Pure & Appl. Chem., Vol. 50, pp. 113-127. Pergamon Press, 1978. Printed in Great Britain.

DEVELOPMENT AND USE OF BIOANALYTICAL SYSTEMS BASED ON MASS SPECTROMETRY WITH IONIZATION AT ATMOSPHERIC PRESSURE

E. C. Horning, D. I. Carroll, I. Dzidic and R. N. Stillwell

Institute for Lipid Research, Texas Medical Centre, Houston, Texas, USA

Abstract - Mass spectrometry with ionization at atmospheric pressure - MS(API) - is a novel form of mass spectrometry in which the ionization process occurs in a reaction chamber external to a quadrupole mass analyser. The reaction chamber is at atmospheric pressure, and ions (and neutral molecules) enter the low pressure region through a small aperture in a metal disc which forms one wall of the reaction chamber. The primary source of electrons is a ^{63}Ni foil or a corona discharge. Positive ions are formed from samples by a series of ion-molecule reactions which usually involve the sequence electrons→carrier-gas ions→reagent ions→sample ions. Negative ions are formed either by direct electron attachment (electron capture) or by ion-molecule reactions. The source will tolerate a variety of gases, organic solvents and reagents. Samples may be introduced in a carrier-gas stream from a gas chromatograph or by evaporation from a platinum-wire probe, or in solution in organic solvents by direct injection or in the stream from a liquid chromatograph. Subpicogram sensitivity of detection has been achieved for both positive and negative ions. The objective of this work is to develop bioanalytical systems which are useful for single-component or multicomponent analysis of biological samples of complex composition. The principles of operation of systems coupling gas or liquid chromatography with MS(API) and a computer have been established.

During the past two decades the changes that have occurred in analytical chemistry are as great as the changes that have occurred in other areas of human endeavour. This is largely due to the development of modern instruments, or more precisely, to the development of analytical systems which are now computer-based and which involve electronic circuitry and electronic sensing devices rather than direct human observation. In the field of organic chemical analysis, as distinct from inorganic and elemental analytical chemistry, there is another significant circumstance which has made possible the development of modern bioanalytical systems. This is the fact that gas-phase analytical methods can be used for very nearly all organic compounds except macromolecules. This combination of circumstances has made it possible to design and use systems that can carry out multicomponent as well as single-component analyses of highly complex samples of biological origin, with unprecedented sensitivity of detection. When applied to environmental problems, the methods are usually referred to as trace organic analysis; in other applications where there is less emphasis on trace analysis, the methods are usually referred to in terms of types of instruments. Systems which are now available are described as GC - MS - COM (a gas chromatograph - mass spectrometer - computer combination) instruments, and some LC - MS - COM (a liquid chromatograph - mass spectrometer - computer) systems are now in use. An LC - GC - MS - COM combination is possible. Since this field of analysis is still undergoing development, this summary should be regarded more as a progress report of recent investigations than as a historical survey. It is helpful, however, to recognize both the nature of the problems under study and the principal changes that occurred as the analytical methodology was developed.

Analytical methods are often characterized in terms of concentration of sample components. For biological systems such as body fluids, many compounds of interest, including many commonly used drugs, are present in concentrations of 1 - 10 μg/ml or higher. The range of 1 - 10 ng/ml or slightly higher is also an important range; many hormones and hormonal drugs are effective at this concentration in plasma. Analyses for organic compounds at concentrations of 1 - 10 pg/ml were until recently not

considered to be important. Femtogram analyses, involving concentrations of 1 - 10 fg/ml, are not yet possible as far as applications are concerned, although an instrumental sensitivity of detection of 30 - 50 fg has been demonstrated. The reason for the current interest in trace analysis for organic compounds lies in the biological phenomenon of concentration build-up in cells or organisms, and in chronic as well as acute toxicity effects. The increase in recognition of the serious nature of environmental biohazards is due to increasing understanding of biological events and to the development of analytical capabilities suitable for the study of these problems.

The most significant step in the early development of bioanalytical systems was the design of a jet-orifice separator which provided a practical solution to the problem of connecting a gas chromatograph to a mass spectrometer. The basic concept of a new type of instrument which could separate and identify organic compounds occurred before 1960 to a number of scientists, notably Bergstrom, Stenhagen and others in Sweden. The design studies by Ryhage in 1958 - 1960 were successful, and the Ryhage (1) "molecule separator" made possible the production of the first commercial combined gas chromatograph - mass spectrometer (LKB 9000). Figure 1 is a schematic diagram of a magnetic-field mass spectrometer with a separator. Since the initial work in this field, quadrupole (electric field) mass analysers have come into general use, and chemical ionization techniques which do not require a separator are also widely used. The initial purpose of combined instruments of this type was to identify organic compounds present in complex mixtures of biological origin, and this is still a major function of current bioanalytical systems. Quantitative techniques were introduced when it was realised that a mass spectrometer could also be used as a highly specific detection and measuring device. Mass spectrometers were used for quantitative purposes in many

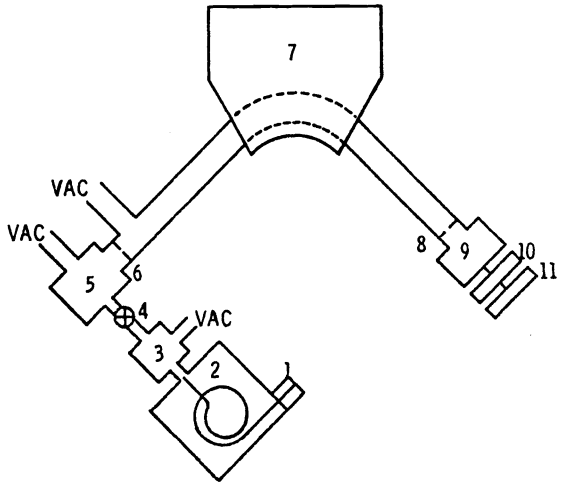


Fig. 1. Schematic diagram of a magnetic-deflection low-resolution GC - MS instrument. The deflection is usually 60° or 90°. The parts or functions indicated are: (1) GC injection, (2) GC column and oven, (3) separator, (4) cut-off valve, (5) ion source, (6) entrance slit, (7) magnet, (8) exit slit, (9) multiplier, (10) preamplifier, (11) amplifier. All earlier designs were based on electron-impact ionization and employed packed GC columns. Current designs usually provide for optional use of electron-impact or chemical-ionization conditions. A separator is not required with a chemical-ionization source. Some current designs permit the use of glass open tubular capillary GC columns. Ion-counting techniques are used in some instruments in place of analogue circuitry.

applications before the development of modern systems, but the critical event for bioanalytical studies was the 1967 - 1968 work of Holmstedt (2) and his colleagues on specific ion detection (now generally called selected ion detection). This technique is now universally used in quantitative work. Other significant changes occurred more gradually. Chromatographic techniques which were developed between 1960 and 1970 are now used everywhere, and it was gradually realized that the analytical scope of these combined instruments included almost all of organic chemistry except for macromolecules. The introduction of laboratory computers led to further changes; most systems are now computer-based or microprocessor/computer-based. This provided new kinds of analytical procedures. The repetitive-scan analytical methods pioneered by Biemann (3), for example, are computer-based analytical methods.

The first significant experiments in mass spectrometry were carried out around 1900, and mass spectrometers have been in use for a long time for analytical applications ranging from research in physics to quality control of industrial products. Bioanalysis, however, is the most recent and most difficult field of analysis in which mass spectrometry plays a major part. The requirements of this field were not met by early designs of mass spectrometers, and consequently it was necessary to carry out additional studies of the theory and practice of mass spectrometry. Until recently, most work with organic compounds was based upon electron-impact ionization, largely because the usual objective was to seek structural information or to confirm identity, and instruments were designed only for positive-ion detection. The current approach is to emphasise quantitative work, particularly under conditions of high sensitivity of detection, and this has led to increased use of chemical-ionization techniques and to the development of negative-ion mass spectrometry. One of these areas of work is atmospheric-pressure ionization (API) mass spectrometry. When this work was started, the accepted view was that the ionization of organic compounds in the gas phase at atmospheric pressure was possible, but that it was unlikely that the process could be controlled or used effectively, that negative ions were not of interest from any point of view, and that high sensitivity of detection could not be achieved in this way. At present, although only a few API instruments are in operation in the world, many of these issues have been resolved.

When chemical-ionization (CI) techniques are discussed, it is usually emphasised that relatively few ions are formed from organic compounds when ionization occurs by a chemical reaction (an ion-molecule reaction) rather than by electron bombardment. While this is an over-simplification of the situation, it is true that most CI conditions are designed to lead, with as few additional ions as possible, to ions such as M^+, MH^+, MR^+, $(MH - N)^+$ or $(MR - N)^+$, where R^+ and H^+ are reagent ions, N is a neutral molecule (water, methanol, trimethylsilanol are frequently eliminated after protonation of hydroxy, methoxy or trimethylsilyloxy groups), and M is the original compound. Electron-impact techniques are also capable of providing simplified mass spectra with only a few ion products, but usually these are not the same ions that are formed under CI conditions. This is illustrated in Figs. 2, 3 and 4.

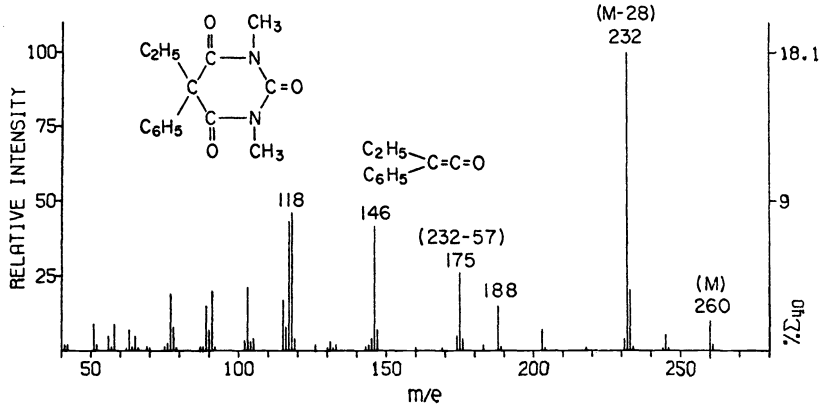


Fig. 2. Electron-impact (70 eV) mass spectrum of the N,N'-dimethyl derivative of phenobarbital. The initial reaction is formation of M^+ radical ions, followed by fragmentation to yield a series of ions. The locations of bond breaking can usually be determined, and the information contained in a mass spectrum of this kind is useful in structural studies. The major ion product from this compound is $(M - 28)^+$, resulting from loss of the ethyl group as C_2H_4.

Figure 2 shows the electron-impact (70 eV) mass spectrum of the N,N'-dimethyl derivative of phenobarbital. This is a typical spectrum containing a number of ions formed, by bond breaking, from M^+. The major product is $(M - 28)^+$ resulting from elimination of C_2H_4. When the electron energy is reduced, the mass spectrum shows fewer ions; Fig. 3, for example, shows a 12-eV mass spectrum for this compound. The cleavage reaction still predominates, and $(M - 28)^+$ is still the major ion, but most ion products are no longer present. Figure 4 shows a CI mass spectrum obtained with methane as both carrier and reagent gas. The major ion product is MH^+. The ions at $(M + 29)^+$ and $(M + 41)^+$ are ions of the MR^+ type formed by addition of R^+ to M. Cleavage reactions observed under CI conditions usually involve the elimination of a neutral molecule from MH^+, and this is most likely due to protonation of the leaving group as the initial step in the ionization process. In effect, both low-energy electron-impact conditions and the usual CI conditions give relatively few ion products, but CI conditions are in general

far more valuable for quantitative analytical purposes than EI conditions. One of the most effective ways to carry out quantitative analyses in biological applications is to use internal reference compounds labelled with a stable isotope (preferably ^{13}C), and these labels must be retained during the analytical process. This will be the case if M^+, MH^+ or MR^+ are the ions involved in the analysis, and this is usually true for $(MH - N)^+$ and $(MR - N)^+$ ions as well. It is often not the case for ions derived by cleavage reactions from M^+ formed under electron-impact conditions.

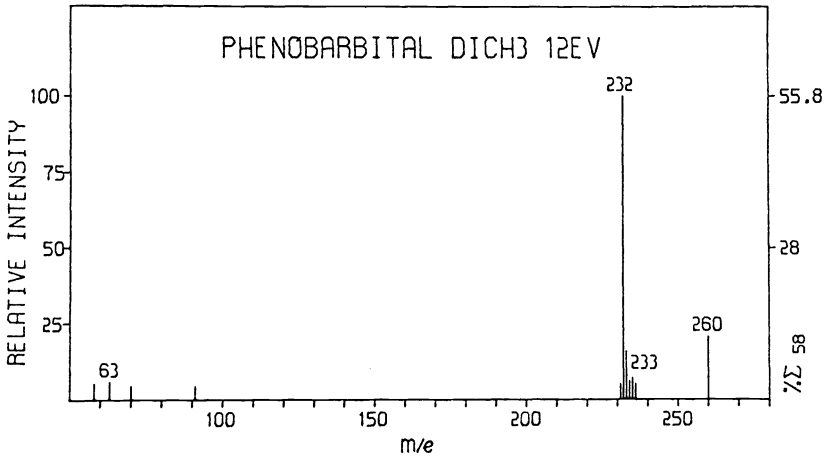


Fig. 3. Electron-impact (12 eV) mass spectrum of the N,N'-dimethyl derivative of phenobarbital. When the energy involved in the initial ionization is relatively low, most fragmentation reactions do not occur, and a simplified mass spectrum is obtained. The major ion product, however, is usually the same as the major product under higher energy conditions. In this instance, the fragment ion $(M - 28)^+$ is the major ion at both 12 eV and 70 eV (Fig. 2). Low-energy electron-impact ionization conditions (10 - 12 eV) are rarely used in practice because the efficiency of ionization is reduced and structural information is lost.

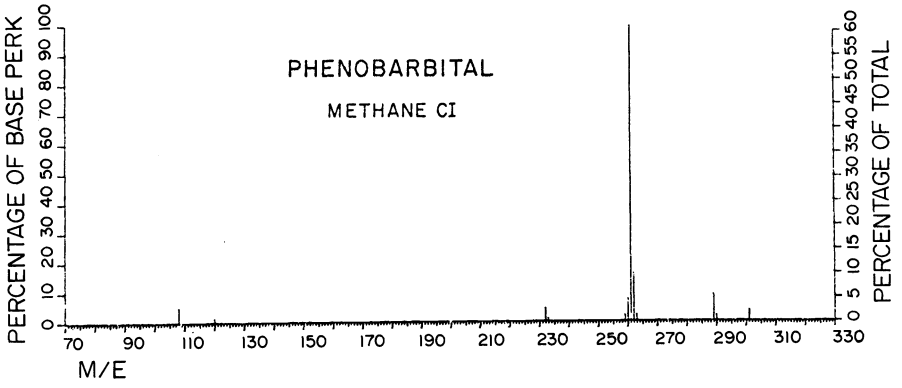


Fig. 4. Chemical-ionization mass spectrum of the N,N'-dimethyl derivative of phenobarbital, with methane as the carrier and reagent gas. The principal reaction is protonation to form MH^+. Ions at $(M + 29)^+$ and $(M + 41)^+$ are characteristic of methane CI spectra, and are due to the addition of $C_2H_5^+$ and $C_3H_5^+$ to form ions of the MR^+ type. When ammonia is the reagent, the principal ion is $(M + 18)^+$ from addition of the reagent ion NH_4^+. The MH^+ ion is also formed with ammonia.

Atmospheric-pressure ionization mass spectrometry (4,5) differs from convential CI mass spectrometry (6) in several ways. The reaction chamber (ion source) is at atmospheric pressure and is external to the high-vacuum region of a quadrupole mass analyser. The ions (reagent- and sample-derived ions) and molecules [carrier gas, reagent(s), sample and solvents if present] enter the mass analyser region through a small aperture. The ions are retained by ion lenses, while the neutral molecules are removed by pumping. Reagent ions are generated with a ^{63}Ni foil or a corona discharge, rather than by use of a heated filament. Both positive and negative ions are detected.

Figures 5 and 6 show two types of ion source used in API studies. The primary ion source is a ^{63}Ni foil (Fig. 5) or a corona discharge (Fig. 6) arranged so that the point of the corona electrode can be placed near the aperture (0.5 mm) or at a distance (4 mm) from it. Samples may be introduced without solvents by means of a platinum wire probe or in the effluent stream from a gas chromatograph. Samples in solution may be injected as in gas chromatography (see Fig. 5) or in the effluent stream from a liquid chromatograph (7 - 9). Virtually all gases, solvents and reagents are tolerated by these sources. The reaction-chamber temperature is usually 100 - 250°, depending upon the compounds under study. Positive ions are formed by a sequence of reactions which are initiated by ionization of the carrier gas: carrier-gas ions → reagent-gas ions → sample ions. The chief ion which would be expected when nitrogen is the carrier gas is N_4^+. This is shown in Fig. 7; the results were obtained with a corona source 0.5 mm from the aperture. Other carrier gases that may be used include helium and argon. When a reagent is present in the carrier gas, or introduced at the source, the usual result is the formation of reagent ions which are both relatively long-lived and relatively reactive (4, 5, 7 - 10).

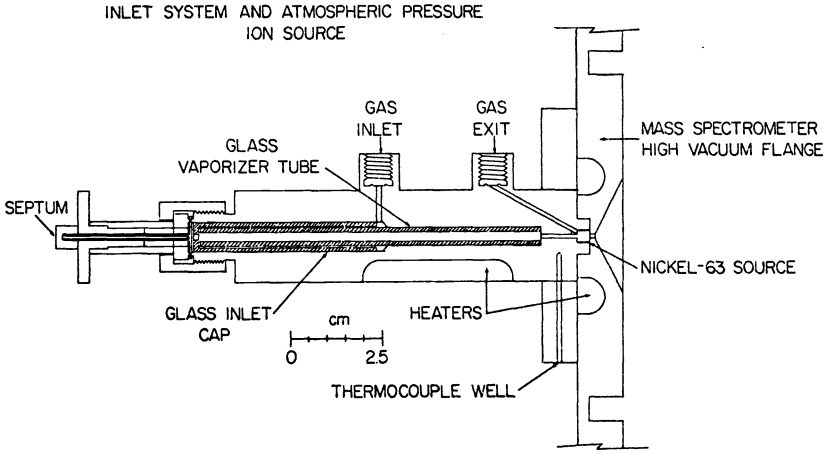


Fig. 5. Diagram of an API vaporizer - reaction-chamber assembly with a ^{63}Ni foil as the primary source of electrons. Samples may be injected in solution as in gas chromatography, or without solvent on a platinum wire as in probe introduction in mass spectrometry.

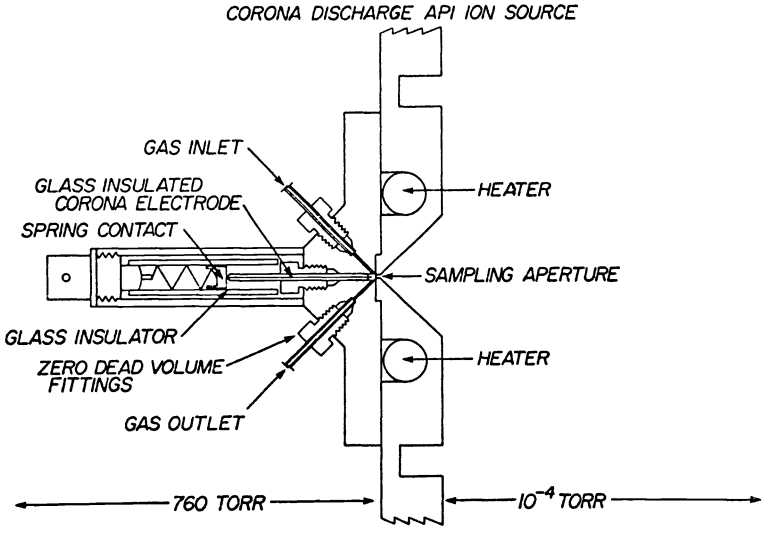


Fig. 6. Diagram of an API reaction-chamber assembly with a corona discharge as the primary source of electrons. The distance of the electrode point from the aperture may be varied from 0.5 to 4 mm. Sample introduction is through the carrier-gas stream of a gas chromatograph.

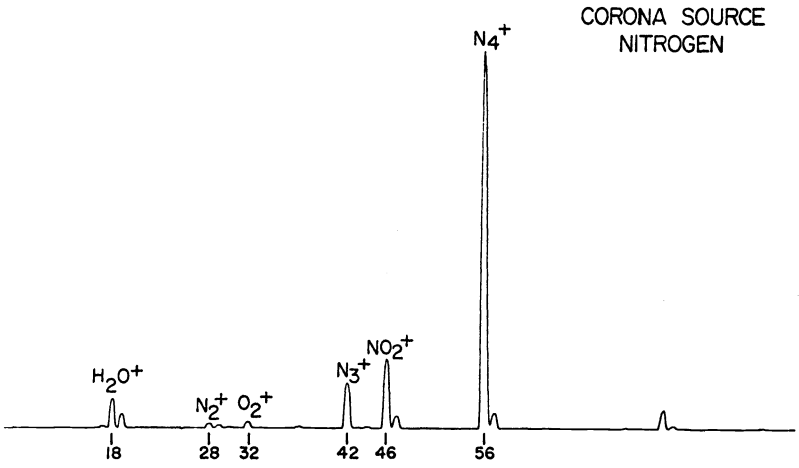


Fig. 7. Mass spectrum of the reagent ions present in nitrogen used as carrier gas with a source assembly of the type shown in Fig. 6, with a 0.5-mm distance. The principal ion is N_4^+. Water, which is always present as a trace impurity in bioanalytical systems, is ionized to form H_2O^+ ions. Subsequent collisions of H_2O^+ with water molecules lead to $(H_2O)_nH^+$ ions which become the predominant water ions when the distance is increased to 4 mm.

Figures 8 and 9 show the reagent ions from isobutane and nitric oxide; in both instances the reagent gas concentration was 0.1% in the carrier gas. Another useful reagent gas is ammonia; the active reagent ion is NH_4^+, and this is always accompanied by the relatively unreactive ion $NH_3NH_4^+$. When organic solvents are used, the reagent ions are derived from the solvent. For example, the ions from benzene are $C_6H_6^+$ and $C_{12}H_{12}^+$; iso-octane forms $C_4H_9^+$ as the principle ion; methanol and ethanol form $(ROH)_nH^+$ ions. In the absence of a reagent or solvent (a condition not used in practice) the ions observed are derived from water, which is always present as an impurity in bioanalytical systems. Product ions formed by reaction of typical reagent ions such as $C_4H_9^+$, NO^+ and NH_4^+ with organic compounds under API conditions correspond to those formed under analogous CI conditions at 0.5 - 1 mmHg. Most compounds containing oxygen and/or nitrogen form MH^+ ions as the principal product. Nitrogen-containing compounds often form relatively stable M^+ ions when NO^+ is used as the reagent ion. In these instances the operation of an API system resembles that of a conventional CI system.

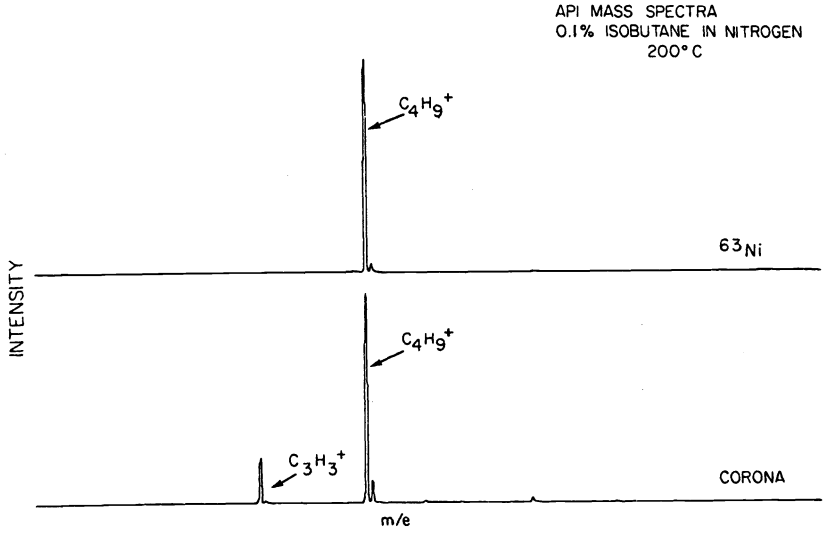


Fig. 8. Mass spectra showing reagent ions generated from isobutane (0.1%) in helium with ^{63}Ni and corona-discharge (4 mm) sources.

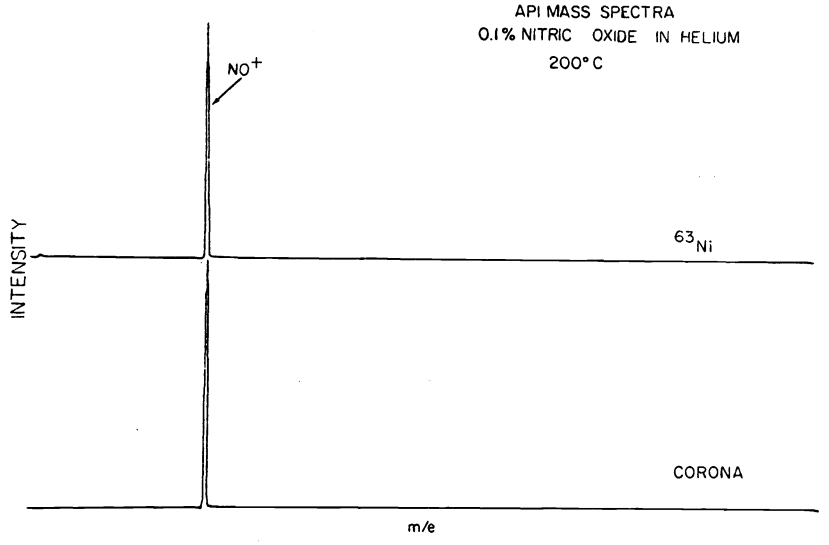


Fig. 9. Mass spectra showing reagent ions generated from nitric oxide (0.1%) in helium with ^{63}Ni and corona-discharge (4 mm) sources.

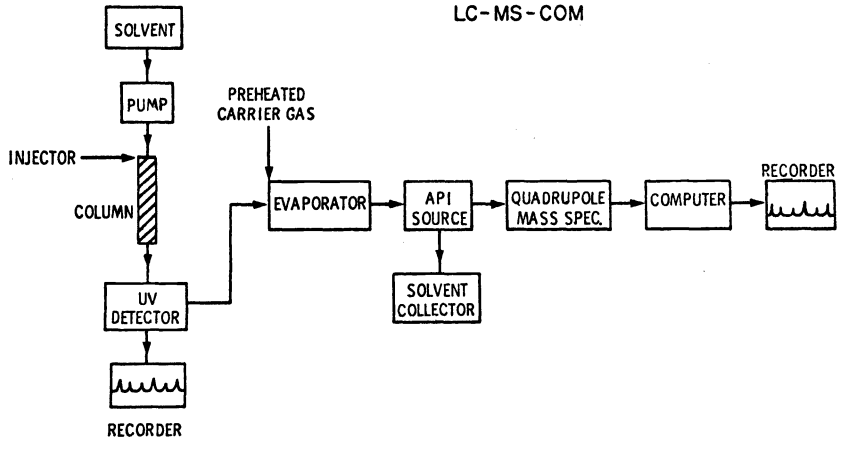


Fig. 10. Diagram of an LC - MS - COM system. The ultraviolet-absorption detector is retained for investigational purposes. This is the liquid-chromatography analogue of a GC - MS - COM system.

One of the significant differences between API and CI systems is that samples in solution, or in the effluent stream from a liquid chromatograph, can be ionized without removal (although vaporization is required) of the solvent. The effluent stream from a liquid chromatograph may be split, or the entire stream may be vaporized and directed through the ion source. Figure 10 shows a schematic diagram of an LC - MS(API) - COM system. Figure 11 shows the detection of three polycyclic aromatic hydrocarbons with this system. The reagent ion was $C_4H_9^+$ from iso-octane, and the ion products were MH^+. Selected ion detection was used to detect each hydrocarbon. Figure 12 shows the mass spectrum of each ion product, obtained while the chromatographic effluent was flowing through the ion source assembly. A system of this kind (Fig. 10) is a direct analogue of existing GC - MS - COM systems. An LC - GC - MS - COM system, while theoretically possible, has not yet been designed. In our work, fractions separated by LC methods are transferred to a GC - MS(API) - COM system after removal of most of the solvents (in order to enhance the detection sensitivity for small amounts of sample components).

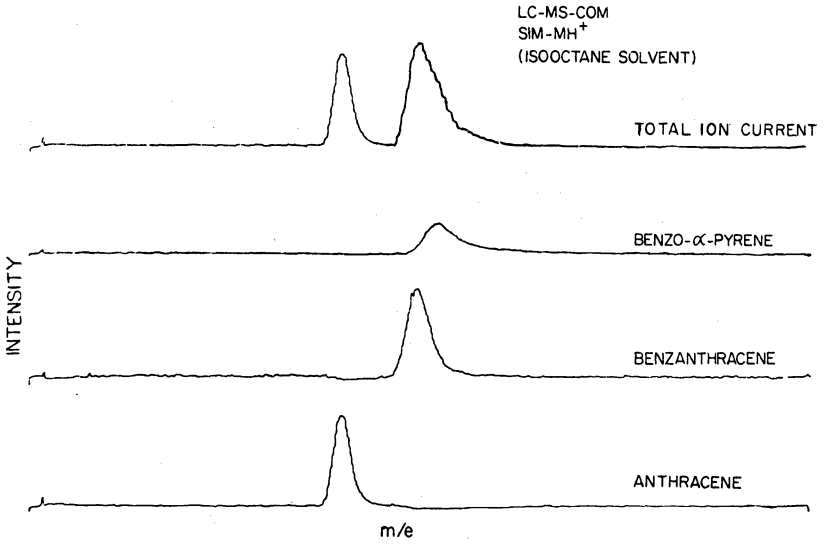


Fig. 11. Analytical separation of three polycyclic aromatic hydrocarbons with an LC - MS - COM system. Each hydrocarbon was monitored as MH^+; when the total ion current and ultraviolet absorption were measured, the separation was not apparent. The solvent was iso-octane.

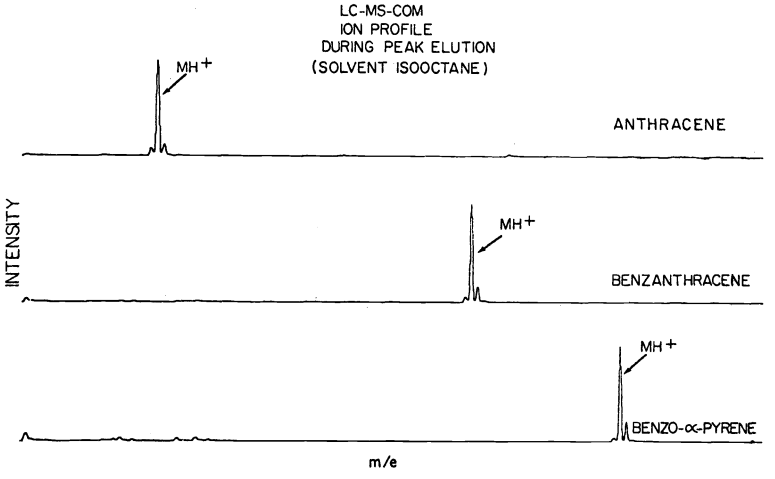


Fig. 12. Mass spectra of the three polycyclic aromatic hydrocarbons used in Fig. 11. These spectra were obtained by scans while the peaks were being eluted. The principal reagent ion from iso-octane is the $C_4H_9^+$ ion, which is also the reactant ion from isobutane (Fig. 8). The products are MH^+ ions.

Many bioanalytical procedures involve small samples, and the compounds of interest are often present in low concentration. Procedures used in environmental analyses generally employ larger samples, but in most instances the concentrations of toxic agents under study would in the past have been considered both unmeasurable and unimportant. Current studies emphasise both sensitivity and selectivity of detection because both are required as the material to be determined decreases in amount. Subpicogram sensitivity of detection has been achieved with an API system for both positive and negative ions, and it may soon be possible to reach subfemtogram (less than 10^{-15} g) sensitivity of detection for organic compounds of this kind. Figure 13 shows the detection of a test substance (2,6-dimethyl-γ-pyrone) as a stable positive

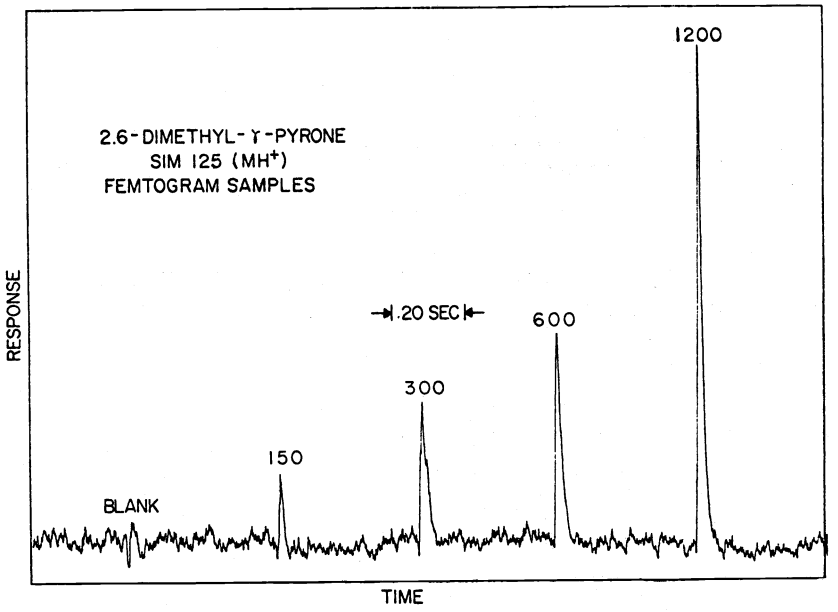


Fig. 13. Limiting sensitivity of detection for 2,6-dimethyl-γ-pyrone observed for an API system with injection in solution. The product ion MH^+ was monitored.

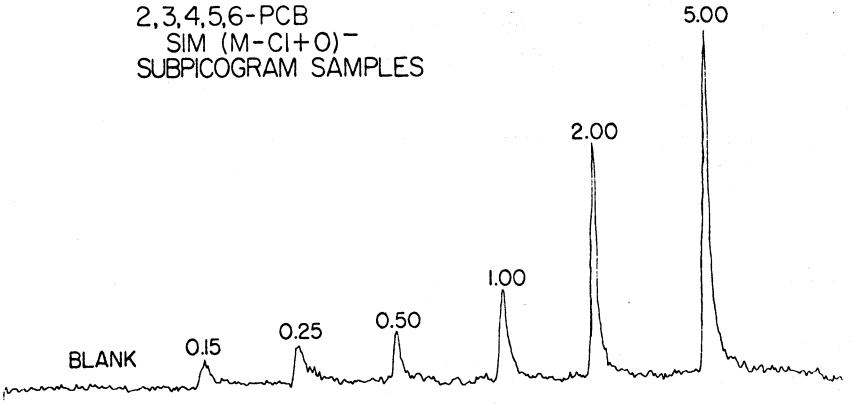


Fig. 14. Limiting sensitivity of detection for a polychlorobiphenyl observed for an API system with injection in solution. Nitrogen containing oxygen was used as the carrier gas; the ion monitored was $(M - Cl + O)^-$.

ion in an API system with nitrogen as the carrier gas. The sample was injected in solution, and ion-counting techniques were used with selected-ion detection to monitor MH^+ ions from the sample. In studies of this kind, it is necessary to use a series of syringes to avoid errors due to adsorption/desorption effects, and to minimize adsorption effects in sample transfer and in vaporization chambers. It is not likely that subpicogram-analysis methods will soon be used routinely, but work with amounts of 1 - 10 pg now appears to be possible. Figure 14 shows the detection of small samples of a polychlorobiphenyl as a phenoxide ion corresponding to $(M - Cl + O)^-$. The same techniques were employed as for Fig. 13, but the carrier gas contained oxygen and the system was used in negative-ion mode. The limiting sensitivity of detection was about the same as that observed for positive ions.

When small amounts of material are studied, numerous impurities or associated compounds will always be present in amounts which may far exceed the amount of the compound(s) of interest. This circumstance makes it necessary to employ detection methods which are selective, and which carry some degree of assurance that the compound of interest is in fact responsible for the detector signal. Electron-capture detection is believed to be highly selective, but in fact the distribution of compounds which form stable negative ions is now so widespread in the biosphere that most environmental samples contain many substances that show a detector response under electron-capture conditions. This is due to past and current use of slowly degradable pesticides, herbicides and fungicides, to the use of chlorination in the treatment of water supplies and to the escape of polychloro and polybromo compounds used in a variety of applications. Mass spectrometric methods provide some evidence of identity, and analytical systems based on positive- or negative-ion formation provide the best current analytical procedures.

Negative-ion formation is of considerable theoretical and practical importance, although this was not recognized until recently. Many substances that are acutely toxic to plants and to lower organisms can be detected by electron-capture gas chromatography. The response of the electron-capture detector is based upon the reaction of these substances with electrons of thermal energy, and the initial step is believed to be the formation of M^- radical ions. Studies of negative-ion formation at low pressure (11, 12) indicate that almost all (if not all) organic compounds will react with electrons of higher energy to form M^- radical ions, and that for many compounds this is followed by loss of a hydrogen radical to yield $(M-H)^-$ ions. Under the usual API conditions where high-energy electrons lose energy rapidly and reach a Boltzmann distribution, and where many ion-molecule collisions occur, ions corresponding to $(M-H)^-$ are observed only for acids that are strong in the gas phase. A useful method of formation of $(M-H)^-$ ions from acids is to use nitrogen as the carrier gas and oxygen as the reagent gas. The superoxide $(O_2)^-$ is strongly basic in the gas phase (13). Figure 15 shows an example of $(M-H)^-$ ion formation for the drugs diphenylhydantoin and phenobarbital.

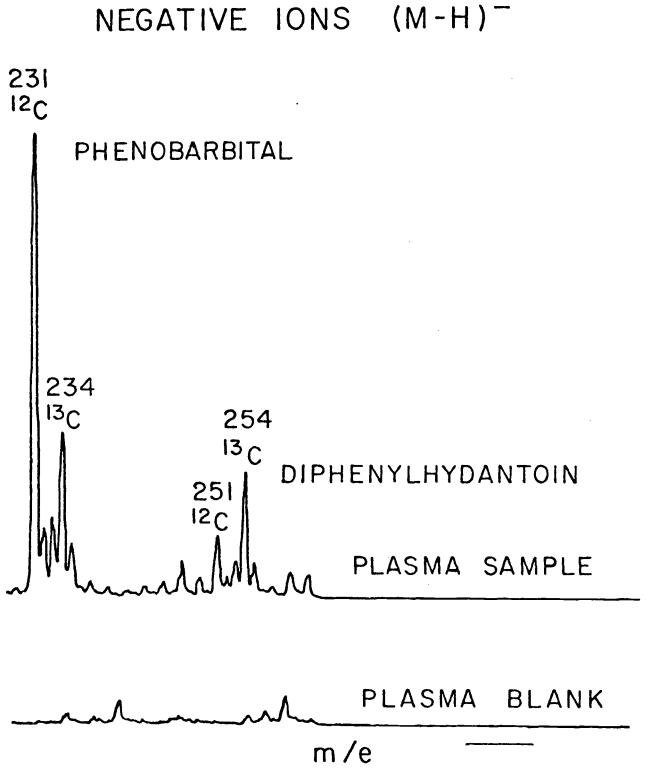


Fig. 15. Analysis of a plasma extract for diphenylhydantoin and for phenobarbital. The ions monitored were $(M-H)^-$ for the two drugs and for the internal reference compounds labelled with stable isotopes. The extract was injected directly.

A few compounds form stable radical M^- ions. Figure 16 shows the electron-capture response and the API negative-ion response for benzil as M^-. In most instances when toxic agents are studied, however, the ion product or products will arise from bond

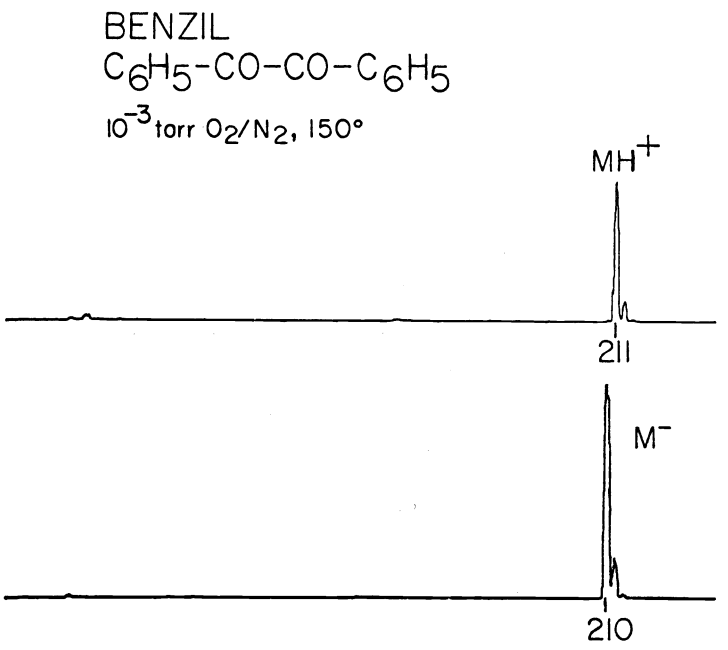


Fig. 16. Product ions from benzil observed under API conditions (^{63}Ni foil) in positive- and negative-ion mode. The carrier gas was nitrogen containing a little oxygen. The ions correspond to MH^+ and M^-. The same product ions are formed in nitrogen alone, and in an argon - methane mixture.

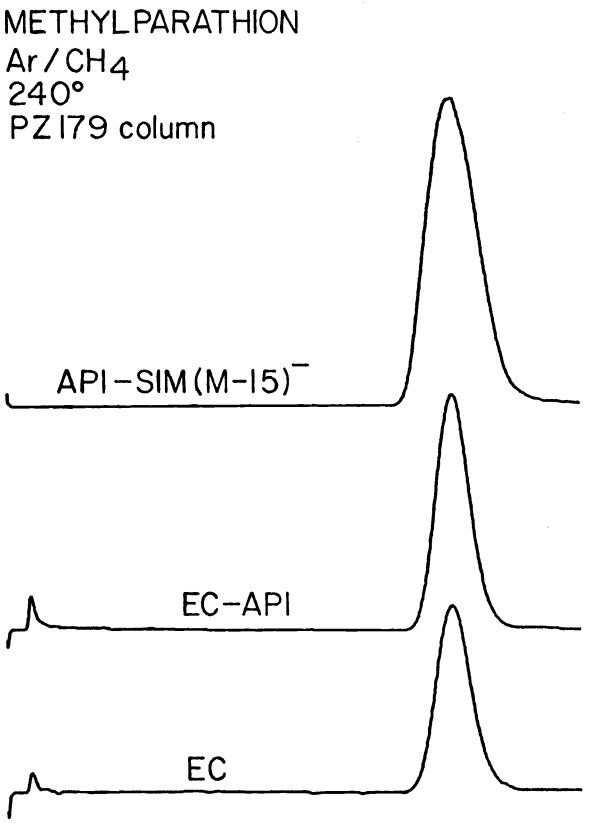
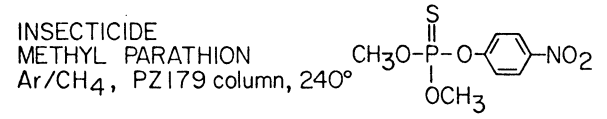


Fig. 17. Monitoring record for methyl parathion observed with an electron-capture detector (EC), with an API source constructed to resemble an electron-capture detector (EC - API), and as $(M-15)^-$ by the API mass analyser (API - SIM). A GC - MS(API) - COM system was used.

breaking with the formation of an anion of a strong gas-phase acid and a neutral radical (which is not detected) (14). The reaction involves the initial formation of M^-, but this ion is not detected under API conditions because it dissociates immediately. The driving force for the reaction arises from the stability of the anion. This effect is shown in Figs. 17 and 18 for methyl parathion. Figure 17 shows the response observed with an electron-capture detector and with an API system in negative-ion mode. The ion monitored was $(M-15)^-$, corresponding to a thiophosphate anion. The ions formed from the parent molecule are shown in Fig. 18. One is a p-nitrothiophenoxide ion and another is a p-nitrophenoxide ion; the others are thiophosphate ions



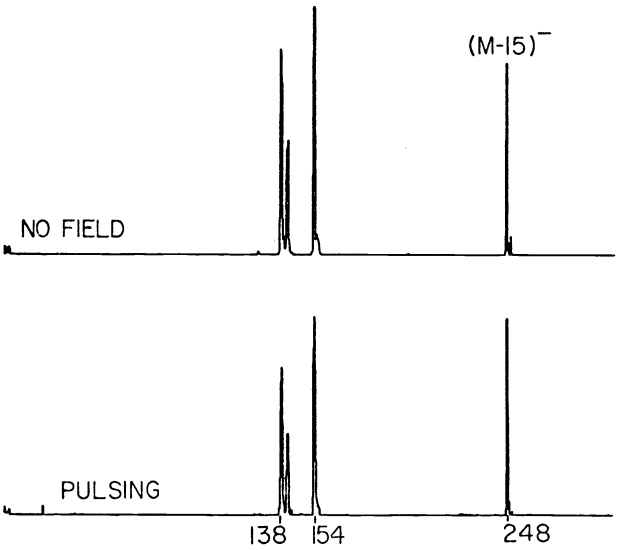
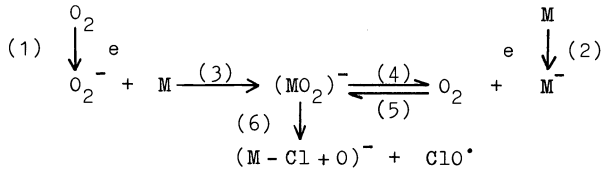


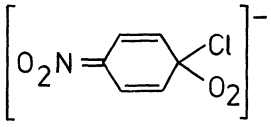
Fig. 18. Negative-ion spectra observed with an API system for methyl parathion. The source was constructed to resemble an electron-capture detector. The product ions were the same under pulsed-field and ordinary conditions. The sample was present in the effluent stream from a gas chromatograph. The ions at m/e = 138, 141, 154 and 248 are due to the nitrophenoxide ion, the phosphate ion resulting from loss of the p-nitrophenyl radical, the nitrothiophenoxide ion, and the phosphate ion resulting from loss of a methyl radical.

formed by loss of methyl and p-nitrophenyl radicals. Ions corresponding to M^- were not observed. The formation of Cl^-, Br^- or I^- ions from many chloro-, bromo- and iodo-substituted compounds is due to the same effect; the M^- ion cleaves to form a stable halide ion and a neutral radical. Reactions of this kind have been called "dissociative electron-capture reactions".

A different and novel replacement reaction has been observed for some aromatic halogen-containing compounds (15). This involves an intermediate structure which corresponds to $(MO_2)^-$. The reactions are believed to be:



When the concentration of oxygen in the reaction chamber is less than that of M, it is possible to detect M^- ions. The $(M - Cl + O)^-$ ions formed under these circumstances involve reactions (2), (5) and (6). As the concentration of oxygen increases, the product ion is increasingly formed by reactions (1), (3) and (6). M^- is not observed with high concentrations of oxygen because reaction (2) is suppressed, and the rate of reaction (6) is greater than that of reaction (4). The nature of the intermediate radical ion $(MO_2)^-$ which is not directly observed is probably



for p-nitrochlorobenzene, and an analogous ion is the probable intermediate from o-nitrochlorobenzene. This view is derived from the observation that m-nitrochlorobenzene yields M^- ions in the presence of oxygen. Polychloroaromatic halides yield polychlorophenoxide ions through a related reaction. In all instances the ion products are anions of strong gas-phase acids.

Most analytical systems of the future will have non-filament sources and will be able to detect both positive and negative ions. Electron-impact mass spectrometry will continue to be used in structural studies, but most quantitative work will probably be carried out with stable ions. Further work is needed to determine the most satisfactory pressure for product-ion formation and to investigate the relationship between source design and pre- and post-aperture ionization reactions. It is likely that it would be desirable to use relatively high pressures in instances involving highly stable ions, and lower pressures for less stable ions.

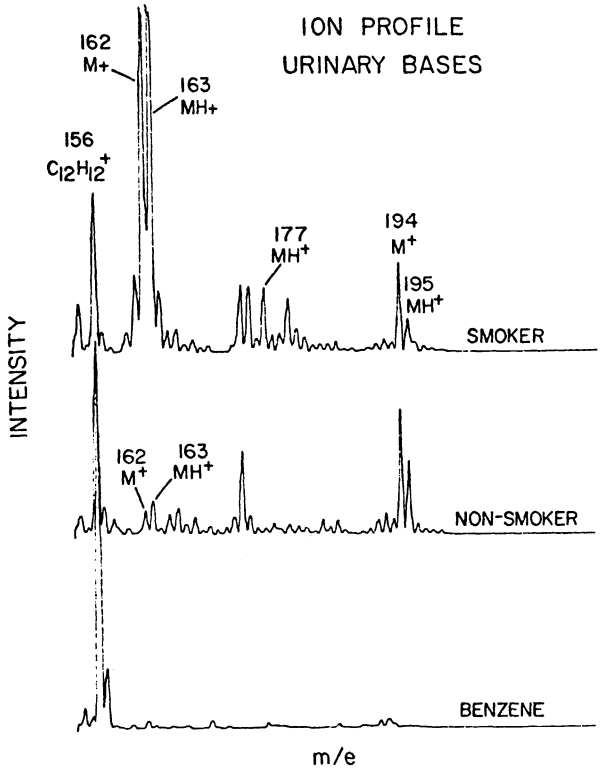


Fig. 19. Analysis of urinary extracts for a smoker and a non-smoker with an API system. The ions at m/e = 162 and 163 are due to nicotine as M^+ and MH^+; both ions are formed when benzene is used as the reagent. Samples from adult non-smokers in an urban environment have about 5% of the nicotine observed for smokers.

Nicotine is a strong gas-phase base, and it also forms a stable M^+ radical ion. This fact has made it possible to detect very small amounts of nicotine under API conditions. Figures 19 and 20 show two analytical results. There is always a high nicotine content in extracts of the urine from smokers. Adults in an urban environment who are

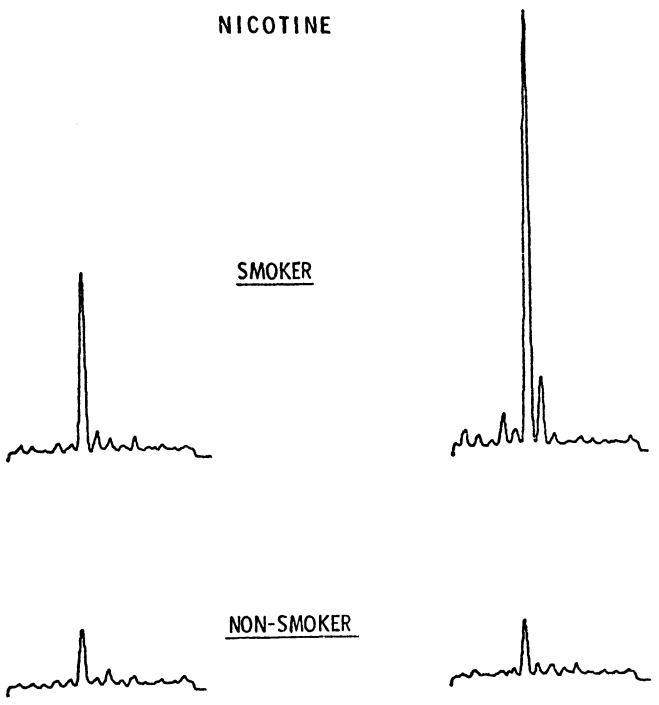


Fig. 20. Nicotine analysis of hexane washings from the fingers of a non-smoker and a right-handed smoker. The extract was injected directly. Non-smokers have less nicotine than smokers and it is approximately evenly distributed. The nicotine is in the lipid layer and is not removed by washing with soap and water.

non-smokers also have nicotine in their urine. The amount is usually about 5% of that found for smokers, and the transfer is by air. Nicotine is also present on the skin of adults in an urban environment. Washings of the fingers show a positive response for both smokers and non-smokers, but for non-smokers the amount is low and is uniformly distributed on both hands. Figure 20 shows an analysis for a right-handed smoker; the amounts on both right- and left-hand fingers is larger than for a non-smoker, and the amount is greater on the hand used in smoking.

Research in mass spectrometry and in the development of bioanalytical systems is generally not considered to involve chemical reactions related to those occurring in cells. This may not be entirely true. Most of the organic compounds released into the environment in order to kill plants or lower organisms, and many organic toxic agents that are released by accident, are detectable under API conditions. These compounds form stable negative ions by reactions that release neutral radicals. The superoxide ion $(O_2)^-$ is capable of effecting these reactions either by charge transfer or by a substitution reaction. In instances where toxicity cannot be ascribed to a specific mechanism, it may be that cell damage is caused by neutral radical formation, with the superoxide ion as a reactant.

This discussion of bioanalytical systems is concerned primarily with the chemical and instrumental aspects of the analytical process. Techniques for sample preparation and the nature of the chromatographic methods employed separately or as part of the analytical system have not been discussed. The major recent changes have been the development of glass open tubular capillary columns for gas chromatography, and the development of high-performance liquid-chromatographic separation methods. These procedures require separate study and involve different considerations. As a consequence of continued research, the analytical methods now employed for bioanalysis are immeasurably more effective than those used in the past, and further studies may be expected to result in better systems, wider areas of application, and increased understanding of biological events.

Acknowledgement - This work was aided by Grants GM 13901 of the National Institute of General Medical Sciences and HL-17269 of the National Heart, Lung and Blood Institute.

REFERENCES

1. R. Ryhage, Anal. Chem. 35, 759 (1964).
2. C.G. Hammar, B. Holmstedt and R. Ryhage, Anal. Biochem. 25, 532 (1968).
3. R.A. Hites and K. Biemann, Anal. Chem. 40, 1217 (1968).
4. E.C. Horning, M.G. Horning, D.I. Carroll, I. Dzidic and R.N. Stillwell, Anal. Chem. 45, 936 (1973).
5. D.I. Carroll, I. Dzidic, R.N. Stillwell, M.G. Horning and E.C. Horning, Anal. Chem. 46, 706 (1974).
6. S.B. Munson, Anal. Chem. 49, 772A (1977).
7. D.I. Carroll, I. Dzidic, R.N. Stillwell and E.C. Horning, Anal. Chem. 47, 2369 (1975).
8. E.C. Horning, D.I. Carroll, I. Dzidic, K.D. Haegele, M.G. Horning and R.N. Stillwell, J. Chromatog. Sci. 12, 725 (1974).
9. E.C. Horning, D.I. Carroll, I. Dzidic, K.D. Haegele, M.G. Horning and R.N. Stillwell, J. Chromatog. 99, 13 (1974).
10. I. Dzidic, D.I. Carroll, R.N. Stillwell and E.C. Horning, Anal Chem. 48, 1763 (1976).
11. M. von Ardenne, K. Steinfelder and R. Tummler, Elektronenanlagerungs-Massenspektrographie organischer Substanzen, Springer, Berlin, 1971.
12. R. Large and H. Knof, Org. Mass Spectry. 11, 582 (1976).
13. I. Dzidic, D.I. Carroll, R.N. Stillwell and E.C. Horning, J. Am. Chem. Soc. 96, 5258 (1974).
14. E.C. Horning, D.I. Carroll, I. Dzidic, S-N. Lin, R.N. Stillwell and J-P. Thenot, J. Chromatog. in press.
15. I. Dzidic, D.I. Carroll, R.N. Stillwell and E.C. Horning, Anal. Chem. 47, 1308 (1975).