Combined Gas Chromatography-Mass Spectrometry in Flavor Research

Methods and Applications

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Combined gas chromatography-mass spectrometry (GCMS) is usually the most efficient approach to identification of volatile flavor components, but some fundamental limitations are imposed by the nature of the samples and by the method itself. Generally, only small quantities of material are available in natural aroma complexes, and ranges of amounts of individual components of the order of 1000:1 may be encountered. Careful selection of chromatographic columns and conditions is a major consideration. Limitations imposed by statistical variation of mass spectral patterns at the scan rates employed may make differentiation of closely related species impossible by mass spectral evidence alone. Chromatographic data may be useful in such cases. Classical chemical techniques may be modified to operate at the microgram level and can be effectively employed with GCMS analysis of reaction products. Applications of these methods in current studies of volatile components from bananas and wood smoke are described. The problems of data acquisition and processing are described in the context of the specialized requirements of flavor chemists.

The complexity of mixtures of volatile compounds obtained from foods and the small quantities in which these compounds are usually recovered necessitate extremely efficient separation methods and sensitive and specific identification techniques. During the past 10 years, application of mass spectrometry to determination of organic structures has become widely accepted. Current literature attests to its wide applicability, both to identification of compounds by comparison of spectra with those of reference materials, and to structural studies in which synthesis of the reference compounds is based on interpretation of the mass spectrum of the unknown compound.

Mass spectra provide information based on the mode of fragmentation of organic compounds which have been ionized, usually by electron bombardment. Fragmentation patterns and specific rearrangements of molecular ions can be used to deduce the arrangement of atoms in a molecule prior to electron bombardment. General approaches to interpretation of mass spectra have been based on both empirical data and detailed knowledge of mechanisms of ion decomposition. Several texts (Beynon, 1960; Biemann, 1962; McLafferty, 1966; Budzikiewicz *et al.*, 1967) provide excellent discussions of interpretation. The present paper discusses instrumental requirements, limitations, and techniques for the application of this powerful tool to the solution of identification problems in flavor chemistry.

Interpretation of the mass spectrum of an unknown compound is always easier when some additional structural information is available to the investigator. Infrared and NMR spectra provide complementary information; chromatographic data also may be helpful in deciding among a number of alternative structures. In many cases, however, only microgram quantities of materials are available for examination. When this is the case, the relatively low sensitivities of the complementary methods make mass spectrometry the only applicable method. The investigator must then rely on this single source of information for all structural data. Mass spectrometry does provide the maximum amount of structural data per microgram of sample. With the present availability of fast-scanning spectrometers, this statement can now be extended. Mass spectrometry provides the maximum amount of structural data per microgram per second. The large numbers of spectra which can be recorded in a short time may aid in interpretation and, on the other hand, lead to some new problems in data processing.

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EFFECT OF SEPARATION AND SAMPLE SIZE

The identification problem in flavor research should be examined from the point of view of the requirements imposed by the samples and by the separation methods. The great ranges of functionality and volatility of compounds which may contribute to food aromas preclude use of a single general analytical approach to all food flavors. Appropriate methods of isolation and pre-fractionation must be selected after examination of every alternative. Gas chromatography has, however, emerged as the most useful final separation method for the majority of flavor investigations. In many cases, adequate separations can be achieved only on the highest efficiency capillary columns. Use of these columns imposes stringent sensitivity requirements on identification methods since high separation efficiency is attained only for sample quantities in the microgram range. The maximum amount of sample which can be applied to high efficiency gas chromatographic columns is limited by overloading of the major components. In some cases, capillary columns can be permanently damaged by injection of samples containing more than 100 μg. of a single component. Figure 1 shows a capillary column chromatogram of an aroma concentrate prepared from bananas. Many of the peaks do not represent single components, but the chromatogram is probably a realistic representation of the complexity of ripe banana odor concentrate. Chromatograms such as this one are now typical in food flavor research, and flavor chemists are no longer surprised by such complex mixtures. The relevant data in Figure 1, from the point of view of this discussion, are the range of peak quantities, the mass flow rates, and the rates of change of these quantities. The major components in such a mixture are usually present in quantities on the order of a few micrograms. For example, the peak eluted at 20 minutes represents approximately 18 μg. of material. This approximation is based on calibration of the GC detector with *n*-amyl butyrate, as are all the quantities stated. The mass flow rate

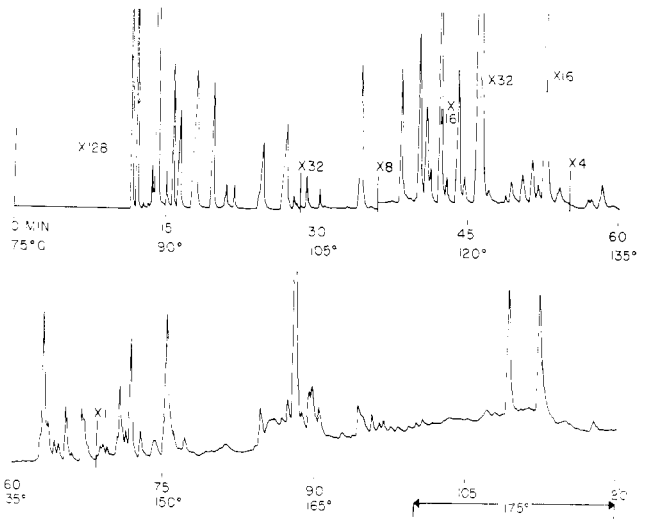

Figure 1. Banana odor concentrate; 0.02-inch I.D. × 500-foot SF 96(50) capillary column; helium flow rate, 4 ml./min.; flame ionization detector; electrometer range, 3 × 10^{-11} amp full scale; sample size, 0.5 μl.

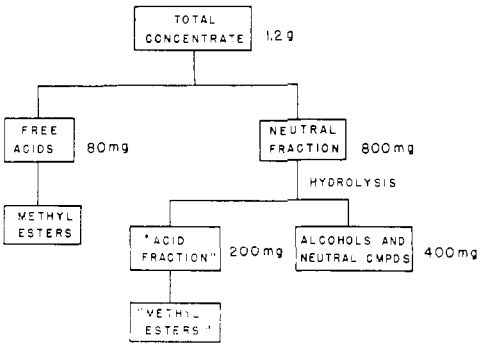

Figure 2. Scheme for analysis of banana odor concentrate

of sample, at the peak maximum, is 1.6 μg. per second. The peak at 43 minutes represents about 1.5 μg. with a maximum mass flow rate of 0.10 μg. per second. The peak at 110 minutes contains 0.23 μg. with a mass flow rate of about 12 ng. per second. The peak at 101 minutes, one of the smallest detectable, contains about 20 ng.; the mass flow rate at the peak maximum is of the order of 0.9 ng. per second. This single chromatogram exhibits a range of component quantity greater than 1000 to 1. The corresponding mass flow rate ratio is 1800 to 1.

Another significant feature illustrated by Figure 1 is the column bleed observed at the higher temperatures. At 175° C. the base line signal is equivalent to that produced by 4.2 ng. per second of amyl butyrate, and is approximately five times the signal measured at the top of the peak eluted at 101 minutes. Since this column bleed consists principally of silicone compounds to which the flame ionization detector is relatively insensitive, the bleed rate is actually higher; silicone compounds are major components of the column effluent.

Wide ranges of component concentrations are common in mixtures of volatiles isolated from foods. Minor components may represent less than 0.1% of mixtures containing more than 100 components. Guadagni *et al.* (1966) found that methyl thiohexanoate, which represented only 0.12% of the mass of hop oil, accounted for 4.8% of the total odor. Minor or trace components cannot be neglected since they may be potent odorants.

The presence of large numbers of components containing a variety of functional groups in widely varying proportions seriously aggravates the separation problems encountered in investigation of volatile flavor compounds. Pre-fractionation is often required to reduce the complexity of mixtures obtained from foods and to simplify the optimizing of chromatographic separations. Certain hazards inherent in the application of such procedures to flavor isolates include the possible production of artifacts, loss of some minor components, and loss of integrity of the natural flavor. Recognizing these hazards, we applied the separation scheme illustrated in Figure 2 to banana flavor concentrates (Wick *et al.*, 1969). Initial infrared and mass spectral examinations indicated that these mixtures contained primarily alcohols and esters. The concentrate was hydrolyzed, and the acids and alcohols were examined separately. The neutral fraction was found to be simpler than the total concentrate but much more complex than the acid fraction. Figure 3 shows a comparison of chromatograms of total concentrate from ripe banana and the

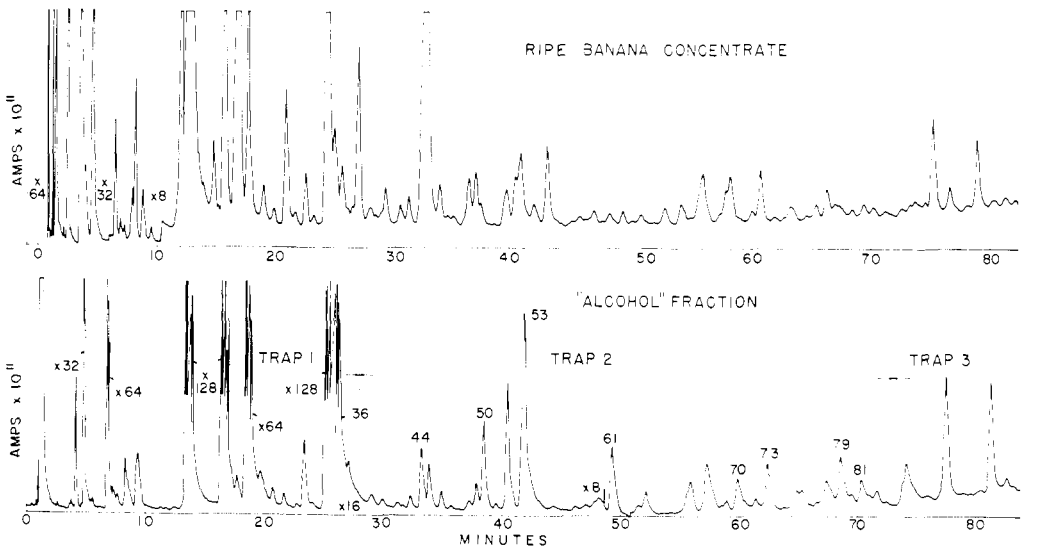

Figure 3. *A.* Ripe banana concentrate. *B.* Alcohol fraction from hydrolysis of concentrate; 0.02-inch I.D. × 50-foot Carbowax 1540 SCOT column; helium flow rate, 4 ml./min.; temperature program, 10 min. at 60° C., to 142° C. at 1° C./min., isothermal at 142° C. (Wick *et al.*, 1969)

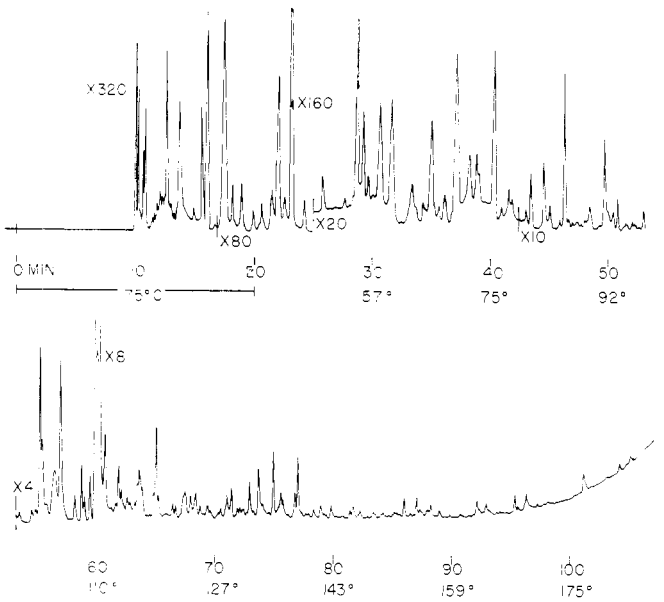

Figure 4. Hardwood smoke vapor phase; 0.02-inch I.D. × 500-foot SF 96(50) capillary column; helium flow rate, 4 ml./min.; electrometer range, 10^{-12} amp full scale; sample size, 80 ml. vapor. (Kornreich and Issenberg, 1968)

neutral fraction from the hydrolysis procedure. A Carbowax 1540 support-coated, open-tubular column (Perkin-Elmer Corporation, Norwalk, Conn.) was used for these separations. Trap numbers indicated on the neutral fraction chromatogram refer to fractions collected from a 6-foot × 0.25-inch O.D. 20% Carbowax 4000 stainless steel preparative column. Trap 2 exhibited a pleasant banana-like aroma which was particularly interesting since it implied that free alcohols or other neutral compounds not affected by hydrolysis were important odor components. These collected fractions were then subjected to combined gas chromatography-mass spectrometry (GCMS) utilizing high efficiency columns with operating conditions optimized for separation of each fraction.

Analysis of wood smoke condensates serves as another example of the utility of this approach (Lustre and Issenberg, 1969). The complexity of wood smoke is indicated in the chromatogram shown in Figure 4. Four 20-ml. samples of smoke vapor from combustion of mixed hardwood sawdust were drawn through a Cambridge filter into a gas-tight syringe. The vapor samples were injected into a capillary column, with the first 12 inches of the column serving as a liquid nitrogen-cooled trap. The peaks in Figure 4 represent the most volatile components of smoke vapor. Major effort was directed toward identification of the less volatile phenolic components of wood smoke condensates, since these compounds have been implicated as significant contributors to smoke flavor (Fiddler *et al.*, 1966; Wasserman, 1966). A phenolic fraction was prepared from an aqueous smoke condensate by fractionation based on acidity (Braus *et al.*, 1952). A chromatogram of a 20-μl. sample of the phenolic fraction was obtained on a packed SE-30 preparative column (Figure 5). Although this chromatogram did not exhibit the degree of separation attainable with capillary columns, it did permit the collection of fractions which were then further separated on more efficient polar and non-polar packed columns used in the GCMS system. Although a variety of capillary and support-coated, open-tubular columns were tried, none were found to be more effective than packed columns for separation of the free phenols.

Chromatography on silica gel columns has been widely used for separation of complex mixtures into fractions consisting of compounds of similar polarity. The microcolumn procedure described by Murray and Stanley (1968) extends this approach to samples in the 5- to 50-mg. range and simplifies recovery of volatile fractions. This method was developed specifically for pre-fractionation of flavor concentrates prior to GCMS analysis and is applicable to a variety of problems in food chemistry.

MASS SPECTROMETRIC LIMITATIONS

In addition to the requirements imposed by sample size and separation methods, certain limitations are inherent in the mass spectrometric method itself. Samples must be sufficiently volatile to be present as vapors in the ion source. In general, any material which is volatile enough to pass through a gas chromatographic column will be vapor in an electron bombardment ion source. Pressures in the range of 10^{-5} to 10^{-7} torr and temperatures between 100° and 250° C. are usually employed. Analysis of samples of low volatility or low thermal stability may require direct insertion into the ion source. A vapor pressure of about 10^{-7} torr at the sample temperature is usually sufficient to produce intense spectra.

Since all organic vapors will be ionized, samples in dilute solution cannot be analyzed directly. This limitation often severely restricts the handling of sample quantities in the microgram range. Solvents may be evaporated prior to introduction of the sample into the spectrometer, but contamination from the solvent or from atmospheric impurities may remain and contribute to the spectra. The ease with which most solvents and solvent impurities can be removed by gas chromatography is a major reason for employing a directly coupled gas chromatograph as an inlet system for the mass

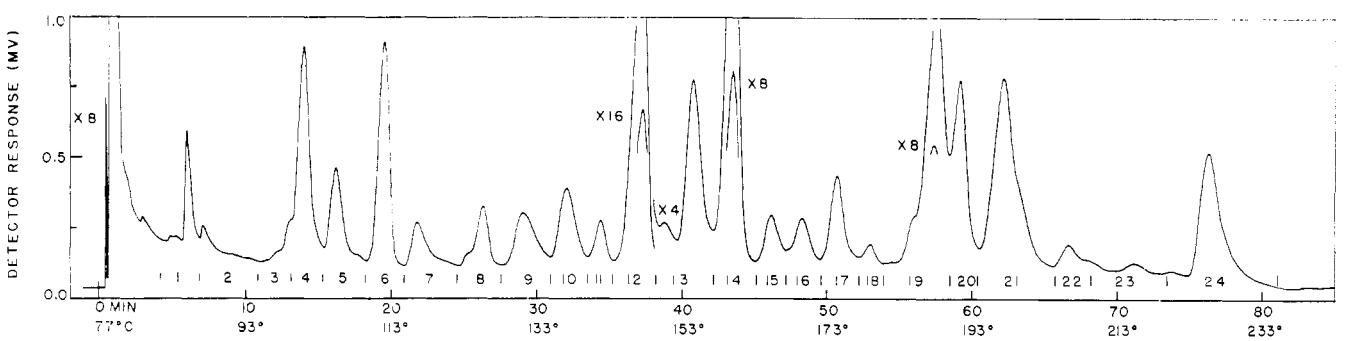

Figure 5. Wood smoke phenolic fraction; 0.25-inch O.D. × 6-foot, 20% SE-30 column; helium flow rate, 60 ml./min.; sample size, 20 μl. (Lustre and Issenberg, 1969)

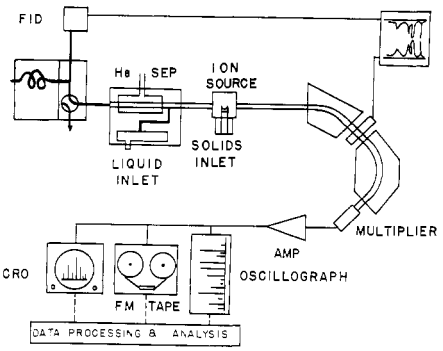

Figure 6. Schematic diagram of apparatus for GCMS

spectrometer. Gas chromatography is necessary, even when spectra of synthetic reference materials are recorded, to ensure a spectrum representative of the pure compound.

The effective sensitivity of a mass spectrometer depends on the method of sample introduction employed and the type of information required. In a recent review, McFadden (1967) stated that identification limits are of the order of 10^{-4}, 10^{-8}, and 10^{-7} to 10^{-8} gram for a batch liquid inlet, direct probe, and direct gas chromatographic coupling, respectively. These values refer to medium resolution spectra, *i.e.*, unit mass resolution at mass 300 to 500. High resolution spectra (resolving power 10,000 to 20,000) may require 1000 times more sample. Our observations agree with McFadden's (1967) requirements. It is difficult to specify minimum sample requirements for the combined GCMS system, since chromatographic peak widths may vary over wide ranges. The flow rate of the sample into the mass spectrometer ion source must be specified. When the mass spectrometer electron multiplier is operated at relatively low gain to minimize noise and background, a sample consumption rate of about 10^{-10} gram per second appears to be a minimum for recording spectra of acceptable intensity. This value refers to the amount of sample actually consumed in the ion source per second. If there are any losses involved, such as those which occur in carrier gas separators used in direct coupling of gas chromatographs with mass spectrometers, the initial amount of sample must be increased to provide the required sample consumption rate. The effect of inlet and effluent stream splitters, when used, must also be considered.

Most compounds of interest as aroma components are gases or liquids at room temperature, but it is possible, and often desirable, to introduce them directly into the spectrometer ion source *via* the direct solids inlet after trapping on gas chromatographic column packing (Amy *et al.*, 1965) or on activated charcoal (Damico *et al.*, 1967). This technique is particularly useful when recording high resolution spectra which may require scanning times too long for direct analysis of the chromatographic effluent, and when extremely small samples are examined and no waste of material can be tolerated.

APPARATUS REQUIREMENTS

Figure 6 is a diagram of the instrument system used in our laboratory for identification of food components and related materials. This diagram illustrates the basic instrument requirements for analysis of the wide variety of samples which can be distilled or extracted from foods. The mass spectrometer is a Hitachi Perkin-Elmer model RMU-7 double-focusing instrument, equipped with a high-gain electron multiplier, repeating scanner, and differential pumping system for the ion source. The instrument is capable of recording high resolution spectra ($M/\Delta M$ = 10,000) for determination of accurate mass and elemental composition of fragment ions. In the subsequent discussion, consideration will be given to applications at medium resolving power, *i.e.*, 300 to 500, for analysis of small samples eluted from gas chromatographic columns in flavor studies. An advantage of using a double-focusing spectrometer with a directly coupled chromatographic inlet is that helium ions can be defocused without significant discrimination against ions of *m/e* greater than 15. An electrode placed just after the energy selection slit will indicate total ion current with insignificant contribution from helium ions. A chromatographic record can be made at this point without resorting to use of low ionization energy to eliminate the helium signal (Ryhage, 1964).

The 16-dynode electron multiplier can produce current gain of about 10^6, which is required for high resolution studies; for medium resolution application, values of 10^3 to 10^4 are used to minimize noise and background. The principal reason for use of an electron multiplier is to reduce the time constant of the output system, rather than to increase over-all sensitivity. If a Faraday cup is used as an ion detector, the amplifier input resistance must be about 10^{11} ohms. When the multiplier is used, the input resistance may be reduced to 10^8 ohms. Thus, a multiplier gain of 1000 provides sensitivity equal to that of a Faraday cup, but the time constant is reduced to about 0.1 millisecond. This value is small enough to provide for scanning a mass decade (*i.e.*, *m/e* 30 to 300) in one second at resolving power 1000 without significant peak clipping or loss of resolution due to the output system (Banner, 1965).

Scanning of spectra is accomplished by varying the magnetic field at constant accelerating voltage. The scanner employed permits repetitive scanning of spectra during elution of chromatographic peaks, with independent control of the rates of magnetic field increase and decrease. This affords continuous observation of spectra on the oscilloscope, while simultaneous permanent recordings are made on either analog (FM) magnetic tape or the conventional oscillographic recorder. A single reel of analog tape can record 4 hours or more of continuously scanned spectra. This is very useful during elution of complex flavor isolates from a chromatographic column. The chromatogram can be recorded simultaneously, and the entire experiment or any portion of it can be reproduced at any time. This method of data recording relieves the instrument operator of the responsibility for deciding the optimum time to record spectra during a long chromatographic separation. It also provides the opportunity to re-examine portions of a chromatographic peak to determine homogeneity. Spectra can be reproduced from tape and examined visually on the oscilloscope prior to permanent recording on the oscillograph.

In addition to providing flexibility in data handling, the analog tape recorder reduces the cost of recording repetitive scans. Much of the data recorded by repetitive scanning during elution of chromatographic peaks is redundant if the peak is homogeneous. If the oscilloscope is used for editing, redundant spectra need not be recorded on paper, thus reducing the work involved in sorting and computing large numbers of spectra. The tapes also may be used as a source of input data for an automatic data acquisition and processing system at any time after recording. The entire GCMS experiment may be reproduced from the tape and processed by any remote computer facility which is capable of on-line operation (Hites

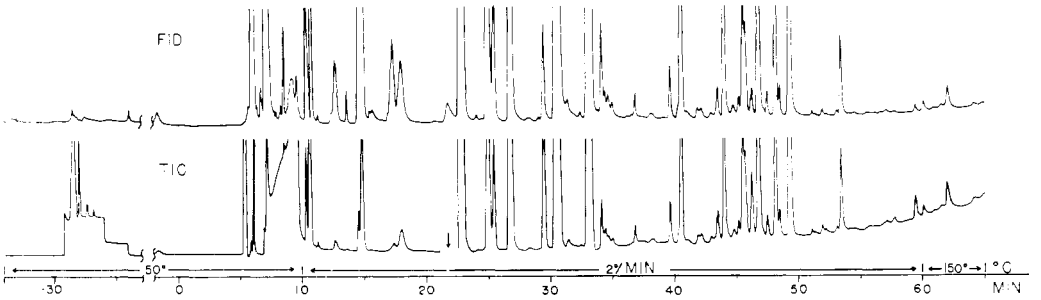

Figure 7. Ripe banana headgas

Upper curve: flame ionization detector; lower curve: total ion current monitor (MS) ionizing current increased from 100 μa. to 200 μa. at 22 min. (↓); 0.02-inch I.D. × 500-foot SF 96(50) capillary column; helium flow rate, 18 ml./min.; sample size, 100 ml.

and Biemann, 1968a). Data lines are not required, since tapes or even the recorder can be transported to a suitably equipped facility. The oscillographic recorder is equipped with 1000-Hz galvanometers which introduce a limiting time constant into the output system. This limit corresponds to a scan rate of about 3 seconds per mass decade at resolving power 300 (Banner, 1965).

During high resolution operation, differential pumping of the ion source is necessary to permit the high source pressures required when very small slit widths (*e.g.*, 5 to 10 microns) are employed, and to maintain low analyzer pressure so that resolving power will not be lost from collisions in the analyzer. Differential pumping is extremely useful for medium resolution work with the chromatographic inlet, since it allows high helium source pressures to be tolerated with minimal loss of resolving power or sensitivity. At a source pressure of 2×10^{-5} torr, the analyzer pressure is about 3×10^{-6} torr. Higher pressures are probably tolerable, but the commercial version of the helium separator provided (Watson and Biemann, 1965) appears to yield optimum efficiency at this source pressure when the helium flow entering the separator is 10 ml. per minute. Optimization of separator geometry and correct pressure balancing (ten Noever de Brauw and Brunnée, 1967; Grayson and Wolf, 1967) could probably increase separator efficiency (ratio of sample amount entering the ion source to the amount entering the separator) from the present value of 10 to 20% (measured from *n*-amyl butyrate) to about 40 to 50%. The latter efficiency range is comparable to that attained by the two-stage Becker jet type separator (Ryhage, 1967). At present 80 to 90% of the sample entering the enricher is lost; it could be recovered if a cold trap were placed in the vacuum line. The partition separator described by Llewellyn and Littlejohn (1966) offers interesting possibilities for application in flavor research. This type of device can be operated with the unused sample vented at atmospheric pressure (Black *et al.*, 1969) for collection or direct sensory evaluation.

The inlet gas chromatograph indicated in Figure 6 is a conventional dual column flame ionization detector instrument (Model 204, Varian-Aerograph, Walnut Creek, Calif.) with a single detector installed. The extra space in the detector oven is used for mounting a splitter, valves, and accessory devices. Capillary columns, support coated capillaries, and $^1/_8$-inch packed columns are employed in the chromatographic inlet, providing the flexibility required for handling the wide ranges of sample concentrations encountered in food flavor studies. To avoid the necessity for splitting very small samples after injection, a minimum volume injector adaptor was constructed for use with 0.02-inch I.D. capillary columns.

For most applications, the flame ionization detector branch of the effluent splitter is sealed, and only the total ion current monitor is used for recording the chromatogram. A conventional detector used parallel with total ion current recording provides a useful check on the operation of valves and auxiliary devices between the column and ion source. It also aids detection of possible sample decomposition or adsorption in the connecting lines or the helium separator. Adsorption of phenolic compounds was observed when a new separator was installed, but the glass surface was deactivated by silanization of the separator and connecting lines (Silyl-8, Pierce Chemical Company, Rockford, Ill.). There is a transport lag of about 5 seconds, at a flow rate of 10 ml. per minute, between the column exit and the ion source. This time difference affords an indication of the optimum time to begin a scan when single spectra are recorded.

A capillary column chromatogram of ripe banana headgas recorded in this manner is shown in Figure 7. Five 20-ml. samples were injected into a trap consisting of a 12-inch coil of capillary column which was removed from the chromatograph oven. The coil was cooled in liquid nitrogen. After injection of the last sample, a 20-minute period was required to establish steady flow and to elute non-condensed gases. Only injection of the last 20-ml. sample is shown in Figure 7. The rectangular deflection in the total ion current record was caused by the large quantity of air. Mass spectra recorded during that period indicated separation of oxygen and nitrogen during elution from the cold trap. At the end of the 30-minute equilibration period, the liquid nitrogen coolant was quickly removed; the coil was then heated with a hot-air blower and returned to the chromatograph oven. The column temperature was programmed from 50° to 150° C. A similar but more elegant system for capillary column analysis of food headspace vapors was described recently by Flath *et al.* (1969). Although this technique is a very useful approach to GCMS analysis of food volatiles, it is subject to even more severe dynamic range limitations than those described for flavor concentrates.

Vapor sampling discriminates against components of relatively low volatility which may be present in low concentrations in the vapor phase but contribute significantly to aroma. Use of large vapor volumes and efficient columns can minimize this problem. The major contribution of direct GCMS vapor analysis will be the determination of whether components identified in concentrates are authentic components of the food or artifacts formed during the concentration procedures. The method is currently being used in studies of banana flavor to determine which of the alcohols and acids identified in hydrolysates are present in the volatile esters.

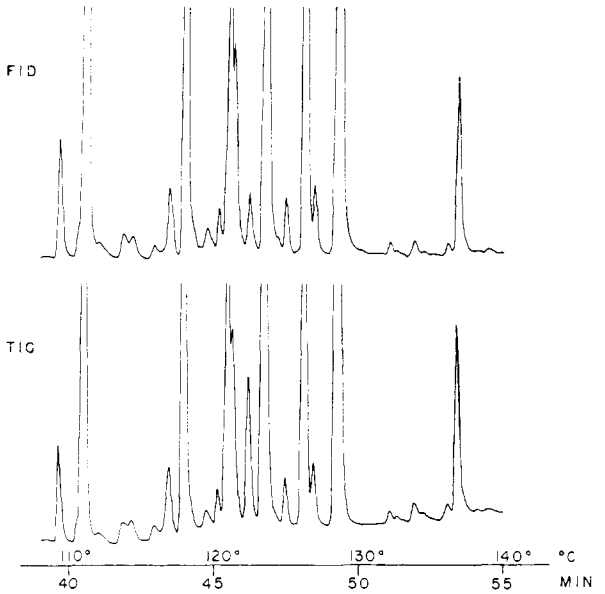

Figure 8. Portion of chromatogram of Figure 7

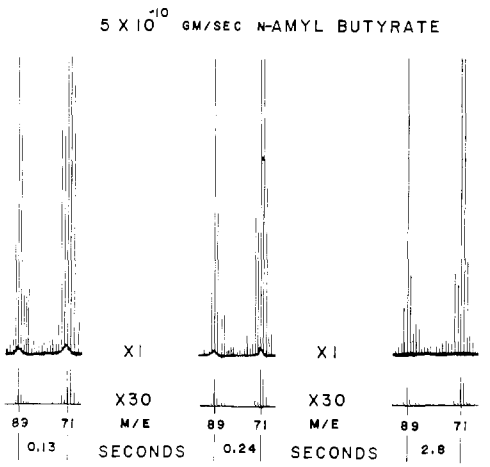

Figure 9. Effect of scan rate on sensitivity

Sample flow rate, 0.5 ng./sec.; scan rates, 1.5, 3, and 30 sec. for *m/e* 20–200

Vapor analysis represents a special case of the pre-fractionation approach described above. Banana flavor concentrates contain significant quantities of volatile alcohols which, since they are relatively water soluble and therefore present in low concentrations in vapor samples (Myers, 1968), do not interfere with identification of the esters.

A portion of the headspace vapor chromatogram is shown in Figure 8. Comparison of the records from the flame ionization detector and the total ion current monitor indicates that chromatographic efficiency is not lost in the heated connecting line (a 1.9-m. × 0.5-mm. I.D. capillary tube), carrier gas separator, or ion source.

Instrument operating conditions are very important because they can influence significantly the quality of data recorded and therefore the amount of information available in mass spectra. Mass spectra are not properties of molecules, but rather records of the way in which they decompose when bombarded by moderate energy (70 eV) electrons. Spectral patterns are influenced by ion source temperature (usually 250° C.), electron energy, and source pressure. Additional variables become important when fast scanning is employed.

Table I. Relative Intensities and Standard Deviation Estimates (S) for Major Peaks in *n*-Amyl Butyrate Spectrum[a]

Scan Time (*m/e* 20–200)	*m/e* 43		70		89	
	%	S	%	S	%	S
30 sec	83.2	2.2	55.7	3.3	66.0	1.9
3 sec	76.0	2.7	54.5	1.2	61.0	2.0
1.5 sec	77.8	7.9	63.4	7.2	71.0	7.6

[a] Sample consumption rate: 2×10^{-8} gram per second.

Figure 9 shows that sensitivity is essentially independent of scan rate in the range shown, provided the sample size is large enough to produce a steady ion beam without significant statistical fluctuations, and provided the output system bandwidth is sufficient to prevent peak clipping. The sample flow rate was 0.5 ng. per second of *n*-amyl butyrate, equivalent to that obtained at the peak of a 25-ng. sample eluted from a chromatographic column with a half-width of 10 seconds and passed through a helium separator with 20% efficiency. The spectra shown in Figure 9 were recorded with sample introduction through the liquid inlet and molecular leak in order to maintain constant sample flow rate. Reservoir pressure was adjusted to provide total ion current equivalent to that measured at the top of a 25-ng. chromatographic peak.

The data shown in Figure 9 were recorded at a multiplier voltage of 1.6 KV, corresponding to a gain of 10,000. About two orders of magnitude greater sensitivity is available at maximum gain. The normal instrument background spectrum may become so intense at higher multiplier gain that the multiplier and amplifiers can be saturated in the absence of sample.

Observation of the ion beam fluctuations led to a brief study of the effect of scan rate on pattern reproducibility. *n*-Amyl butyrate was used as the test sample, and intensities of the *m/e* 43, 70, and 89 peaks relative to the *m/e* 71 peak were measured from spectra recorded repetitively at scan rates of 1.5, 3, and 30 seconds for the range *m/e* 20 to 200. Six spectra were recorded at each scan rate. Sample flow rate of approximately 2×10^{-8} gram per second was established by use of the molecular leak inlet and maintained constant during the recording of each spectrum. Results obtained at multiplier gain of 10^3 are presented in Table I. Mean relative intensities and standard deviation estimates are shown for the three major peaks at a sample flow rate corresponding to a 1-μg. sample. These results indicate that when small samples are examined at high scanning speeds, discrepancies on the order of 10% for relative intensities of individual peaks occur when spectra of unknown compounds are compared with reference spectra recorded under identical conditions on the same instrument. Additional error may be introduced when spectra of unknowns are compared with data recorded under different experimental conditions in different laboratories. Some of these discrepancies, such as those caused by collision processes at the high helium pressures employed, use of different ionizing energies, variation of ionization chamber temperature, and instrumental mass discrimination are usually reproducible. Electrical noise and statistical effects are random, and though the over-all spectral pattern may be helpful for general characterization, discrimination between isomers which give similar mass spectra may be impossible. When these difficult problems arise, the inherent precision of the mass spectrometer can be utilized only by lowering the scanning speed and by limiting the output system bandwidth to reduce noise.

Additional information is often readily obtainable and should be employed to aid interpretation of mass spectra recorded during separation of a complex mixture. Chromatographic retention data can be recorded simultaneously and used to resolve some ambiguities. Since many compounds which occur in flavor isolates are unsaturated, hydrogenation may be a useful technique. Simple catalytic hydrogenators have been described (Beroza and Sarmiento, 1966; Mounts and Dutton, 1967) for use between the injection port and column of a gas chromatograph. This approach facilitates characterization of pure materials or of mixtures with only a few components. It is difficult to apply to identification of components of complex mixtures since it converts many of the isomers originally present to the same product, thus resulting in uncertainty in relating product peaks with peaks in chromatograms obtained prior to hydrogenation. The ideal location for a micro vapor-phase catalytic hydrogenator is between the chromatographic column and mass spectrometer. Under this condition, if quantitative hydrogenation occurs, peaks appearing on the flame ionization or total ion current chromatogram have the retention times of the original components of the sample, but yield spectra of the hydrogenated products. Teeter *et al.* (1966) applied this approach to quantitative analysis of alpha olefins, using a gas chromatograph mass spectrometer combination. The method should also provide valuable supplementary structural information if spectra of unreacted and hydrogenated products are compared. Such data can be particularly useful in analysis of compounds containing both rings and double bonds. The empirical formulas of such compounds, determined by mass spectrometry, show the number of rings plus double bonds. Hydrogenation of double bonds under conditions which prevent ring opening can eliminate this ambiguity in spectrum interpretation (Biemann, 1962).

Figure 10 is a schematic diagram of the micro-reactor system used in some initial studies of catalytic on-line hydrogenation. The entire apparatus was placed in the detector oven of the inlet chromatograph and maintained at a temperature of 145° C. The switching valves provide a means for bypassing the reactor when spectra of original material are recorded. In the initial experiments, the flame ionization detector was not used and its branch of the splitter was sealed. The reactor contained 10 to 15 mg. of 1% neutral palladium catalyst on Chromosorb W (Beroza and Sarmiento, 1966).

Application of the micro-reactor during separation of a mixture of hexen-1-ols is shown in Figure 11. Increased retention times and significant tailing are evident in the peaks after passage through the hydrogenator. This is probably caused by the large excess of Chromosorb present. Each alcohol was present in amounts between 0.2 and 0.6 μg. The order of elution is: *trans*-3-hexen-1-ol, *cis*-3-hexen-1-ol, *trans*-2-hexen-1-ol, *trans*-4-hexen-1-ol, and *cis*-4-hexen-1-ol.

Spectra recorded during elution of each peak with the column effluent by-passing the hydrogenator are shown in Figure 12. Spectra of peaks 5 and 6 are consistent with published spectra of *trans*-4- and *cis*-4-hexenols, respectively (Honkanen *et al.*, 1963). Peak 4 (*trans*-2-hexenol) was not well separated from peak 5 and the spectrum shows a major contribution from the *trans*-4-isomer at *m/e* 67. Peak 3 contains *cis*-3-hexenol. Although well separated from all other components, this spectrum does not agree with data published on the spectrum of this compound (Honkanen *et al.*, 1963). In the spectrum shown, the base peak is at *m/e* 67 and the *m/e* 41 peak has about 80% relative intensity. These ratios

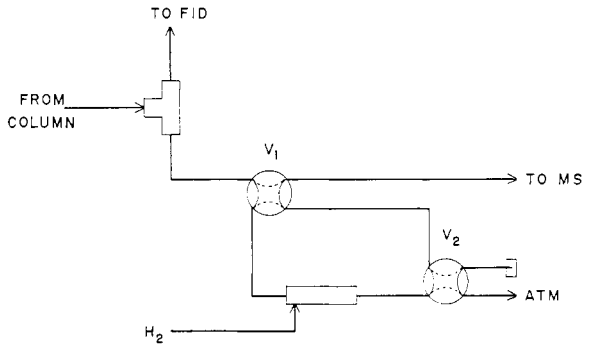

Figure 10. Schematic diagram of on-line microhydrogenator

V_1, and V_2, model 2011 switching valves (Carle Instrument Company, Anaheim, Calif.)

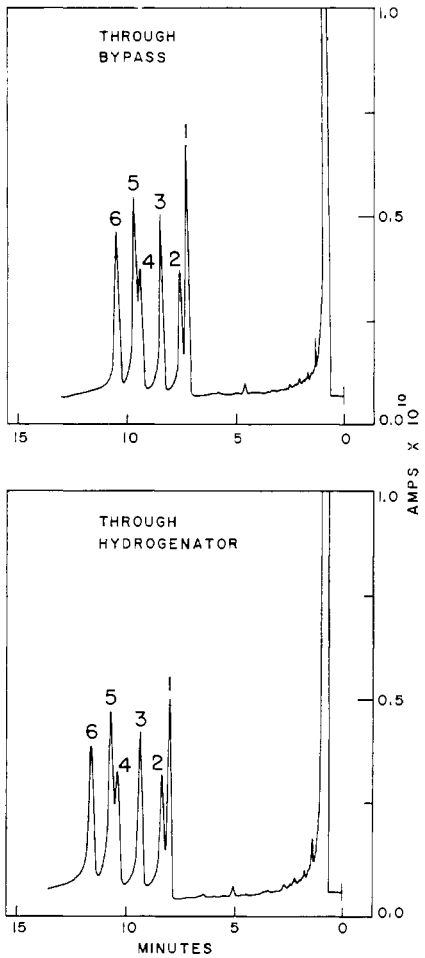

Figure 11. Effect of on-line hydrogenator on separation of C_6-alcohols

0.02-Inch I.D. × 50-foot Carbowax 1540 SCOT column; helium flow rate, 10 ml./min.; hydrogen flow rate, 20 ml./min.; column temperature 100° C., TIC detection. Peak identification: 1. *n*-hexan-1-ol; 2. *trans*-hex-3-en-1-ol; 3. *cis*-hex-3-en-1-ol; 4. *trans*-hex-2-en-1-ol; 5. *trans*-hex-4-en-1-ol; 6. *cis*-hex-4-en-1-ol

are in reverse order of those reported by Honkanen *et al.* (1963). Peak 2 contains *trans*-3-hexenol; this spectrum is more consistent with the published data. Peak 1 in the chromatogram was *n*-hexanol. These data indicate that peaks 2 through 6 contain C_6 alcohols with one double bond. Double bond position in these isomers can be determined from the mass of the most intense peak in spectra scanned at slower

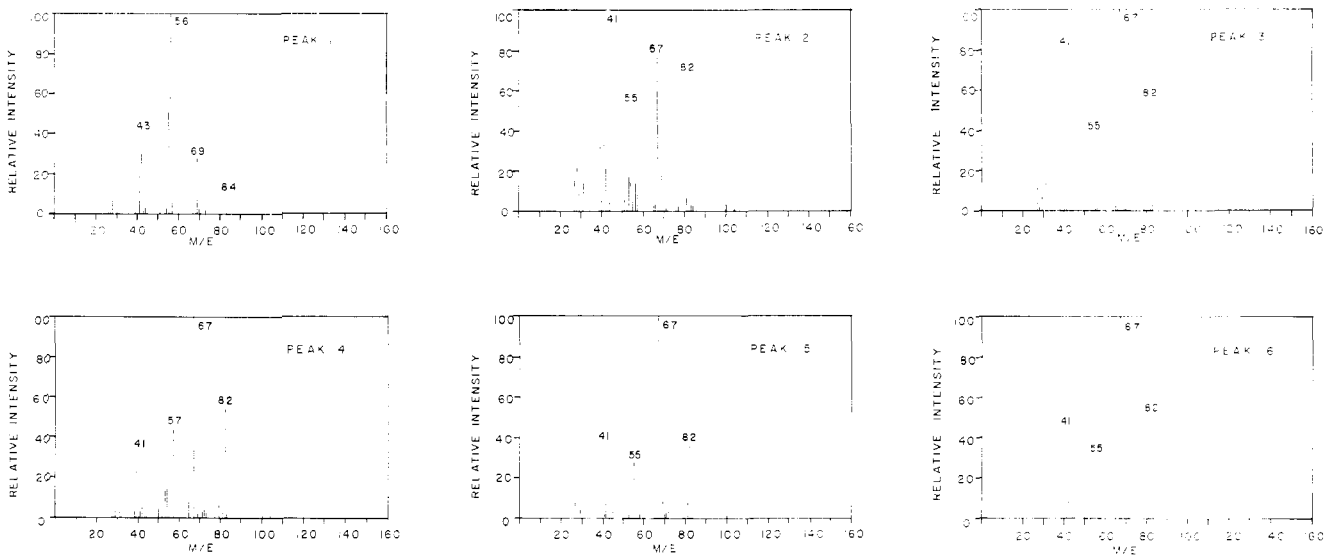

Figure 12. Mass spectra recorded during elution of C_6-alcohols. Hydrogenator by-passed. Peak identification same as Figure 11

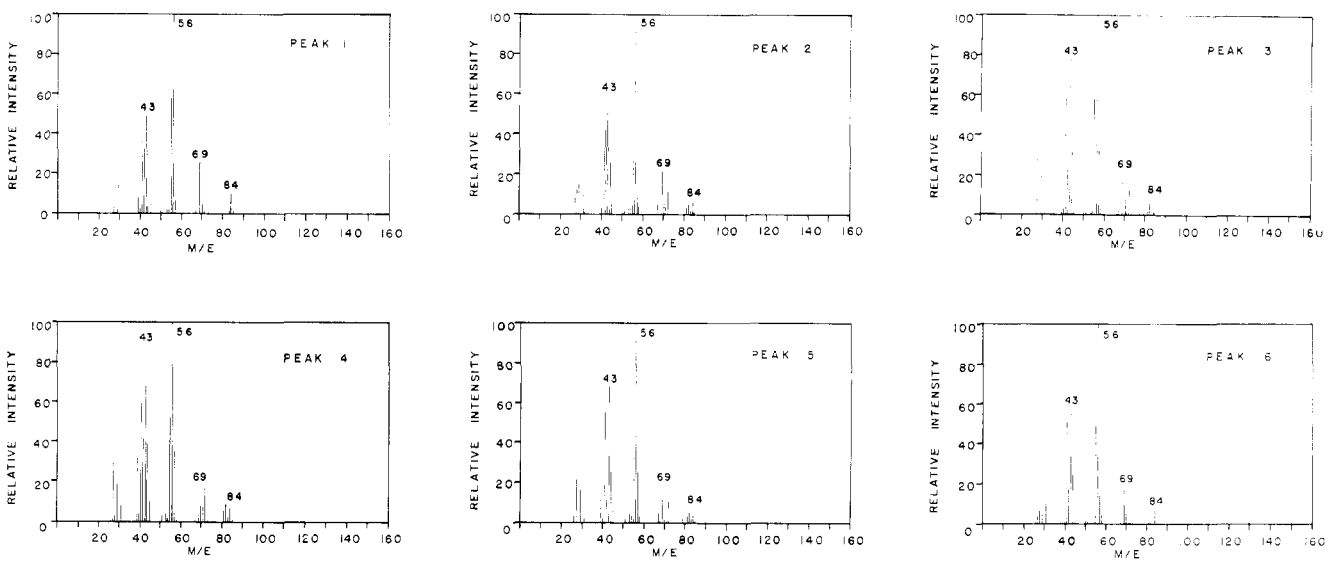

Figure 13. Mass spectra recorded during elution of C_6-alcohols. Column effluent passed through hydrogenator. Peak identification same as Figure 11

speeds, but at the 1.5-second scan rate, statistical variations prevent positive identification of each isomer. The same mixture of hexen-1-ols serves to illustrate results obtained when the micro-reactor was used. Spectra obtained for each peak in the mixture are shown in Figure 13. All can be identified as *n*-hexanol, though some spectra still exhibit small peaks at *m/e* 82, due to the presence of unreacted material. These data show the need to utilize information from all available sources to achieve identification. After determination by mass spectrometry that all of these peaks contain hexenols which are reduced to *n*-hexanol, and that each peak is homogeneous, gas chromatographic retention data may be employed to refine the characterization. This approach was used in the identification of *cis*- and *trans*-4-hexen-1-ol in hydrolyzed banana concentrate (Wick *et al.*, 1969).

The on-line hydrogenator is a generally useful tool in GCMS analysis of flavor components. Its major limitation is that temperatures high enough to prevent sample condensation but below the level at which appreciable hydrogenolysis occurs must be maintained. In studies of phenolic compounds isolated from wood smoke condensates (Lustre and Issenberg, 1969) it was necessary to use batch hydrogenation of gas-chromatographically purified fractions. Three components with molecular weight 194 and similar mass spectra were detected during GCMS analysis of the phenolic fraction. All three were reduced to 2,6-dimethoxy-4-propylphenol by catalytic hydrogenation at room temperature. Hydrogenation of samples consisting of less than 1 mg. of purified materials is feasible, since reactions can be performed in dilute solution with separation of the reaction products during GCMS analysis. The three phenols were identified as 2,6-dimethoxy-4-allylphenol, 2,6-dimethoxy-4-*cis*-propenylphenol, and 2,6-dimethoxy-4-*trans*-propenylphenol on the basis of mass spectra of products produced by hydrogenation and by micro-ozonolysis (Beroza and Bierl, 1967). Ozonolysis is applicable to sample quantities of a few micrograms of unsaturated compounds in dilute solution; the reaction products may be conveniently examined by GCMS. Many synthetic and degradative reactions can be modified for application to sample quantities in the microgram range. With GCMS analysis of reaction products, much of the uncertainty inherent in identification based entirely on interpretation of mass spectra of un-

known compounds can be eliminated. This approach is particularly useful when spectra of reference compounds are not available and synthesis is impractical.

DATA ACQUISITION AND PROCESSING

Continuous scanning of the mass spectrometer during elution of components from a gas chromatographic column yields maximal information regarding the composition of mixtures of volatiles isolated from foods. When chromatographically purified fractions are examined by the GCMS system, continuous scanning will often reveal the presence of unresolved components. The quantities of data generated by this mode of operation usually prohibit its routine use in laboratories not equipped with on-line data acquisition systems. Repetitive scanning at 3-second rate during a 60-minute chromatogram will yield 1200 spectra.

Special purpose data acquisition systems may be employed for digital tape recording of spectra with subsequent computer processing (Hites and Biemann, 1967; Merritt *et al.*, 1968). Abrahamson (1967) and Hites and Biemann (1968a) have reported the use of medium-size computers which can process the spectra as well as perform analog-to-digital conversion. These systems are versatile and effective, but their cost is high (five to ten times the cost of a medium resolution mass spectrometer). Though many analytical instruments may be monitored with such systems, time-sharing is not feasible while a fast-scanning mass spectrometer is operating. The high data rates, 3000 to 10,000 data points per second, usually prohibit on-line operation of other instruments. The computer is totally committed to handling the mass spectrometer output and much of the capacity and flexibility of the computer system is wasted.

The small general-purpose computer is adequate for acquisition and processing of the mass spectral data required by most flavor research laboratories. Such a machine can be used for control of mass spectrometers in which the scan function is determined by a voltage setting, as well as for acquisition and data processing (Reynolds *et al.*, 1968). For magnetically scanned spectrometers, complete closed-loop control is not yet feasible. With adequate auxiliary storage capacity, the small computer can perform most of the processing of mass spectra required in repetitive scanning GCMS applications. The minimum operations which must be performed are: computation of mass, subtraction of background, calculation of relative intensities, and storage in a form suitable for further rapid recovery and processing. At the required digitizing rates, some of these operations can be performed during the 100- to 300-μsec. interval between data points, thus reducing the quantity of data which must be stored. The table of mass and intensity data which characterizes each spectrum may be stored on magnetic tape for future manipulation or plotting. Spectra of components which are not completely resolved by the gas chromatograph may be separated by the computer (Hites and Biemann, 1968a), increasing the effective separating power of the combined system. When spectra recorded from a single chromatographic peak are found to be identical, they can be averaged, and the precision of the mass spectral data increased.

Small computers have not yet been used for comparing spectra of unknown compounds with collections of reference spectra. It is likely that some of the searching systems developed for use with large machines (Abrahamson, *et al.*, 1966; Crawford and Morrison, 1968; Hites and Biemann, 1968b) can be modified to operate with small computers. Searching efficiency and speed might be reduced and some human control might be required, but the small computer can handle the majority of mass spectral data acquisition and processing tasks.

At present, the cost of small computer systems adequate for mass spectral data acquisition and processing appears to be prohibitive for many laboratories using mass spectrometry for identification of flavor components. This situation is similar to that which prevailed just a few years ago with respect to mass spectrometers. It will not be many more years before the computer is considered an indispensable laboratory instrument.

The availability of GCMS systems has made one part of the flavor chemist's job much easier. It is now possible to record spectra of sub-microgram quantities of flavor components with a minimum of prior separation and handling. In most flavor investigations, use of this system does not simplify the fundamental problem of relating chemical composition to flavor, but it provides a more realistic insight into the complexity of the material being studied. Combining a gas chromatograph with a mass spectrometer yields the most powerful single tool available to the flavor chemist. But even when a computer is added to the system, GCMS will produce only vast quantities of uninterpretable data unless all other chemical, instrumental, and sensory methods are considered and applied whenever appropriate to the solution of flavor problems.

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