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OPTIMISATION OF CONTINUOUS FLOW FAST ATOM BOMBARDMENT MASS SPECTROMETRY FOR BIOANALYSIS

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SUMMARY

Fast atom bombardment mass spectrometry (FAB-MS) has been employed very successfully over the last seven years for the analysis of polar and thermally-labile compounds which are frequently encountered in bioanalysis. The recently developed method of continuous flow FAB-MS (CF-FAB) as a liquid chromatography–MS interface and ionization method has opened up a new area of research in the analysis of biomolecules such as peptides and proteins. Ion suppression effects observed in standard FAB analysis seem to be reduced, and increased signal-to-noise gives better sensitivity for quantification. The optimisation of CF-FAB on the MAT90 magnetic sector mass spectrometer will be discussed. Applications in bioanalysis, *e.g.*, peptide analysis in conjunction with linked scan techniques, will also be reported.

INTRODUCTION

Fast atom bombardment mass spectrometry (FAB-MS) has been employed very successfully over the last seven years for the analysis of polar and thermally labile compounds[1] which are frequently encountered in bioanalysis. The recently developed method of continuous flow FAB-MS[2] (CF-FAB) offers a number of advantages over conventional FAB-MS, especially in the analysis of peptides and protein digests[3,4]. These are: more rapid analysis, the ability to monitor processes such as enzyme digestions and increased signal-to-noise ratio giving better sensitivity for quantification[5].

Potential applications for CF-FAB at Sittingbourne Research Centre (SRC) include directly coupled liquid chromatograpy (LC)–CF-FAB-MS of polar metabolites, antibiotics from fermentation broths, biosurfactant and peptide analysis. The analysis of peptide and protein digests by CF-FAB will allow enzyme digestions to be followed in real time with prior on-line reversed-phase high-performance liquid chromatographic (HPLC) separation of components[6].

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The CF-FAB probe was installed on the Finnigan MAT90 magnetic sector spectrometer in August, 1988. The initial aims were to optimise the performance of the CF-FAB method as follows. (i) To make the technique viable for peptide analysis with picomole sensitivity. (ii) To examine the feasibility of obtaining sequence information from peptides run by CF-FAB using linked scanning techniques on the MAT90 instrument.

The CF-FAB probe for the MAT90 instrument at SRC was the first delivered by Finnigan in Europe, with the exception of two prototype probes under development in collaboration with Finnigan MAT. The MAT90 is an ideal instrument on which to install the device because it is fully microprocessor controlled, facilitating easy programming of a sequence of scanning modes *i.e.*, normal spectrum acquisition followed by B/E linked scans [keeping the ratio of magnet to electric sector (B/E) constant] of the parent ions.

A number of features were critical in optimising the conditions for a stable flow and good sensitivity in the CF-FAB technique. These were (i) wettability of the probe tip; (ii) temperature of the probe tip; (iii) flow-rate of the solvent; (iv) solvent composition.

These important factors and their interrelations were examined using model peptides, and are discussed in this report.

EXPERIMENTAL

Design and operation of the CF-FAB probe

The probe, shown in Fig. 1, consists of a hollow shaft that is capped with an angled probe tip, through which a 0.7 m × 0.075 mm I.D. fused-silica capillary is passed. This is allowed to protrude by approximately 0.2 mm beyond the tip. The tip

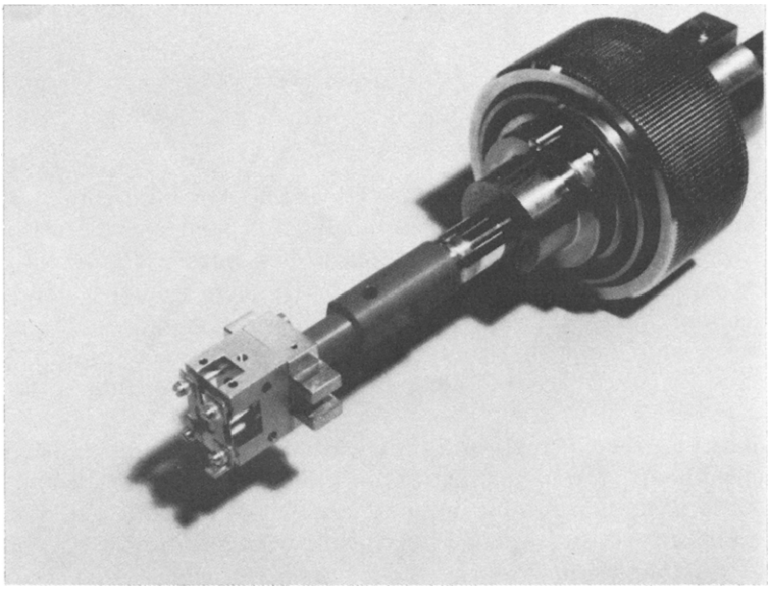

Fig. 1. Continuous flow FAB probe for the Finnigan MAT90 spectrometer.

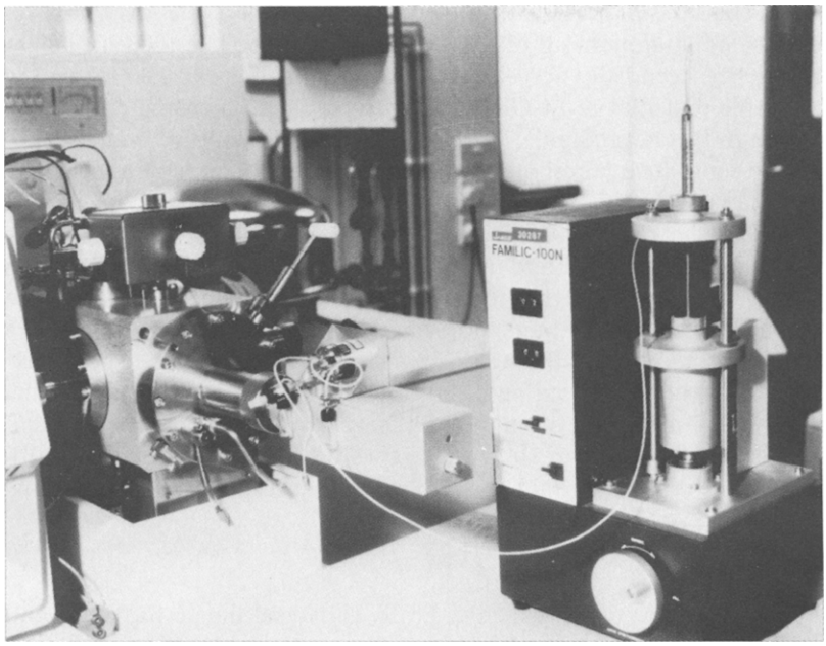

Fig. 2. CF-FAB probe in position on the MAT90 instrument.

is in contact with a paper wick, which is located inside the source ion volume directly below the probe tip. The capillary is connected to a Rheodyne injection valve (Model 7410) for injection of 0.5-μl amounts. The continuous flow solvent is provided by a suitable pump (a Jasco Familic–100N) normally operated at 2–5 μl/min as shown in Fig. 2.

The FAB gun is operated at 8 keV and 1 mA current with Xenon bombarding gas, as with conventional FAB.

The two novel design features of the Finnigan MAT CF-FAB probe are (i) a paper wick below the probe tip to soak up the excess solvent running down the probe; (ii) an adjustment screw at the base of the probe to alter the position of the capillary tip in-situ (Fig. 2).

Reagents

Bradykinin, bradykinin potentiator B and renin substrate tetradecapeptide were obtained from Sigma. The samples were dissolved in the same solvent mixture as used for the continuous flow carrier solvent *i.e.*, typically glycerol–water–methanol–trifluoracetic acid (10:45:45:0.2, v/v).

RESULTS

Stability

Stable operation of the probe can be defined as the condition where a constant ion current is obtained from ions sputtering from the tip under steady solvent flow conditions, *i.e.*, 2–5 μl/min. This is achieved when the rate of evaporation of the

solvent from the probe tip is in balance with the pumping speed of the MS source. If this is not the case, boiling/bubbling of solvent occurs, giving rise to peak height fluctuations. It has been generally agreed[7] that fluctuations in peak heights within ± 10% are acceptable, but that most CF-FAB systems take approximately half an hour to settle down to this level. Stable operation was very difficult to obtain on the MAT90 system and reproducible response for repeat 50-ng injections of undecanoic acid was poor as shown in Fig. 3. The upper trace represents the selected mass trace for the $[M - H]^-$ ion, showing variation in peak height of ± 50%. The lower trace represents the total ion current trace and indicates the instability. The mass spectrum obtained, however, shows good signal-to-noise ratio. Repeat injections could be made every 2–3 min with no significant memory effects with dilute solutions, although with sample quantities in excess of 100 ng slight memory effects become apparent.

The Rheodyne injector supplied had a fixed internal loop of nominal volume 0.5 μl. Use of 1-, 2- and 5-μl sample volumes to fill this internal loop gave inconsistent results. Only with sample volumes > 10 μl were reproducible ion current traces obtained. It was concluded, therefore, that dead volumes within the valve precluded its use with < 10 μl sample volumes.

Modifications

Two modifications were made, which radically enhanced the performance and stability of the CF-FAB probe. (i) The Rheodyne valve supplied by Finnigan MAT was replaced by a Model 7125 with a 10-μl external loop. A better model, the 8125 with lower dead volumes, is to be ordered. (ii) The length of capillary within the probe was increased to 1.1 m of 0.05 mm I.D. This may have acted as a flow restrictor applying a back pressure to the pump and greatly increased stability.

The effect of the above allowed stability to ± 10% for full day operation.

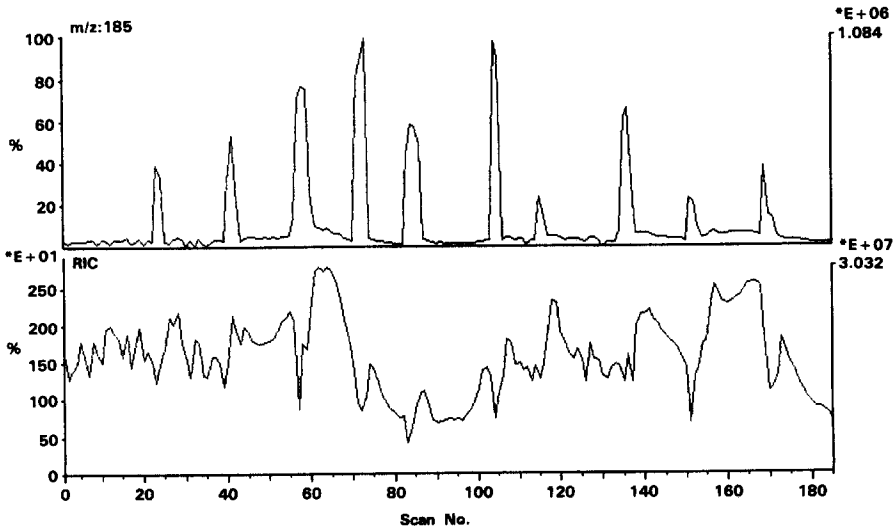

Fig. 3. Repeat injection of 50 ng of undecanoic acid.

Wettability of the probe tip

Even coating of solvent from the capillary onto the probe tip was critical. Acid etching of the surface was found most effective. Wettability varied with solvent composition, methanol–water mixtures being better than water or water–acetonitrile.

Role of the wick

The wick, situated below and in contact with the probe tip, consists of a wad of compressed filter paper approximately 6 mm diameter and 3 mm depth. The role of this wick is to ensure an even thin film of solvent across the probe reducing the formation of droplets thus stabilising the ion current produced. In addition, evaporation takes place from the flow of solvent rather than on the end of the capillary, therefore reducing sample memory effects. A deterioration in stability was observed when the wick was omitted. The wick was replaced when saturated with solvent. In practice this depended upon solvent composition and flow-rate and more critically upon the glycerol content. Using 10% glycerol in the solvent the wick was replaced daily.

Probe tip temperature

The probe tip is heated indirectly from the source. Its temperature was not easy to control. A temperature of approximately 40°C at the tip was required to avoid freezing of the solvent within the capillary tip. The design of this could be improved to allow direct heating and faster response to changes. Lower probe temperatures resulted in decreased ion currents and reduced stability. Reoptimisation was necessary with changes in solvent composition, however, adjustment of capillary position had a greater effect than change in probe temperature.

Solvent flow-rates

Flow-rates of 1–7 μl/min were evaluated. 5 μl/min and above led to sputtering of the liquid surface formed on the tip, giving rise to unsteady ion currents and very high source pressures $> 10^{-3}$ Torr. Low flow-rates led to a diffusion of sample and broad peak shape. 3–4 μl/min was found optimum for most solvent systems.

Solvent composition

Initial solvents of water, water–methanol, acetonitrile–water were made up with 20% glycerol. It was desirable to reduce the glycerol content to make the solvent more suitable for HPLC columns. A reduction to 10% glycerol was tolerated with no reduction in sensitivity. Optimising the capillary position for different solvent compositions was essential to retain a wet film of solvent on the probe tip at the position where the atom beam impinges. This could be easily carried out on this probe design.

Model peptides

A range of peptides *i.e.*, bradykinin, bradykinin potentiator B, renin substrate in the 1000–2000 dalton molecular weight range could be run by CF-FAB at the 1–2-pmol level. Fig. 4 shows the $[M + H]^+$ of bradykinin for 2 pmol injected. For comparable spectra from the conventional FAB probe nanomol of sample are required. Mixtures of three peptides all at the 40-pmol level gave very similar responses, unlike conventional FAB where suppression effects are known to occur.

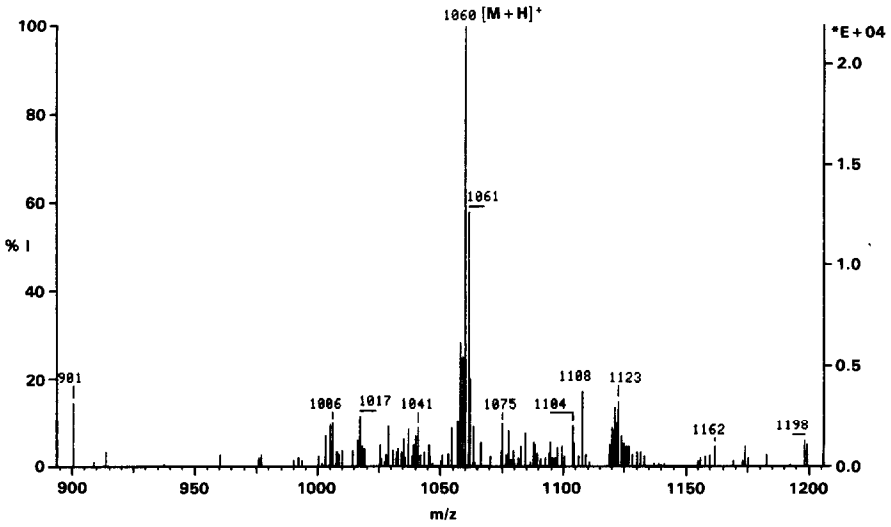

Fig. 4. CF-FAB spectrum of bradykinin (2 pmol).

Linked scans

Collision-induced fragmentation could be obtained on the peptide molecular ions, by the introduction of helium into the first field free region gas cell (between the source and the magnetic sector). Daughter or fragment ions formed could be analysed by a B/E constant scan from the parent ion. This was done from the conventional FAB probe (Fig. 5) and from the CF-FAB probe (Fig. 6). 100 pmol of sample was utilised for the CF-FAB, whereas approximately 10 nmol was required for the conventional FAB analysis to give comparable signal-to-noise ratios. Comparing the B/E spectra of bradykinin potentiator B run under conventional FAB conditions (Fig. 5)

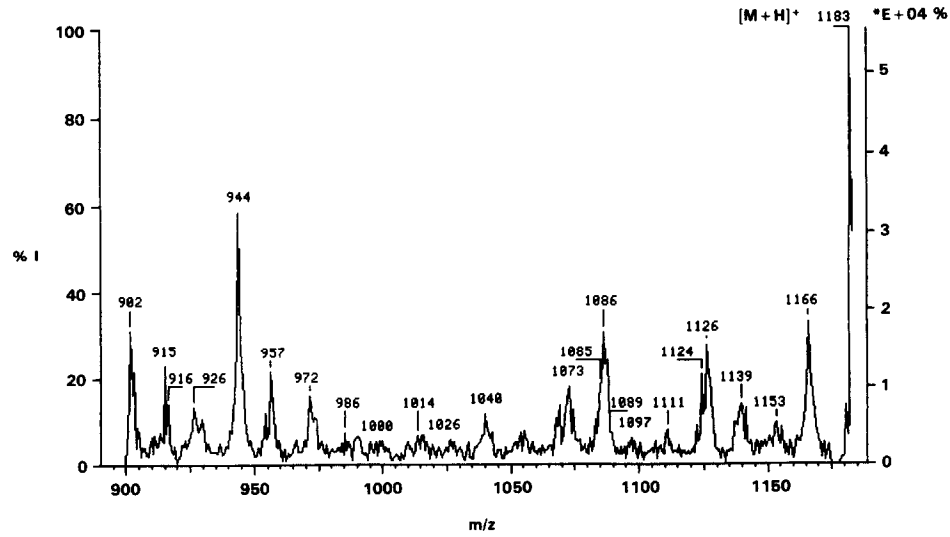

Fig. 5. B/E linked scan of bradykinin potentiator B from conventional FAB probe (10 nmol).

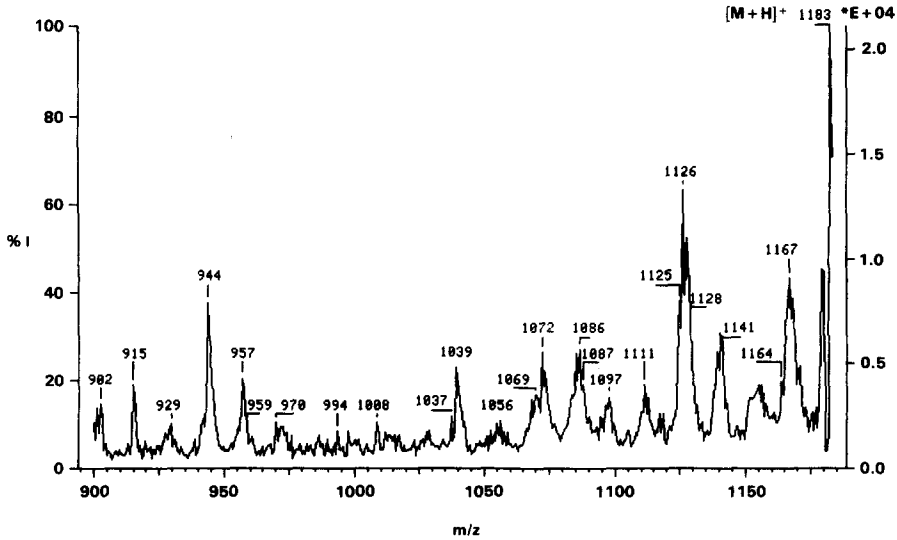

Fig. 6. B/E linked scan of bradykinin potentiator B from CF-FAB probe (100 pmol).

and from the CF-FAB (Fig. 6) the same daughter ions are present, however, intensities do vary. Significant fragment ions could be rationalised by amino acid cleavages from N- or C-termini, and according to fragmentation behaviour of peptides in collision dissociation mode[8–10]. Some assumptions have been made about amino acid side chain losses. Table I lists the peaks observed and assignment has been made with the Roepstorff and Fohlman nomenclature[11].

TABLE I

FAB B/E LINKED SCAN ANALYSIS OF BRADYKININ POTENTIATOR B (900 > 1183 μ)

pGlu-Gly-Leu-Pro-Pro-Arg-Pro-Lys-Ile-Pro-Pro.

m/z	*Assignment*[a]
1183	$[M + H]^+$ or protonated molecular ion
1166	B11
1139	MH − Arg side chain
1138	A11
1126	MH − Leu side chain
1086	C10
1073	Y10
1069	B10
1040	A10
972	B9
957	MH − Leu, Arg, Lys side chain
944	A9
902	Y8

[a] Using Roepstorff and Fohlman nomenclature.

DISCUSSION

These initial results indicate that CF-FAB can be used to analyse polar and thermally labile compounds encountered in bioanalysis and the following preliminary conclusions can be drawn. (i) It can be 100 times more sensitive than conventional FAB; (ii) may be used with a range of solvents compatible with HPLC systems; (iii) provides a rapid method for analysis of samples injected via a loop into the solvent flow; (iv) is compatible with linked scanning techniques.

Further work is continuing with on-line coupling to HPLC, linked scanning techniques and optimisation of performance for a wider range of compounds.

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