charging on the trap plates should be

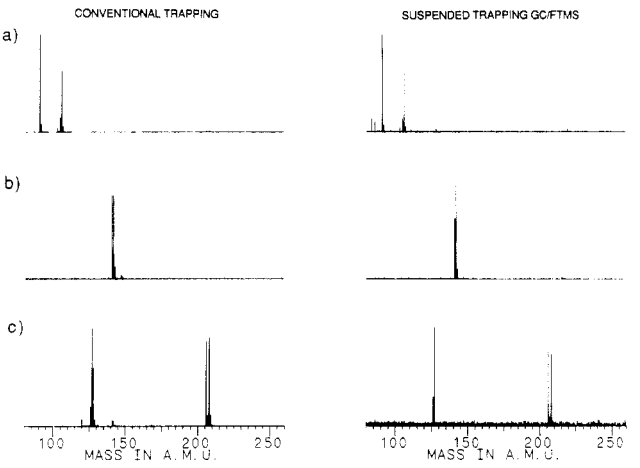

Figure 5. Suspended trapping GC/FTMS and conventional trapping FTMS spectra of *p*-xylene, methylnaphthalene, and bromonaphthalene in parts a, b, and c, respectively. Suspended trapping spectra are from the three-component mixture analyzed by GC/FTMS to yield accurate mass data in Table I. Ionization parameters included a −21.5-eV beam energy, 30-ms beam, 40-μA beam current, and 10-ms suspended trapping delay. Conventional trapping spectra are for pure samples introduced through a variable leak valve and detected with −21.5-eV beam energy, 10-ms beam, and 10-μA current.

representative of the ion population formed during the electron beam. If this population represents a significant fraction of the ions detected, then mass discrimination would be reduced proportionally.

Summary. A suspended trapping FTMS pulse sequence suggested as a means to eliminate space charge effects in spectra acquired from fluctuating neutral analyte populations is applied to GC/FTMS. By combination of intense ionization conditions with an appropriate suspended trapping delay, the working range extends from low picogram detection limits to the capacity of the GC column. GC/FTMS detection of complex mixtures should now be feasible. An additional benefit of the suspended trapping event is that the shift in cyclotron frequency associated with ion Coulombic repulsion is minimized so that a single mass calibration table is sufficient to generate data point limited low ppm error accurate mass measurement over the range of neutral concentrations.

LITERATURE CITED

(1) Laude, D. A., Jr.; Johlman, C. L.; Brown, R. S.; Weil, A.; Wilkins, C. L. *Mass Spectrom. Rev.* **1986**, *5*, 107–166.
(2) Ledford, E. B., Jr.; White, R. L.; Ghaderi, S.; Wilkins, C. L.; Gross, M. L. *Anal. Chem.* **1980**, *52*, 2450–2451.
(3) White, R. L.; Wilkins, C. L. *Anal. Chem.* **1982**, *54*, 2211–2215.
(4) Sack, R. M.; Gross, M. L. *Anal. Chem.* **1983**, *55*, 2419–2421.
(5) McIver, R. T., Jr.; Hunter, R. L.; Bowers, W. D. *Int. J. Mass Spectrom. Ion Processes* **1985**, *64*, 67–77.
(6) Kofel, P.; Allemann, M.; Kellerhaols, Hp.; Wanczek, K. P. *Int. J. Mass Spectrom. Ion Processes* **1985**, *67*, 97–103.
(7) Kofel, P.; Allemann, M.; Kellerhaols, Hp.; Wanczek, K. P. *Int. J. Mass Spectrom. Ion Processes* **1989**, *87*, 237–247.
(8) Giancaspro, C.; Verdun, F. R.; Muller, J. F. *Int. J. Mass Spectrom. Ion Processes* **1986**, *72*, 63–71.
(9) Wang, T. L.; Ricca, T. L.; Marshall, A. G. *Anal. Chem.* **1986**, *58*, 2935–2938.
(10) Jeffries, J. B.; Barlow, S. E.; Dunn, G. H. *Int. J. Mass Spectrom. Ion Processes* **1983**, *54*, 169–187.
(11) Wang, T.-C.; Marshall, A. G. *Int. J. Mass Spectrom. Ion Processes* **1986**, *68*, 287–301.
(12) Hunter, R. L.; Sherman, M. G.; McIver, Jr., R. T. *Int. J. Mass Spectrom. Ion Processes* **1983**, *50*, 259–274.
(13) Stafford, G. C.; Taylor, D. M.; Bradshaw, S. C.; Syka, J. E. P. *Proceedings of the 35th ASMS Conference on Mass Spectrometry and Allied Topics, Denver, CO. May 24–29*, 1987; pp 775–776.
(14) Laude, D. A., Jr.; Beu, S. C. *Anal. Chem.* **1989**, *61*, 2422–2427.
(15) Ledford, E. B., Jr.; Ghaderi, S.; White, R. L.; Spencer, R. B.; Kulkarni, P. S.; Wilkins, C. L.; Gross, M. L. *Anal. Chem.* **1980**, *52*, 463–468.
(16) Ledford, E. B., Jr.; Rempel, D. L.; Gross, M. L. *Anal. Chem.* **1984**, *56*, 2744–2748.
(17) Rempel, D. L.; Huang, S. K.; Gross, M. L. *Int. J. Mass Spectrom. Ion Processes* **1986**, *70*, 163–184.
(18) White, R. L.; Onyiriuka, E. C.; Wilkins, C. L. *Anal. Chem.* **1983**, *55*, 339–343.

(19) Shomo, R. E., II; Marshall, A. G.; Weisenberger, C. R. Anal. Chem. 1985, 57, 2940-2944.
(20) Rempel, C. L.; Ledford, E. B.; Sack, T. M.; Gross, M. L. Anal. Chem. 1989, 61, 749-754.
(21) Johlman, C. L.; Laude, D. A., Jr.; Wilkins, C. L. Anal. Chem. 1985, 57, 1040-1044.
(22) Sack, R. M.; McCrery, D. A.; Gross, M. L. Anal. Chem. 1985, 57, 1290-1295.
(23) Laude, D. A., Jr.; Johlman, C. L.; Brown, R. S.; Ijames, C. F.; Wilkins, C. L. Anal. Chim. Acta 1985, 19978, 67-77.
(24) Francl, I. J.; Sherman, M. G.; Hunter, R. L.; Locke, M. J.; Bowers, W. D.; McIver, R. T., Jr. Int. J. Mass Spectrom. Ion Processes 1984, 54, 189-199.

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Carbon-Isotopic Analysis of Dissolved Acetate

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Heating of dried, acetate-containing solids together with oxalic acid dihydrate conveniently releases acetic acid for purification by gas chromatography. For determination of the carbon-isotopic composition of total acetate, the acetate-containing zone of the chromatographic effluent can be routed directly to a combustion furnace coupled to a vacuum system allowing recovery, purification, and packaging of CO_2 for mass-spectrometric analysis. For analysis of methyl carbon, acetic acid can be cryogenically trapped from the chromatographic effluent, then transferred to a tube containing excess NaOH. The tube is evacuated, sealed, and heated to 500 °C to produce methane by pyrolysis of sodium acetate. Subsequent combustion of the methane allows determination of the ^{13}C content at the methyl position in the parent acetate. With typical blanks, the standard deviation of single analyses is less than 0.4‰ for acetate samples larger than 5 μmol. A full treatment of uncertainties is outlined.

INTRODUCTION

Acetate, a product of anaerobic microbial metabolism and an intermediate in the degradation of organic matter, is a substrate for both methanogenic and sulfate-reducing bacteria. Variations in the carbon-isotopic composition of sedimentary methane have been related to the distribution of acetate between these pathways of degradation (*1*, *2*). Further study of this segment of the carbon cycle will be aided by techniques allowing determination of the isotopic composition of sedimentary acetate and of isotope effects associated with its production and degradation.

Although numerous techniques are available for measurement of concentrations of acetate in sediments and in aqueous media (*3-9*), reports of the carbon-isotopic composition of acetate in natural solutions are much less common (*10-12*). Because accurate measurement of low concentrations of acetate is difficult, vacuum distillation (*13*) and passive diffusion (*14*) have been employed to concentrate acetate in samples. However, the sample-size requirement for isotopic analyses generally limits the application of current isotope procedures to samples with acetate concentrations greater than 100 μM (*10-12*). Because concentrations of acetate are less than 50 μM in most marine and freshwater sediments (*8*, *15*, *16*), isotopic analyses of acetate in these environments have not been achieved. We have developed techniques in which the carbon-isotopic composition of acetate and its methyl carbon can be measured in aqueous solutions at concentrations down to 20 μM. In this procedure, separation of acetate from other organic components, combustion of the acetate, and collection of the resultant CO_2 are combined.

Quantitative distillation of acetic acid from acidified aqueous solutions is impractical. Water and acetic acid co-distill; quantitative removal of acetic acid requires quantitative removal of water from the sample and little is gained. Accordingly, we first evaporate water (after adjusting pH to prevent loss of acetic acid), then evaporate acetic acid from acidified residual solids. Acidification must be accomplished with an acid that (1) is strong enough to protonate acetate quantitatively to acetic acid, (2) will not superprotonate acetic acid, forming an involatile cation, and (3) is nonaqueous. Gaseous HCl will protonate solid acetate salts, but HCl is much more volatile than acetic acid. Evaporation of a mixture of NaCl and acetic acid yields HCl, not acetic acid. In contrast, oxalic acid dihydrate (pK_{a1} = 1.23, pK_{a2} = 4.29 at 25 °C) can protonate acetate and has other advantages. As a solid, it can be mixed with an acetate-containing powder with almost no reaction occurring until the oxalic acid is melted (mp = 101.5 °C). A mixture of acetate and oxalic acid dihydrate, when heated to melt the oxalic acid dihydrate and dissolve the acetate, will yield acetic acid by the reaction

$$CH_3CO_2Na + HOOCCOOH \rightarrow HOOCCOONa + CH_3COOH \quad (1)$$

The acetic acid can be volatilized and removed from the sample with the residual solids remaining in the reaction vessel. The purified acid can be handled as a gas in a vacuum line (*13*) and transferred to combustion tubes by cryogenic distillation (*11*).

EXPERIMENTAL SECTION

Apparatus. The acetate-preparation system consists of a reaction vessel, a gas chromatograph, a combustion furnace, and a glass vacuum system (Figure 1). A disposable 6 cm × 9 mm o.d. Pyrex tube attached by an O-ring-sealed compression fitting (Cajon Ultratorr) to a U-trap at the inlet of a gas chromatographic column serves as a reaction vessel for liberation of acetate from samples. A Hewlett-Packard 5700A gas chromatograph was fitted with a 2.5 m × 4 mm i.d. Pyrex column packed with 80-100 mesh Porapak Q and was conditioned at 200 °C for 24 h prior to use. An external, dual-filament (gold sheathed tungsten), thermal conductivity detector (Gow-Mac, Model 10-301) is maintained at an operating temperature of 100 °C and a filament current of 150 mA. The flow rate of the carrier gas, helium, is 20-25 mL/min;

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