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132. Support from the National Science Foundation and the Department of Energy and comments from J. Amy, K. Wood, B. Freiser, and N. Delgass are acknowledged.

Hyphenated Techniques for Analysis of Complex Organic Mixtures

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Techniques in which a separation device is combined with a detector, such as gas chromatography–Fourier transform infrared spectroscopy (GC-FTIR) and GC–mass spectrometry (GC-MS), have already become well accepted analytical tools. Accordingly, the present article will be confined to those techniques in which two or more detectors are used in addition to a separation device. The vehicle for this discussion will be the relatively new method of GC-FTIR-MS.

About 15 years ago, in one of the earliest papers on the GC-FTIR technique, Low and Freeman (*1*) made the suggestion that addition of a mass spectrometer might yield an even more valuable tool for the analysis of mixtures. Practical realization of this suggestion was delayed until advances in computer technology permitted it. One reason was the demanding data acquisition requirements of on-line spectrometers (signal digitization rates of 30 to 100 kHz for GC-IR and 100 kHz to 5 MHz for GC-MS) and the equally demanding data reduction needs. Furthermore, significant sample mismatch problems arose because of the substantially different requirements of the gas chromatographic separation method and each of the two spectrometric techniques. Thus, it is not surprising that 8 years passed before an analytical system resembling that suggested by Low and Freeman was demonstrated. Even then, the system described (*2*), which included pyrolysis GC, mass chromatography, elemental analysis, and infrared spectrometry "on-the-fly" (that is, with the sample passing through) was not the general-purpose tool for mixture analysis visualized by the earlier workers. In fact, it was 1980 before there was a successful demonstration of a GC-FTIR-MS linkage providing full mass and infrared spectral information on eluting mixture components (*3*, *4*).

For obvious reasons, methods involving integration of multi-instrument arrays have recently come to be known as hyphenated techniques (*5*, *6*). The GC-MS (*7*) and subsequently the GC-MS-COM, when a laboratory computer was added, probably provide the earliest and best known example. It is interesting that computers, and even multiple computers, have become ubiquitous and that their presence in linked analysis systems is no longer noted in the hyphenated

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designations. In this article I will focus on a number of considerations common to such analytical approaches and on the remarkable advances that have made possible the new multidimensional hyphenated method, GC-FTIR-MS (*8*).

Why Link Instruments?

Considering the possible experimental difficulties, it is reasonable to ask whether the benefits of linked instrument systems outweigh the disadvantages of the more complex procedures required. Certainly, one of the primary motivations for establishing such linkages is the analyst's desire to increase the discriminating power of analytical systems. It is well established that joint use of separation devices (such as gas chromatographs) in conjunction with spectrometers lessens the development effort that would otherwise be needed to overcome inherent chromatographic separation limitations related to component overlap for complex mixtures (*9*). The multidimensional information thus made available can greatly ease the task of differentiating and identifying mixture components that are partly or completely fractionated by the separation. In fact, incomplete purification of individual components need not be a difficulty, as the analyst can compensate by use of complementary selective detection methods or multiple detectors. In principle, these analytical approaches can be used with multiple samples of mixtures of interest, analyzed at different times. In practice, because of data management and experimental constraints, simultaneous separation with serial or parallel analysis is usually the method of choice.

For example, in the relatively common GC-MS and GC-FTIR systems, three-dimensional information (GC retention time, mass, and relative abundances for MS and GC retention time, frequency, and transmittances for FTIR) is routinely obtained on mixture components as they are separated. Although initial work in GC-MS and GC-FTIR was with packed columns, advances in technology permitting faster scan speeds and improved sensitivity have made possible the use of capillary columns for GC-MS with scan rates of 3 to 4 Hz for either magnetic sector instruments (*10*) or Fourier transform mass spectrometers (*11*). Similarly, although the first report of GC-FTIR described the use of a packed column (*12*), advances in detectors, digital electronics, and interferometer designs have now made capillary GC-FTIR with scan rates as high as 20 Hz a reality (*13*). As a result, high-resolution chromatographic separations can be achieved and spectrometric information obtained on quantities of complex mixture components that would be difficult to isolate in sufficient amount for convenient transfer to nonlinked spectrometers.

Summary. Advances in computer technology as well as analytical instrumentation have made practical analytical systems combining various separation methods and multiple spectrometric detectors. Systems consisting of a separation device and a detector, such as gas chromatography–mass spectrometry (GC-MS), are already widely used. Newer methods employing two or more detectors in addition to a separation device are exemplified by gas chromatography–Fourier transform infrared spectroscopy–mass spectrometry (GC-FTIR-MS). A GC-FTIR-MS system has been used in different configurations for the analysis of two relatively complex mixtures of about 30 compounds. Complementary information obtained with such a multi-instrument system shows that it is a potentially powerful tool for complex mixture analysis.

Thus, even for minor components one can eliminate the ambiguities in cross-correlation of spectral information that could result if separate, rather than parallel or serial, measurements were made. It has been amply demonstrated that such multisource information is a powerful and specific tool for identification and structure elucidation (*14–20*).

Sample Requirements for GC-MS and GC-FTIR

Sample requirements for GC and either FTIR or MS are much different. Because of the sensitivity limitations of FTIR, it is desirable to have the greatest possible concentration of samples in the infrared cell to obtain the best spectra; this conflicts with the requirement for capillary GC of small sample size to avoid column overloading. Similarily, in GC the mobile phase gas pressures of 760 torr or more conflict with the pressures of 10^{-3} torr or less required by most mass spectrometers. Both problems have been solved (or, at least, minimized) by using a combination of approaches. Either the separation method can be changed to meet the spectrometer requirements, or the spectrometer can be modified to match the chromatographic conditions. In the first case, the separation method is made less efficient through the use of packed columns, which permits larger analytical samples but degrades the resolution and produces broader eluent peaks than in capillary columns. For MS this requires both use of a separator to remove much of the carrier gas, while enriching the stream in organics, and high-speed pumping of the MS source. For GC-FTIR, packed columns and either flow or stopped-flow methods have been successfully used to permit analysis of samples on a time scale appropriate for the spectrometer (*21, 22*). The second option, which is preferable, is to improve spectrometer speed or sensitivity so that high-resolution capillary GC separations can be performed.

For GC-FTIR, two key advances are the basis for most present-day commercial systems. These are the development by Azarraga (*23*) of high-transmittance gold-plated Pyrex lightpipes (sample cells) and the introduction of high-sensitivity mercury–cadmium telluride (MCT) detectors (*13*). With the fabrication techniques pioneered by Azarraga (*23*) and guidelines for lightpipe volumes developed by Griffiths (*24*) and others (*25*), it is now possible, with modern high-speed GC-FTIR systems and narrow-band MCT detectors (4000 to 750 cm^{-1}), to obtain on-the-fly infrared spectra for tens of nanograms of eluting compounds (*13*). However, optimal quantities are generally in excess of 100 ng.

Modern high-resolution mass spectrometers, using scan speeds of 3 to 4 Hz (*10*) or Fourier transform methods (*11*), now make possible GC-MS analyses with mass spectral resolution much greater than the 500 to 1000 usually possible with quadrupole mass spectrometers, which operate at the same or slightly higher scan rates. With the lower carrier gas flows required by capillary GC, separators are not commonly required and effluent can be introduced directly into the ion source.

Thus, GC-FTIR measurements at the 10-ng level (spectral resolution, 4 to 8 cm^{-1}) and GC-MS measurements at the subnanogram level (spectral resolution, $> 10^3$; valley definition, 10 percent) are now nearly routine. Furthermore, although FTIR is still less sensitive, its sensitivity has been improved by at least

two to three orders of magnitude in the past 10 years and now approaches that of the least sensitive mass spectrometers. This is desirable if direct-linked GC-FTIR-MS is to be practical for general-purpose mixture analysis.

As a result of the technological advances described above, it is now possible to successfully link a gas chromatograph either serially or in parallel with an FTIR and an MS (using an effluent splitter in the latter configuration). In this way complete infrared and mass spectra of GC effluents are obtained on-the-fly and nearly simultaneously. Figure 1 shows the two possible arrangements in block diagram form. Although a single computer could be used for data acquisition, instrument control, and data analysis, all GC-FTIR-MS systems thus far demonstrated have had separate computers for the FTIR and MS subsystems.

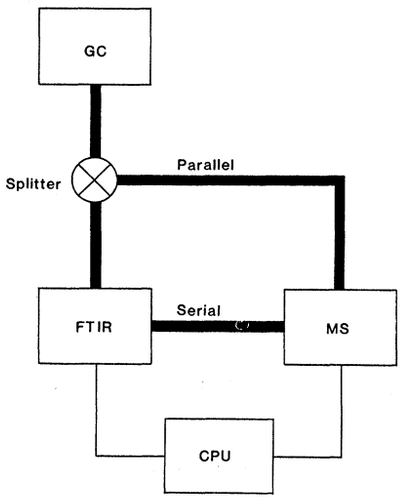

Fig. 1. Block diagram of alternative approaches to direct linkage of GC-FTIR-MS systems. In practice, two separate computers have been used, rather than a single one as depicted here.

Direct-Linked GC-FTIR-MS

In early papers describing the operation of GC-FTIR-MS systems, the utility of the complementary information thus available was demonstrated by using simple test mixtures, first with packed GC columns and a high-resolution double-focusing mass spectrometer (*4*) and later with a support-coated open tubular (SCOT) GC column and a low-resolution quadrupole mass spectrometer (*26*). Test mixtures difficult to analyze by either FTIR or MS alone were chosen to demonstrate the potential power of the combined approach for compound identification. For example, in the first study (*4*), a mixture of *o*-, *m*-, and *p*-xylene was separated and infrared and mass spectra were obtained for each of the eluting components. Computer-readable library searches were performed to retrieve the five nearest matches from files of 2300 vapor-phase infrared spectra and about 32,000 mass spectra. Because the mass spectra of the xylenes were indistinguishable, all three isomers appeared among the best five matches for each component. However, only a single isomer appeared among the best five infrared spectra found in the library searches in each case. When the unknown's identity was assigned by manual comparison of search lists, using coincidence of infrared and MS list entries as the requirement for assignment, 12 of 16 test compounds in this study were correctly identified. For the unidentified compounds, either search list coincidence was absent or spectral information was not obtained because of sensitivity limitations. In no case did a coincidence yield an incorrect identification (*4*).

By following precisely the same manual cross-correlation procedure, using the same two data bases, a similar six-component test-mixture, containing the xylene isomers as well as other compounds, was analyzed by other workers (*26*). In that study, in which a SCOT column was used for the separation, two of the xylenes were incompletely separated. When cross-correlation of the top four matches from the library search was carried out on the five resulting GC peaks, use of the coincidence criterion resulted in correct identification of the four pure components. Furthermore, the unresolved *m,p*-xylene peak yielded both constituents in both the infrared and MS searches. Subsequently, the first serial linkage of a GC-FTIR with a quadrupole MS was demonstrated for analysis of an almost identical seven-component test mixture, with equal success (*27*). These results clearly supported the expectation that complementary spectral information, when available, would markedly strengthen the analyst's ability to identify unkown compounds. Nevertheless, an experienced spectroscopist could object that these simple model studies are unremarkable and, in any event, fail to yield results superior to those that could be obtained by visual inspection of the mass and infrared spectra. Thus, application to more complex mixtures containing many components and more representative of actual mixture analysis problems was required to provide convincing evidence of the value of the new analytical system.

Analysis of "Real" Complex Mixtures

It is in the analysis of complex mixtures that the use of complementary techniques in hyphenated systems is expected to be most powerful. For such mixtures, manual examination, verification, and comparison of five or more library spectra of each type for each component is the most time-consuming aspect of the analysis. For example, even a moderately complex mixture of 25 components could require examination of as many as 300 spectra if only the five closest library spectra from the MS and infrared libraries were considered. It might be argued that a spectroscopist could easily resolve any single specific ambiguity by a careful examination of individual spectra. In the simpler cases (such as the first-eluting component of the lacquer thinner sample discussed below) this could well be true. However, one goal of linking separation and detection systems is to significantly enhance both speed and reliability of component identification. By joining the instruments and introducing computer interpretation, it is ensured that infrared and mass spectra corresponding to the same sample component are being analyzed. From the results with simple test mixtures such as those discussed above, it appears that automated tests for search list congruence could greatly decrease the data analysis time without compromising the quality of the results.

The utility of complementary GC-MS and GC-FTIR information obtained separately on industrial wastewater (*28*) and a 21-component priority pollutant sample (*29*) has been noted by Shafer and coworkers. In the latter case, 19 of the 21 components were correctly identified by manually combining the results of infrared and MS library searches. Identity assignments were made on the basis of appearance in both lists. It is especially important to recognize that, regardless of the search algorithm or spectral method used, the closest library match cannot be assumed to yield the correct identity of the unknown. The first published report of use of linked GC-FTIR-MS systems for samples of similar complexity appeared in 1982 and presented results of studies of both known (peppermint oil) and unknown (a commercial lacquer thinner) samples containing at least 28 and 30 components, respectively (*30*). For the peppermint oil analysis a splitter was interposed between the GC, a Nicolet 7199 FTIR instrument, and a Kratos MS-80 double-focusing mass spectrometer, permitting parallel FTIR and MS

Table 1. GC-FTIR-MS analysis of lacquer thinner. Compound type is the structural feature most common in the two search output lists. The specific compounds listed in the FTMS and FTIR columns are not those with the highest similarity index on their respective searches. Instead, they are the compounds considered to be most similar to one another when the search lists were compared. [Reprinted from (*30*) with permission of the copyright holder]

Peak	Compound type	FTMS	FTIR	CI molecular weight	Identity
1	CH_3CO-R	1-Dimethylaminoacetone	Acetone	58	Acetone
2			Methanol	32	Methanol
3	*sec*-Alcohol	Isopropanol	Isopropanol	60	Isopropanol
4	CH_3CO-R	3,4-Dimethyl-2-hexanone	2-Butanone	72	2-Butanone
5	*tert*-Alcohol	3,7-Dimethyl-3-octanol	3,7-Dimethyl-3-octanol		3,7-Dimethyl-3-octanol
6	Substituted alkane	3-Methylhexane	3,3-Dimethylpentane		
7	Substituted cyclopentane	1,1-Dimethylcyclopentane	Methylcyclopentane	98	1,1-Dimethylcyclopentane
8	Alkane	Heptane	Hexane		Heptane
9	Substituted cyclohexane	Methylcyclohexane	Methylcyclohexane	98	Methylcyclohexane
10		*sec*-Butyl acetate	Isobutanol	74	Isobutanol
11	Alcohol	3-Methyl-1-butanol	3-Methyl-1-pentanol	102	3-Methyl-1-pentanol
12	Substituted cyclopentane	1,1,2-Trimethylcyclopentane	Butylcyclopentane	112	1,1,2-Trimethylcyclopentane
13	Substituted benzene	Toluene	Toluene	92	Toluene
14		2,2-Dimethyl-1,3-propanediol	Butyl acetate	116	Butyl acetate
15	Substituted alkanes	2,3,3,4-Tetramethylpentane	2,4-Dimethylhexane		
16		6-Decen-5-one	2,5-Dimethyl-2-hexene	112	2,5-Dimethyl-2-hexene
17	Large alkane	2-(5-Cyclohexyl) undecane	2,6,10,14-Tetramethylpentadecane		
18	Substituted cycloalkane	1,1,3,4-Tetramethylcyclopentane	*cis*-1,3-Dimethylcyclohexane	126	1,1,3,4-Tetramethylcyclopentane
19	Alkene	2,3,6-Trimethyl-4-octene	2,5-Dimethyl-2-hexene		2,5-Dimethyl-2-hexane
20	Substituted alkane	3-Methylheptane	3,3-Dimethylheptane	114	3-Methylheptane
21	Substituted alkane	3,4-Dimethylheptane	3,3-Dimethylheptane		Dimethylheptane
22	Substituted alkane	3-Ethylheptane	3-Ethylpentane		
23			2,4-Dimethylhexane		
24	Substituted alkane	2,2,4-Trimethylheptane	2,2,4-Trimethylhexane		
25		Xylene	Butylcyclopentane	106	Xylene
26	Alkane	3,3-Dimethylhexane	Octane		
27		Xylene	2-Butoxyethyl acetate	106	Xylene
28	Bicyclic alkene	Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)	Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl	136	
29	2-Ethoxy ester	2-Ethoxyethyl acetate	2-Ethoxyethyl acetate	132	2-Ethoxyethyl acetate
30		Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)			

analysis. Lacquer thinner was analyzed with a system linking the GC, FTIR, and a Nicolet FTMS/1000 Fourier transform mass spectrometer in a serial linkage (*31*).

Results of Complex Mixture Analysis

In preliminary studies of complex mixture analysis with directly linked GC-FTIR-MS systems, the key question we wished to answer was whether a high level of reliability in identification of mixture components could be obtained with this analytic approach. The results obtained with the two mixtures chosen for the first studies were encouraging. As expected from the results of the earlier model studies, highly reliable identifications were achieved for compounds that were present in the spectral data bases searched. Fortuitously, the complex mixture we chose to study initially consisted of relatively common and well-known materials. As a result, most of the compounds (known and unknown) in these mixtures were represented in the data bases we used. This permitted evaluation of the library search procedure without the complications that would arise if compounds to be identified were not represented.

Peppermint oil analysis (30). As shown in Fig. 2, peppermint oil is a moderately complex mixture of about 30 compounds that are present in a wide range of relative concentrations (judging from the uncorrected flame ionization detector trace). In our study, a 0.1-μl splitless injection onto a 35 m by 0.44 mm Carbowax 20M SCOT column was used for the separation, followed by on-the-fly infrared analysis with a Nicolet 7199 FTIR spectrometer and parallel analysis of approximately 1 percent of the effluent split at the GC outlet and routed to a Kratos MS-80 mass spectrometer, operated at an MS resolution of about 1000. As is often the case in naturally occurring mixtures, peppermint oil is a mixture of closely related compounds, including stereoisomers, which are difficult or impossible to identify by exclusive use of either infrared or mass spectral information. Thus, it was expected to be a challenging test of the combined spectral approach. By paying careful attention to minimizing dead volumes in the transfer lines (64 μl for the FTIR line and 80 μl for the MS-80 transfer line), it was possible to eliminate virtually any loss in GC resolution. The lightpipe used was a commercial gold-plated Pyrex tube 42 cm long with an

inner diameter of 1.2 mm and a total volume of 0.5 cm^3.

In the peppermint oil analysis, seven components with relative concentrations of 3 percent or more were correctly identified by use of the library search coincidence criterion. Seven components present as less than 0.5 percent each of the total mixture did not yield infrared spectra with high enough signal-to-noise ratios to permit library searches. For the remaining dozen compounds, searches did not include the correct identity in either the infrared or mass spectral search lists but did include similar compounds. This kind of result does give useful clues to functional groups and structures, even though it is inadequate for positive identification. Our conclusion from this study was that the analysis worked well for major components for which adequate FTIR sensitivity was available, but that improved sensitivity would be required if significantly better performance were to be achieved. Accordingly, an improved lightpipe (12.6 cm long with an inner diameter of 2.25 mm) was fabricated according to guidelines of Griffiths (*24*), and FTIR sensitivity improved by about a factor of 4. The improved lightpipe was used for the lacquer thinner analysis.

Lacquer thinner analysis (30). This mixture was analyzed after making three significant changes in the instrument configuration. Although the GC and FTIR were the same as in the previous study, a Nicolet FTMS/1000 Fourier transform mass spectrometer was linked serially with the FTIR rather than in parallel, as had been the case when the MS-80 mass spectrometer was used. As a consequence, a 0.5-cm^3 dead volume (the lightpipe) was introduced before the MS. This was compensated by using a 30-m, 0.323-mm inner diameter, 1-μm film DB-1 fused silica column coupled with the 35-m SCOT column from the peppermint oil analysis. Although the lightpipe was of different dimensions than that used previously, the nominal volume was the same and afforded a fourfold improvement in FTIR sensitivity. Substitution of the FTMS instrument, which operates on different principles (*11, 31, 32*) than the double-focusing mass spectrometer used earlier, did not affect the data analysis procedure, as comparable mass spectra are produced.

Even though GC resolution obtained at the FTMS instrument was degraded from that at the FTIR, it was still better than the resolution required for successful analysis of this 30-component mixture. Table 1 summarizes the results of this analysis. Because there was no difficulty in collecting both electron impact (EI) and methane chemical ionization (CI) mass spectra with the FTMS instrument (*11, 33*) all three measurements, EI and CI mass spectra and FTIR spectra, were used in a complementary fashion to make compound identifications. Therefore, in cases where there was no match between the infrared and mass spectral search lists and where closely related compounds appeared in each, the molecular weight obtained by CI was used to resolve ambiguities. One such case was the eleventh-eluting GC peak, for which 3-methyl-1-butanol (by FTMS) and 3-methyl-1-pentanol (by FTIR) were the most similar search list entries. A CI measurement yielded a molecular weight of 102, identifying the compound as the latter. In another case, a search of the library spectra for a spectrum corresponding to that of peak four had one of the dimethylhexanones among the top five results, while the FTIR search gave 2-butanone as the most similar result. Methane CI showed that the molecular weight was 72 and allowed identification of the unknown as 2-butanone. As a final example, consider the minor and incompletely resolved components corresponding to peaks 25 and 27. Here, the infrared spectra were so poor that the two mass spectral measurements (EI and CI) were needed to identify the substances as xylene isomers. Thus, the chief difficulties have arisen from the need for greater sensitivity to identify minor components (*30*). In all, 18 of the components were unambiguously identified by use of the infrared and mass spectral information and ten of the remainder were classified with respect to major structural features. In the remaining two cases only one type of spectral information (MS or infrared) was obtained for each, so although an identification was possible for one of these, it was not done by use of complementary information. Because of the improved infrared sensitivity, it was possible to obtain vapor-phase FTIR spectra on all but one of the compounds separated, using a 0.2-μl splitless GC injection. Accordingly, 29 of the 30 components of this unknown lacquer were identified with a high degree of certainty.

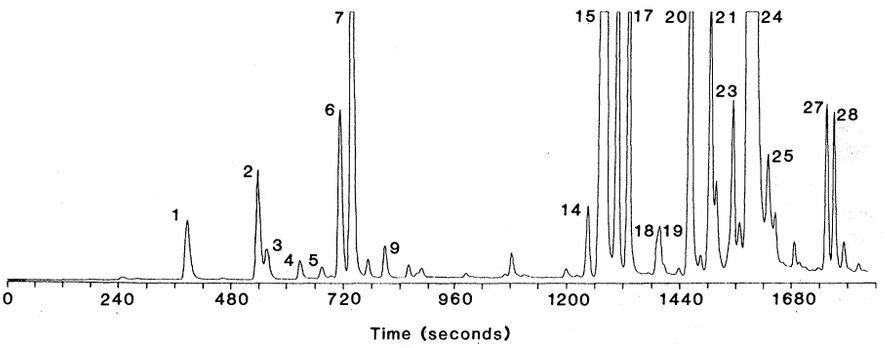

Fig. 2. Flame ionization detector trace for peppermint oil separation. [From (*30*), with permission of the copyright holder]

Data Analysis for GC-FTIR-MS

Several conclusions have emerged from the studies described thus far. First, it is clear that hyphenated systems such as those described have enormous potential for adaptation to fully automated computer-controlled analysis systems. Second, computer-readable library searches are extremely effective in exact as well as qualitative identification of unknowns, when used in a complementary fashion. However, present data bases are limited in size and in the types of compounds represented. Further, many of the spectra included are of dubious quality. Substantially larger and more reliable data bases will be required to realize the promise demonstrated by these first studies. Third, unique computer representations of all data base members will be needed to allow computer comparison of search results. Use of the unique *Chemical Abstracts* registry numbers of data base concordances would allow automatic determination of coincidence of MS and infrared search outputs. However, more general qualitative comparisons may require use of connection tables or similarity indices derived therefrom (*34, 35*). Finally, concatenation of data bases to allow combined searches (analogous to linkage of instrumentation to obtained combined spectral information), as recently demonstrated by Isenhour and co-workers (*20, 36*) or application of qualitative pattern recognition procedures, as suggested years ago (*15*), may be a worthwhile

alternative to the methods described here. Certainly, use of additional complementary information, including other physical and spectral properties, would be expected to strengthen the analytical power of hyphenated systems.

Other Hyphenated Methods for Organic Analysis

Not all the hyphenated methods presently (or soon to be) in existence can be reviewed here. An excellent survey of several of the possibilities was published a few years ago, prior to the realization of several of them (*5*). More recent promising developments include the demonstration by Shafer and Griffiths (*37*) of supercritical fluid chromatography-FTIR-UV-flame ionization detection, using series coupling of the detectors with a wide-bore fused silica capillary column and supercritical CO_2 as the mobile phase. Developments in hyphenated mass spectrometry methods continue to be rapid, and tandem quadrupole and double-focusing mass spectrometers have already made possible MS-MS, MS-MS-MS, and GC-MS-MS (*38*). Using a Fourier transform mass spectrometer, workers in Nibbering's laboratory recently demonstrated MS-MS-MS-MS (*39*). In our laboratory, liquid chromatography-FTMS-MS is under investigation, as is supercritical fluid chromatography-FTIR-FTMS (in collaboration with Shafer and Griffiths).

Concluding Remarks

The analytical possibilities of hyphenated instrumentation have only begun to be explored, and analytical chemists are on the threshold of impressive new developments brought about by advances in separation science, computer science, and spectrometry. I believe that within the next 10 years directly linked FTIR-FTNMR-MS systems will be developed and operated under computer control to analyze organic mixtures. Furthermore, the analyst may have a choice of GC, supercritical fluid chromatography, or LC as the separation device preceding the IR-NMR-MS system. Single or multiple computers with memory sizes well in excess of a megabyte will be standard. These systems will incorporate both color video and multicolor plotters for data display. Computational speeds will be at least 20 to 30 times those of present-day VAX 11/780 computers and will permit Fourier transform and other data analysis in real time, so that completion of the analytical report will coincide with completion of data acquisition. Such developments may well make possible achievement of our long-term goal of a fully automatic analysis system for organic mixtures.

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40. I thank the National Science Foundation for support under grants CHE-79-10263, CHE-80-18245, and CHE-81-13612. I would also like to acknowledge the contributions of my co-workers, especially G. Giss, G. Brissey, R. White, S. Steiner, and E. Onyiriuka.