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Improved Detection Limits in an Organic Mass Spectrometer using a Combination of Matrix Free FAB and Photodiode Array Detection

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Scanning mass spectrometers suffer from the disadvantage of monitoring only one mass at any particular time in contrast to mass spectrographs [1] which allow the simultaneous detection of an extended mass range. Historically such mass spectra were recorded by directing the ion beam onto a photograhic plate which was subsequently developed and interpreted. The introduction of electronic devices such as the photodiode array enabled the development of imaging detectors capable of real time readout, so that a range of masses can be detected and rapidly processed using a computer system. This work uses such a detector in combination with a double focusing MS50RF mass spectrometer for the parallel detection of a limited mass range.

The technique of FAB -MS is an extremely useful tool for the analysis of involatile, polar molecules, however the need for a relatively involatile matrix does introduce problems with background peaks or chemical noise. It is possible to obtain mass spectra of compounds without a liquid matrix in order to eliminate this background and therefore reduce the ultimate detection limit, but under the conditions of primary ion flux used in FAB (which is of the order of microamperes), such signals are extremely transient. The purpose of this work was to see whether useful data could be obtained from a dry sample using an instrument with a standard FAB source, scanning magnetic sector analyzer and an array detection system providing parallel detection of a partial mass spectrum.

Of course, static SIMS using a dry sample is a well established technique which pre-dates the introduction of FAB and liquid SIMS. While we appreciate that a high

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intensity FAB gun and a magnetic sector analyzer are non-ideal choices for these experiments, such instrumentation is in widespread use. Conversely pulsed ion guns and suitable time-of-flight analyzers are less commonly available, particularly in routine analytical laboratories. The aim of these experiments is to demonstrate that an array detector can not only alleviate some of the difficulties of running dry samples on an organic sector instrument but also provide the level of sensitivity achieved in conventional SIMS.

Experimental

The array detector developed for the MS50RF, shown schematically in Figure 1, is an electro-optical system providing parallel detection of a 4% wide mass window with sufficient gain to detect the arrival of individual ions.Two microchannel plate (MCP) multipliers mounted in a chevron configuration are positioned along the angular refocus plane of the mass spectrometer. Ions arriving at the face of the first MCP cause the emission of secondary electrons. This electron image is intensified by the two MCPs and subsequently converted into photons by a phoshor screen. The resulting light image is transmitted out of the vacuum system along a fiber-optic bundle which has a photodiode array (Reticon) attached to the end face. The light intensity is recorded via the self scanning Reticon. A more detailed description of the detector may be found in reference 2.

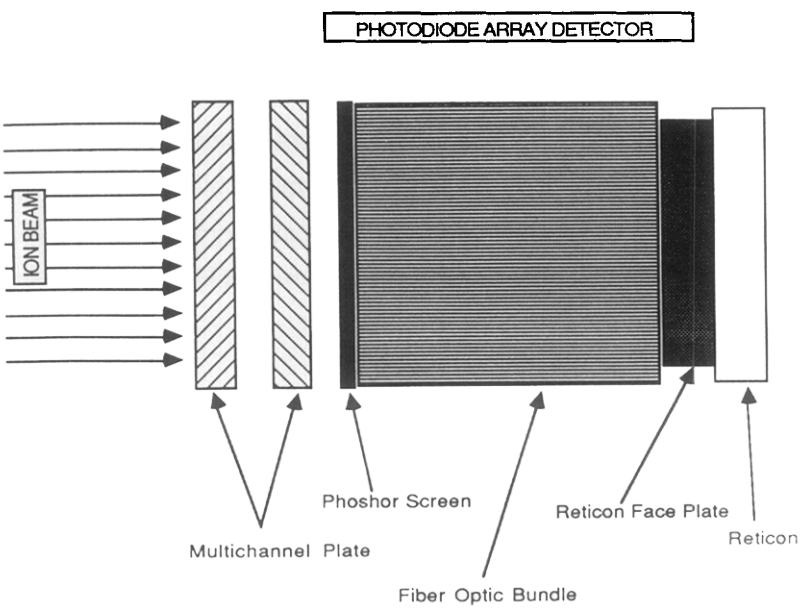


Figure 1.Schematic diagram of the ion detector.

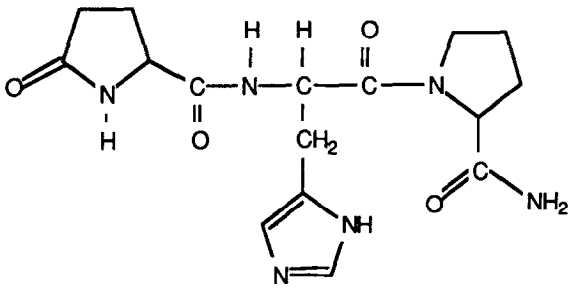


Figure 2.Structure of thyrotropin releasing hormone (TRH).

The mass spectrometer was a Kratos MS50RF double focusing instrument with a mass range of 10000 daltons at 8 kV. The ion source was a standard Kratos FAB source fitted with a model B11NF saddle field fast atom gun (Ion Tech Ltd., Teddington, UK). Data collection and processing were performed by a model TN-1710 multichannel analyzer (Tracor Northern, Middleton, WI), in conjunction with a Kratos DS 90 data system. Under normal operating conditions the values indicated on the fast atom gun control unit were 40 μ A beam current at an energy of 8kV. For these experiments, the unit was operated at the minimum levels at which a stable discharge could be maintained, typically 8 μ A at 4kV using xenon.

Samples of the blocked tripeptide pyroGluHisProNH$_2$, (thyrotropin releasing hormone, TRH), Fig 2 were analyzed by FAB using a variety of instruments as summarized in Table 1.

The relatively poor sensitivity of this compound to FAB analysis may be related to it being a moderate to weak base as determined by its pKa values. The most basic site is at the NH on the imidazole ring which has a value of approximately 7.

The FAB experiments were conducted using the same conditions with glycerol as a matrix. The detection limit for this peptide was determined by the ability to identify the sample peaks from the background spectrum of the matrix material and this problem becomes increasingly more serious at low mass. The MS/MS experiment was

Table 1

Ionization mode	Instrument	Minimum Detectable Quantity
FAB	MAT 731	1.0×10^{-6} g
FAB	MS 50	1.0×10^{-6} g
FAB	VG 70-70S	1.0×10^{-6} g
FAB MS/MS	VG 70-70SQ	1.0×10^{-7} g

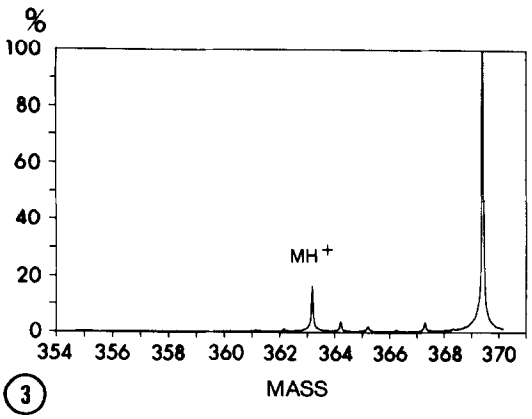
performed by monitoring the daughter ion spectrum of the MH^+ ion at m/z 363 and at the 100ng level it was possible to distinguish several significant daughter ions in the spectrum of the 363 peak from the daughter ion spectrum of the glycerol background peak. The recent development of the continuous flow sample probe for FAB [3] has demonstrated that signal-to-chemical noise can be significantly improved and provides one approach to the problem of increasing sensitivity. An alternative strategy is not to use a matrix material but instead coat the sample on the target surface from solution and evaporate to dryness.

Weighed amounts of dry TRH sample were taken up in methanol and diluted with water. Solvent blanks were prepared in parallel using identical apparatus and reagents. Measured aliquots of sample solution were deposited onto a gold plated probe tip and evaporated to dryness under a rough vacuum. The standard procedure for cleaning the probe tip using nitric acid to etch the surface followed by rinsing with Analar water proved to be inadequate for dry FAB experiments of quantities which give approximately monolayer surface coverage. The signal from the peptide was masked by intense background signals which presumably originate from residual material left by the cleaning procedure. In order to eliminate this problem the etched probe tip was rinsed in water, dried and exposed to the FAB beam at full intensity until the initial burst of secondary ion emission had fallen to a stable level, (typically 10 to 30 seconds of irradiation).

For the analysis of dry samples in SIMS, the primary ion beam flux is approximately a factor of 10^3 less than that used in the FAB source. The problem with not using a matrix material is the transient nature of the secondary ion yield , however this is less important when combined with some form of parallel ion detection.

Results and Discussion

The calibration of the mass scale was achieved using CsI as a reference compound and for data acquisition the magnet was set to transmit a mass of approximately 360 daltons. The integrated spectrum at the molecular ion region of TRH , Figure 3, for 1 μg of sample in a glycerol/thioglycerol (50:50) matrix shows the protonated molecular ion at mass 363 together with the protonated glycerol trimer at m/z 369. In the dry FAB experiments a very strong molecular ion was obtained for 1 μg of sample. At lower sample quantities it was possible to obtain spectra at the 10 n g and 1 n g levels as can be seen in Figures 4 and 5a. Clearly in the 10 n g experiment a distinct MH^+ peak is present together with the isotope peak at m/z 364. The spectrum for 1 n g of sample shows the protonated molecular ion at 363 as well as background



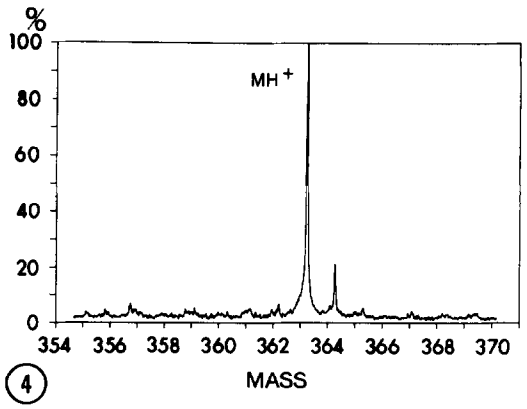
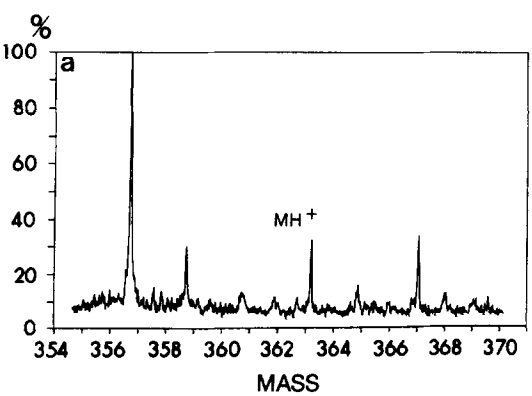


Figure 3. 1µg TRH in liquid matrix.

Figure 4. 10ng TRH (dry).

ions at m/z 357 and 367. These ions can be seen in the solvent blank spectra in Figure 5a also in the 10 n g case but with a very much reduced intensity. All these spectra were acquired with an integration time of 8 seconds. The FWHM resolution is seen to be approximately 5000 and is limited by the spatial resolution of the component parts of the detector. The measured mass of the MH^+ for TRH was 363.180 compared with the calculated mass of 363.178.

The non-linear signal response below the 10 n g level may well be related to the surface coverage of the TRH on the target area. Computer modeling of the peptide for minimum energy conformation produces the geometrical dimensions of approximately 10 by 7 angstroms from which an estimate can be made for the number of molcules required to give monolayer coverage of the target surface. Interestingly 10 n g of TRH corresponds to about one monolayer coverage assuming uniform surface distribution. Addtional experiments using a greatly reduced target area will confirm this is the



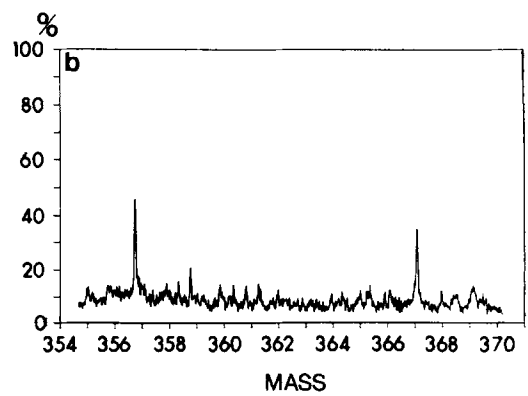


Figure 5a. 1ng TRH (dry).

Figure 5b. Solvent blank spectrum for 1ng experiment.

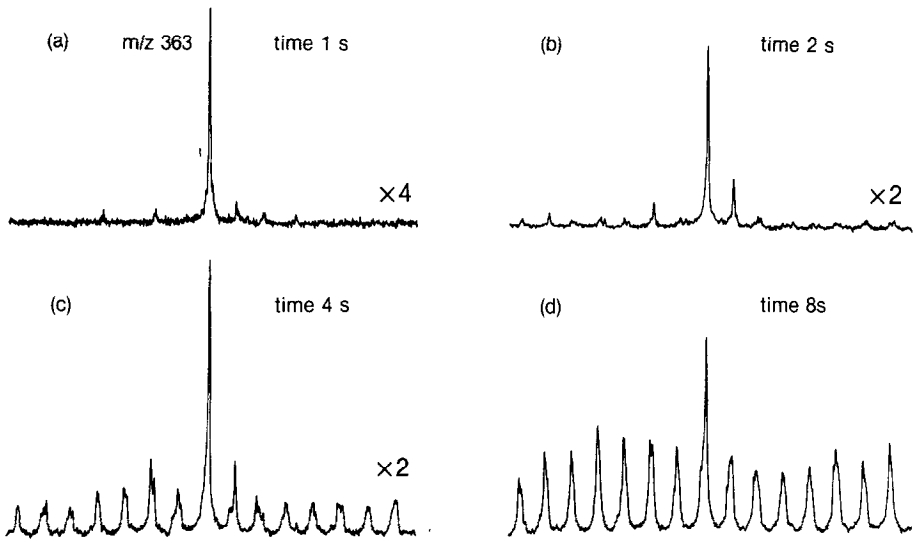


Figure 6. Variation of spectrum with time.

reason for the non-linear signal response. It is quite possible that the non-linear response from sub-monolayer loadings of sample is due to the preferential formation of $[M+Au]^+$ adduct ions rather than the protonated species as described by Colton[4], and further work will investigate this possibility.

These spectra were all obtained with a source slit width of 0.0127 mm in order to attenuate the secondary ion flux and obviously a much lower primary ion beam flux in the nano ampere range will reduce the secondary ion yield and therefore allow the source slit width to be increased. It should be noted that no smoothing of the raw data peaks was used and the only data processing was the subtraction of fixed pattern Reticon noise.

When the amount of sample loaded onto the probe tip corresponded to a few monolayers, the sample signal was observed to decrease rapidly during the first few seconds of exposure. After 10 seconds the spectrum became indistinguishable from that of the clean probe tip, however if much larger quantities of peptide were loaded, the fall off in sample signal was followed by the emergence of an intense uniform background spectrum which is presumed to originate from radiation damaged sample molecules. The appearance of the dry sample spectrum can be clearly seen to change within the first 8 seconds of irradiation as can be seen in Fig. 6 for a sample loading of 1 μ g. The spectrum after 1 second, Figure 6a, shows only the TRH peaks at m/z 363 and 364, however after two seconds the characteristic background spectrum with a peak at every nominal mass has begun to appear, Figure 6b, and proceeds to increase

with time, such that after 8 seconds the background peaks are approximately 50% the intensity of the MH^+ peak, Figure 6d. On closer examination, the background peaks can be seen to be unresolved triplets.

Further experiments will evaluate this detector in conjunction with the continuous flow FAB probe.

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