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ARTICLE

Fast atom bombardment of solids as an ion source in mass spectrometry

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A new ion source for molecular structure determination of thermolabile and involatile compounds by mass spectrometry is presented together with some preliminary results from peptides, glycoside antibiotics, organometallics and vitamin B_{12} and its coenzyme.

THERE have been several attempts to overcome the necessity of presenting the sample to the mass spectrometer in the gas phase, before ionization. This limits the range of applicability of the technique due to thermal lability and involatility of numerous types of compounds, especially in the case of high molecular weight substances of biological and biomedical importance. This deficiency has been overcome by field desorption of ions[1], sputtering of ions from surfaces by bombardment with fast ions[2], and with heavy fission products from the radioactive decay of ^{252}Cf (ref. 3) and the use of lasers to desorb ions from surfaces[4].

Most of these methods have serious deficiencies, however. Field desorption is a difficult technique experimentally, necessitating the conditioning of fragile emitters, producing highly transient mass spectra, which in many cases seem to be dominated by thermal degradation of the material. The ^{252}Cf plasma desorption source, although producing spectacular results, presents many difficulties, while laser-induced desorption of ions is in its infancy.

Perhaps the most tractable method has been to use the sputtering of ions by fast ion bombardment (SIMS)[2,5] to produce both positive and negative ion mass spectra of small molecules. Again, there are several difficulties: (1) the ions produced have large kinetic energy spreads[6], thus limiting the resolution available in simple mass spectrometers, and (2) the use of fast ions as the sputtering medium induces surface charging of insulating materials, and also introduces problems if the beam is to be steered into the high voltage regions of the ion sources of modern large mass spectrometers.

We have largely overcome problem (2) by using a beam of fast neutral atoms as our primary sputtering medium.

Experimental method

Our apparatus consists of a cold cathode discharge ion source producing Ar^+ of controllable energy between 1 and 8 keV. The emergent beam is focused and passes through a collision chamber containing a high pressure (10^{-3}–10^{-4} torr) of Ar gas. Resonant charge exchange occurs with little or no loss in forward momentum. This results in a beam emanating from the chamber which consists of a mixture of Ar atoms with approxi-

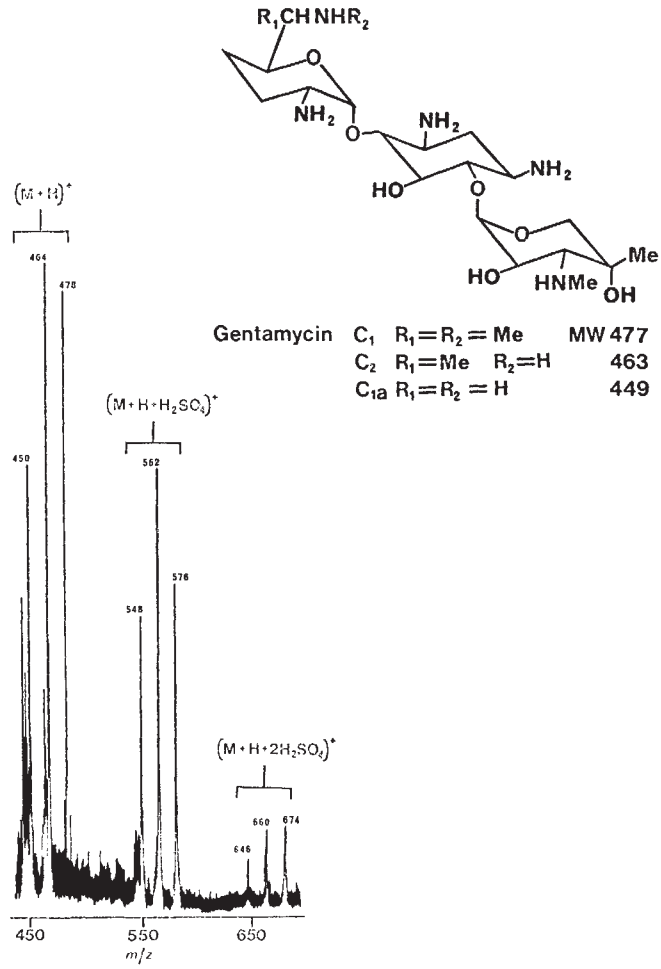
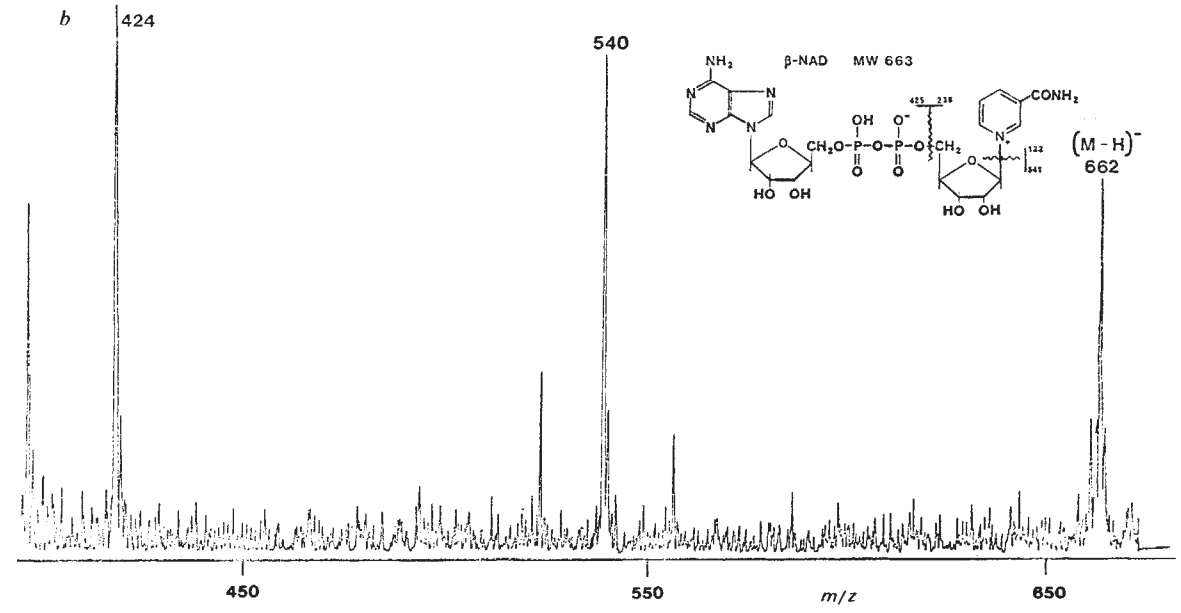


Fig. 1 Positive ion FAB mass spectra of a glycoside antibiotic: pseudomolecular and sulphate adduct ions of the gentamycins. MW, molecular weight.



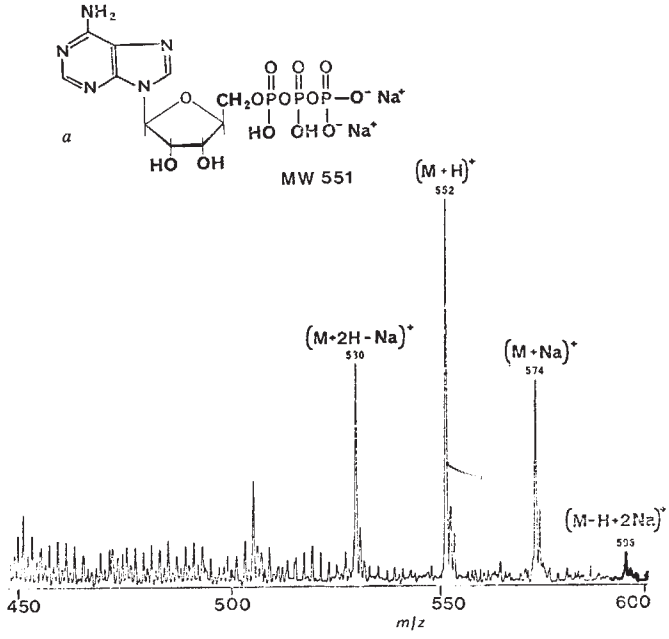


Fig. 2 FAB mass spectra of some nucleotides. *a*, Positive ions from ATP sodium salt; *b*, negative ions from NAD.

mately the same kinetic energy as the original Ar^+, and Ar^+ which has not been charge exchanged. The latter is cleansed from the beam by a set of electrostatic deflector plates. The whole system is differentially pumped to maintain an adequate vacuum in the mass spectrometer ion source. To minimize the surface damage, the intensity of the pure Ar atom beam is maintained at $\sim 10^{10}$–10^{11} atoms per cm^2 of target per s.

To help alleviate problem (1) we have modified the ion source region of an AEI MS902 double focusing mass spectrometer to accept our atom gun. The system has been adapted so that materials can be introduced by deposition from solution on to a copper stage outside the vacuum and inserted through a standard vacuum lock system into the MS902 source to intercept the fast atom beam. The ions produced by the sputtering mechanism are then accelerated to the normal spectrometer potential and pass into the analyser region of the instrument. With such a system we have produced moderate resolution mass spectra from a wide variety of molecules which have previously proved difficult. Using the double focusing instrument we have also carried out accurate mass measurements, and using the Barber–Elliott technique[7] have observed first-field free region metastable transitions of ions produced by the sputtering phenomenon.

Results

To determine the limitations of this ion source we initially studied polyhydroxylated materials such as oligosaccharides and, in particular, glycoside antibiotics. These compounds have previously been extensively studied, although with difficulty, by electron impact, using suitably protected derivatives, and by field desorption of the virgin materials. Using fast atom bombardment (FAB), even the salts of these compounds can be studied by depositing the sample from solution on to the copper stage. Figure 1 shows the high mass region of gentamycin sulphate. This material is a mixture of three compounds differing by CH_2 units in the A-ring side chain and we can detect each component of the mixture quite easily.

Two general features of these spectra must be noted, the first being the observation of an $(M+H)^+$ parent ion. It is a characteristic of this ion source that odd electron molecular ions are rarely detected in any abundance as a primary species. The analogy may be drawn to chemical ionization in that as a rule, in positive ion FAB, even electron $(M+H)^+$ ions are produced, and $(M-H)^-$ in the negative ion mode. The second feature is that high sensitivity is obtained for such a low primary sputtering beam intensity. The intensity of the pseudomolecular ion peaks of the glycosidic antibiotic gentamycin is of the order of 10^6 ions s^{-1}.

Commercial samples of these materials are often in the form of sesquisulphates or hydrochlorides which have proved to be almost impossible to characterize by any other method in mass spectrometry.

Next let us consider simple mono- and dinucleotides. The mononucleotides have been investigated by making their trimethyl silyl (TMS) derivatives and using electron impact ionization. Some field desorption work has also been reported on the underivatized monophosphates[8]. There seems little problem in producing mass spectra from these molecules in an underivatized form using the FAB ion source, either as the free acids or as their sodium salts. Figure 2*a* shows the FAB spectrum of the sodium salt of ATP. We have also obtained spectra of similar quality from the dinucleotide salts of NAD and FAD, the former being illustrated in Fig. 2*b*. The fragmentation obtained from these molecules is shown and reveals considerable structural information.

Another class of compounds, the sulphonic acid derivatives of aromatic naphthalene and anthraquinone systems, are of interest in the dyestuffs industry and have been extensively investigated using field desorption mass spectrometry which has proved to be of little value. We have obtained good spectra from molecules containing up to five sulphonic acid groups, as both free acids or their sodium and potassium salts. Figure 3 shows a typical spectrum which can be obtained from these compounds; considerable fragmentation of the molecule takes place which is easily explained on the basis of the structure of the original material.

The ability to sequence peptides has probably been one of the most spectacular successes in structural mass spectrometry. Careful choice of derivatives were necessary when using conventional methods of ionization. The existing techniques have gained increasing popularity, and have led to the sequencing of not only moderate-sized molecules such as the enkephalins[9], but, after suitable enzymatic cleavages, to the amino acid

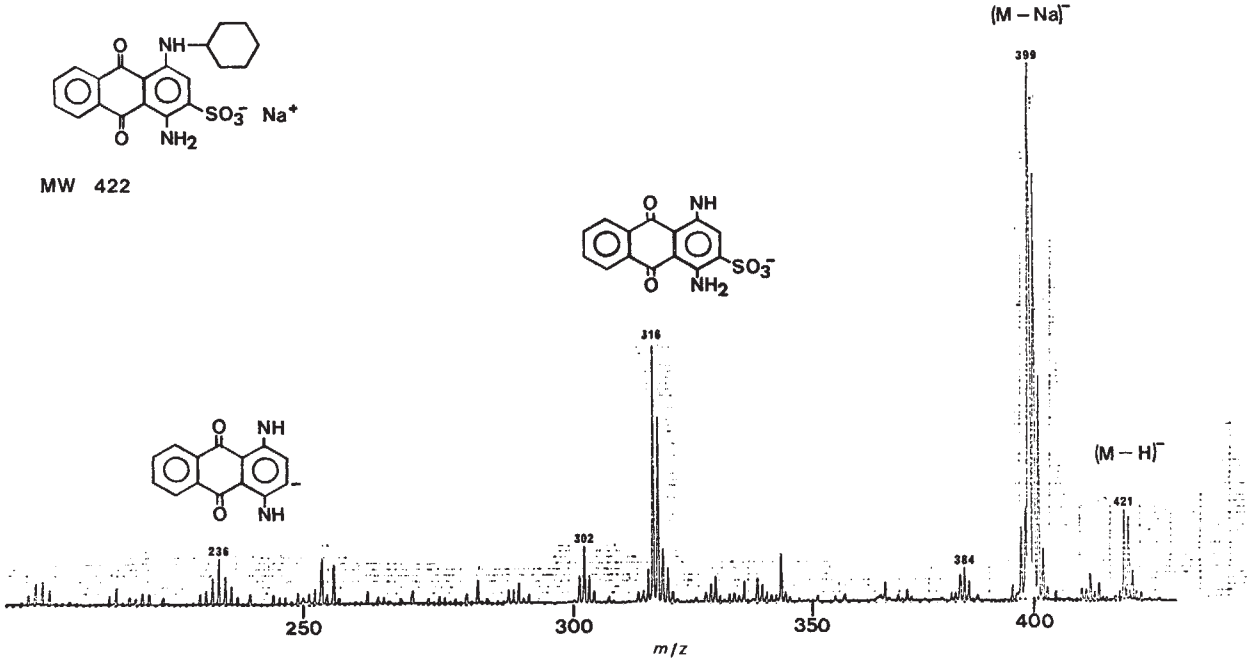


Fig. 3 Negative ion FAB mass spectrum of anthraquinone disulphonic acid.

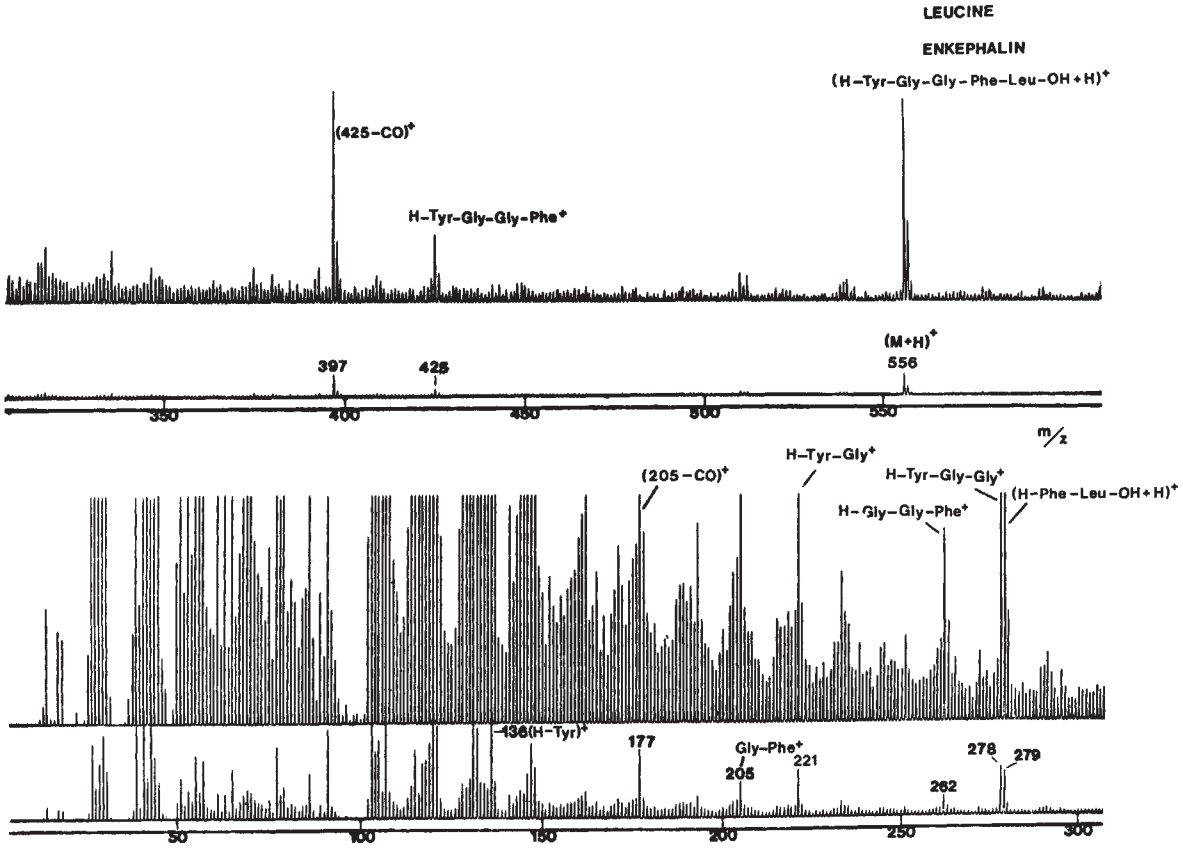


Fig. 4 Positive ion FAB mass spectra of the oligopeptide leucine enkephalin.

sequence of some proteins[10]. We have concentrated on investigating the FAB mass spectra of underivatized peptides, with a view to determining the size of peptide amenable to this method and the sequence information contained within the spectra. Figure 4 shows the positive ion mass spectra of leucine enkephalin on which the sequence ions are indicated. Similarly for bradykinin, the FAB spectrum revealed comprehensive sequence information with cleavages being observed for both the C-terminal and N-terminal ends.

The largest underivatized peptide that we have investigated is human gastrin 1 of molecular weight 2,096. This peptide gave reasonable 'pseudo-molecular' ion intensity, $(M+Na)^+$ at m/z 2,119, but lack of sensitivity with our present equipment has prevented detailed examination of the fragmentation of this molecule. From these preliminary investigations it seems that sequencing underivatized peptides at least as large as gastrin 1 may be possible.

We have also studied the fragmentation pathways which give rise to the major peaks found in the FAB mass spectrum of small peptides as revealed by standard metastable detection methods. Figure 5*a* shows such 'metastable' transitions occurring in the positive ion FAB mass spectrum of the tripeptide, H-Ala-Leu-Gly-OH and the transitions are interpreted. Alternatively, the Barber–Elliott method[7] of metastable scanning on the same molecule is shown in Fig. 5*b* with the interpretation of the various transitions interpreted. This sort of information is crucial for understanding the mass spectra from any class of compounds, and we believe that this is the first demonstration that sputter ion sources produce 'molecular' species with internal energy, sufficient to produce ion decompositions in the gas phase, entirely analogous to the other ionization methods.

We now consider some organometallic systems. The first example is a rhodium complex, the positive ion spectrum of which is shown in Fig. 6*a*. This is of interest for two reasons. First, the molecule is thermally labile and water sensitive, and gave no mass spectrum of any worth by conventional methods. Second, it is produced as an oil, demonstrating that the FAB ion source can accommodate liquids. It is also of interest that the oil produced a 'non-fading' spectrum without modified sample preparation.

Our final examples of organometallic systems are the vitamin B_{12} complexes. Schiebel and Schulten[11] describe B_{12} as a 'milestone' in mass spectrometry. We have successfully obtained mass spectra not only from cyanocobalamin but also from the hydroxy and methyl derivatives. The FAB mass spectrum of cyanocobalamin is shown in Fig. 6*b*. It is interesting to compare our spectra with field desorption spectra. These are dominated by a set of peaks at m/z 914 and m/z 932, with the parent peaks being very small. In our opinion, the field desorption mass spectrum is dominated by thermal degradation which we do not observe. In addition to these cyanocobalamins, we have been able to obtain the first mass spectrum of the coenzyme of vitamin B_{12} as shown in Fig. 6*c*. The interpretation of our spectra will be discussed elsewhere.

Discussion and conclusion

FAB mass spectrometry has immense possibilities for extending the mass range of materials accessible to mass spectrometric analysis, especially when the usual mass spectrometric facilities are available. However, certain points and generalities concerning the characteristics of this ion source need clarifying.

First, sample preparation and manipulation are essentially very simple. The material is deposited from solution or a slurry on to the sample stage, requiring no previous generation of delicate substrates, as in field desorption. During our work we have noted, as others have reported[12] that in many cases the spectra obtained are transient; rapid 'fading' ensues with a half life for the pseudomolecular ion of the order of a few minutes. The origin of this fading is obscure, being possibly a mixture of surface damage by the primary atom beam, and surface contamination by the residual gases from the relatively poor vacuum obtainable in the ion source region in most conventional large mass spectrometers.

We have, however, largely overcome this effect by judicious use of solvent and support systems, paying particular attention

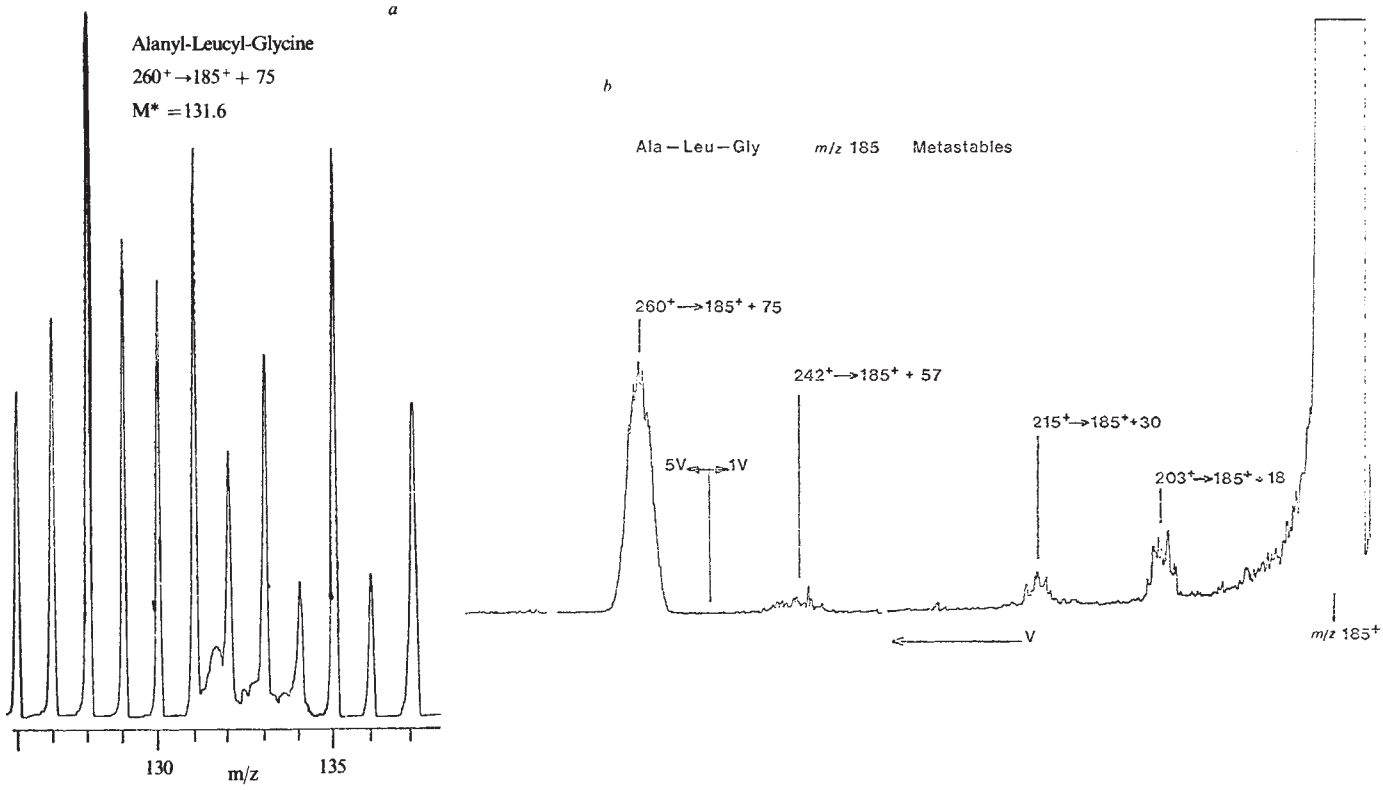


Fig. 5 Metastable ions in FAB mass spectra: *a*, second-field free region metastable ions in H–Ala–Leu–Gly–OH; *b*, Barber–Elliott metastable scan showing the precursor ions of m/z 185^+ in H–Ala–Leu–Gly–OH.

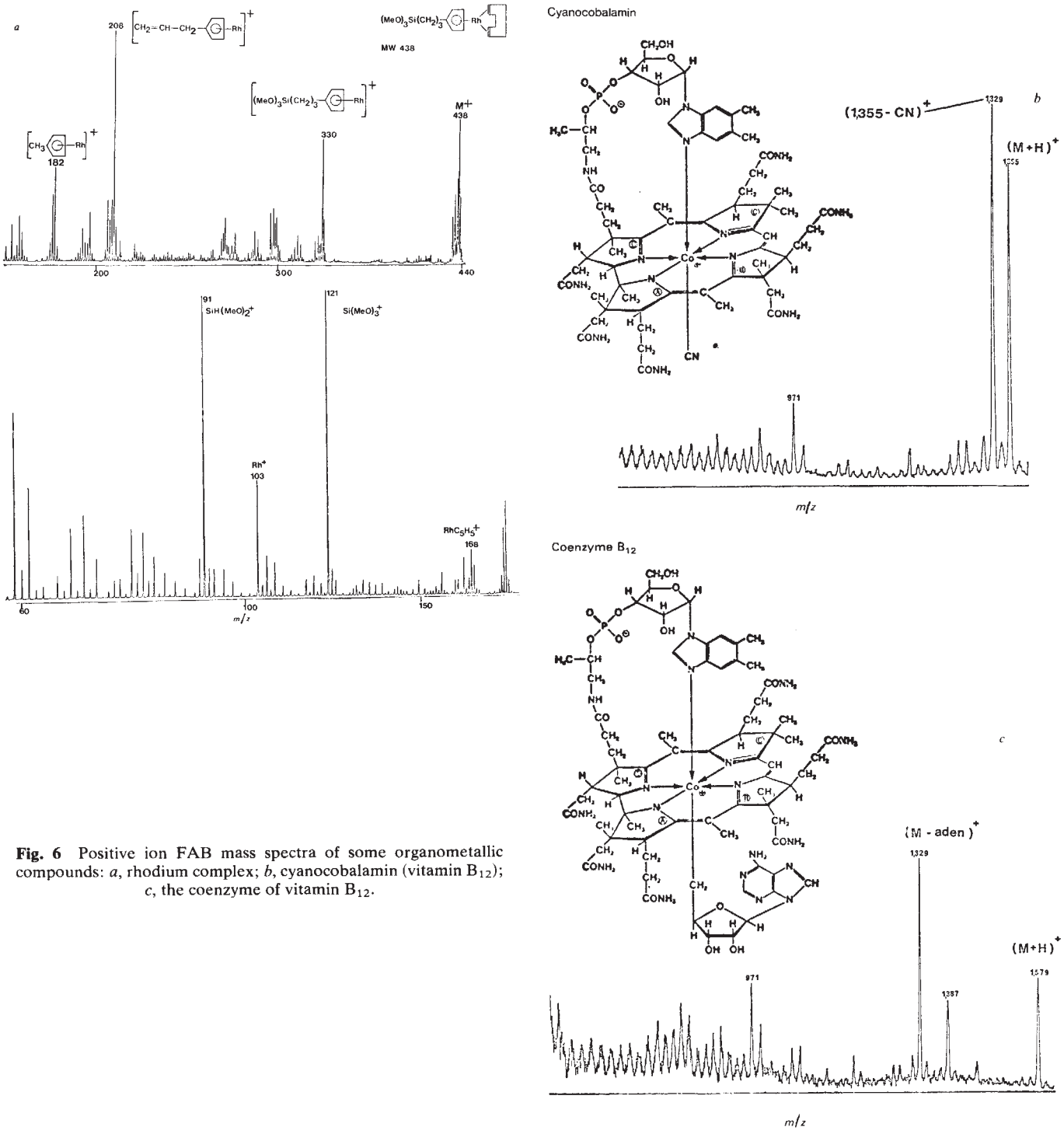


Fig. 6 Positive ion FAB mass spectra of some organometallic compounds: *a*, rhodium complex; *b*, cyanocobalamin (vitamin B_{12}); *c*, the coenzyme of vitamin B_{12}.

to the viscosity and volatility of the medium from which the sample is deposited on the stage. By this means we have preserved samples, with no depletion of 'parent' ion sensitivity over periods of hours.

One disadvantage of this ion source is that the ions produced have large kinetic energy distributions and most of the double focusing instruments obtainable commercially are not fully energy focusing. Indeed our instrument whilst performing fully first order focusing in terms of direction and energy, has a large second order energy aberration. If full use were to be made of this, and other high kinetic energy ion sources, then fully corrected second order focusing geometries must become standard in the next generation of high performance mass analysers.

Improvements must be made to the vacuum systems used in conventional organic instruments, such that ultimate pressures in the ion source and related regions of instruments should be in the region of 10^{-8} torr or better. As sputter ionization methods are surface sensitive, they require an environment which makes surface contamination minimal.

We do not believe that we have yet nearly realized the full mass range capability of this ionization method. Indeed, using simple polymers as samples, we have covered the full mass range of our existing instrument (4,000 daltons) but this at a serious reduction in sensitivity due to the large reduction in ion accelerating voltage (a factor of 4) and consequent poor ion extraction efficiency from the source itself. High field strength magnets are available commercially, which partly alleviate this problem.

If, however, we consider a target specification as a mass range of 5,000–10,000 daltons at present source extraction efficiencies, then the physical size of the next generation of fully-energy-corrected instruments must be reconsidered.

A generalization of the ionization process, and other sputter sources, is that genuine odd-electron molecular ions are rarely observed.

Even-electron pseudomolecular ions of the type $(M+H)^+$ or $(M-H)^-$ are invariably observed. We have observed large M^+ ions in polynuclear aromatic systems, but metastable analysis has revealed that these arise by unimolecular dissociation of the $(M+H)^+$ species.

In many cases, especially for carbohydrates, we can stabilize the 'molecular' species by deliberate alkali ion addition to give an even-electron $(M+Na)^+$. Compounds presented to the source in the form of alkali metal salts, for example, carboxylic acid salts of the type R.COONa, give rise in the positive ion spectra to $(RCOONa_2)^+$. Such observations indicate that considerable ion–molecule interactions must take place during the collisional process of sputtering, and we may therefore draw some analogy to chemi-ionization as the predominant means of producing the ions we see. A simple, molecular dynamic treatment of the sputtering phenomenon[13] suggests that the initial impact by the fast particles creates a growing cavity within the lattice which contains a high density gas of randomly distributed particles. A secondary effect due to the dendritic growth of collision chains within the lattice is considerable surface layer peeling, where whole layers of the material seem to be able to separate from the rest of the solid.

Within the high density gas cavity, both positive and negative ions, neutral ions and electrons can be formed in the initial process by 'percussive' ion sputtering mechanisms. Here a molecule is placed on the high repulsive potential energy side of its potential energy hypersurface by the initial impact, and can then decay by surface crossings into dissociation products which are ion pairs or any other combination of ions, neutral species and electrons. We thus have a system in which a high probability exists that ion–molecule reactions occur in the high density cavity with subsequent chemi-ionization of neutral species which are being peeled from the surface. This, we believe, is the essence of the ionization processes occurring in this and other similar ion sources.

We conclude that the FAB ion source has the following advantages. (1) Ionization occurs from the solid, so no sample volatilization is necessary. (2) Sample preparation is easy compared with derivatization techniques or field desorption experiments. (3) There is high pseudomolecular ion sensitivity together with structurally significant fragmentation. (4) A very high mass range of molecules are now accessible.

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LETTERS TO NATURE

Hard X-ray spectrum of Cygnus X-1

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Cygnus X-1, a strong X-ray source in a binary star system, is generally considered a good candidate for a black hole[1]. Its X-ray emission provides the best method of studying the physical processes near the collapsed object. The average energy spectrum (in the 'low' state) is remarkably stable[2] and has been interpreted as the result of comptonization, that is Compton scattering of optical or UV photons in a very hot plasma[3–5]. We have measured the low-state spectrum on 26 October to 18 November 1977, over a wide energy range (3 keV–8 MeV). This is the first measurement to cover such a broad energy range and the first long-term average measurement above 300 keV. This spectrum agrees well with a single temperature comptonization model at low energy, but shows a significant excess at high energy ($E > 300$ keV).

Our observations were made with two instruments on the HEAO 1 satellite, in the spacecraft's scanning mode, in which the X-ray detectors scanned great circles on the sky once every 30 min, covering the entire sky in six months. The lowest-energy data (3–40 keV) came from one of the xenon proportional counters of the GSFC Cosmic X-ray experiment[6] (A2), during 3–8 November 1977. Higher-energy information (15 keV–8 MeV) came from six different NaI scintillation counters in the UCSD/MIT Hard X-ray and Low-Energy Gamma-Ray experiment[7] (A4). We obtained spectra from both of this experiment's low-energy detectors (15–180 keV), three of the four medium-energy detectors (100 keV–2 MeV), and the high-energy detector (200 keV–8 MeV) by a point-summation technique. Detector response functions were calculated for the proportional counter and the 15–180 keV scintillators by analytical models, and for the other scintillators by a Monte-Carlo simulation. Figure 1 shows the resulting composite spectrum for all detectors from both experiments. The proportional-counter spectrum has been multiplied by a factor of 1.3 to make the best match in the overlap range (15–40 keV). This is allowable because Cyg X-1 is variable and the proportional counter data were taken only during a small portion of the total observing time.

The strong X-ray source Cyg X-3 is only ~8° from Cyg X-1, close enough to confuse measurements made by detectors with wide fields of view. Fortunately, the 15–180 keV scintillators had a small field of view ($1.2° \times 20°$), thus we were able to obtain independent observations of Cyg X-1 and Cyg X-3 in this energy range. These showed that the emission of Cyg X-3 was negligible compared with that of Cyg X-1 above 100 keV. Thus we have rejected all data from the medium- and high-energy scintillators below 100 keV. The proportional counter also had a small field of view ($3° \times 3°$).

As Cyg X-1 is variable, we must show that the spectrum did not change during our observations. Figure 2 shows the history of the intensity of the Cyg X-1flux, as measured by one of the 15–180 keV scintillators. There is no significant linear trend in these data. We compared the 15–180 keV spectra from 14 different two-day intervals, fitting each with a comptonization spectrum. At the 95% confidence level, our measurements were consistent with no variation in the spectrum shape. Our 95% confidence limits for the r.m.s. variation in kT_e and γ are