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CONTINUOUS-FLOW FAST ATOM BOMBARDMENT MASS SPECTROMETRY

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SUMMARY

The continuous-flow fast atom bombardment probe performs equally well with or without a high-performance liquid chromatography column producing clean spectra containing little or no background noise. Its function as a liquid chromatography–mass spectrometry interface for labile and involatile samples has been illustrated with reference to dansylated amino acids. The versatility of the new probe has been exemplified by on-line enzymatic peptide sequencing.

INTRODUCTION

The advent of fast atom bombardment (FAB)[1] has made mass spectrometry (MS) accessible to many classes of compounds that are too labile or involatile to be amenable to more conventional ionization techniques. Samples are dissolved in a liquid matrix with a suitably low vapour pressure *e.g.* glycerol, and a few microlitres of this solution placed on the tip of a direct insertion probe which is then introduced into the ion source. Bombardment of this sample by a beam of energetic xenon atoms causes the sputtering of products which include abundant quasi-molecular ions.

FAB is a simple and effective method and is widely applicable, for example to biopolymers, but does have some shortcomings. First, the significant matrix levels give rise to a relatively high chemical noise in the spectra. Second, conventional FAB is not well suited to analyses, where rapid changes in analytes are expected *e.g.* liquid chromatography (LC)–MS or the direct analysis of enzyme reaction mixtures.

Several approaches have been made to overcome these shortcomings by continually introducing liquid samples into the FAB ionization source of a mass spectrometer. Ito *et al.*[2] have reported a capillary inlet device terminated by a mesh frit for the direct connection of a microbore high-performance liquid chromatography (HPLC) column to a FAB source; the purpose of the frit being to disperse the mobile phase and concentrate the sample and matrix. More recently, a continuous-flow FAB probe which allows a continuous flow of solution to be introduced directly into a FAB source has been described[3]. This paper presents data obtained with the continuous-flow FAB probe, illustrating its use as an LC–MS interface, and as a device for continually monitoring reaction mixtures.

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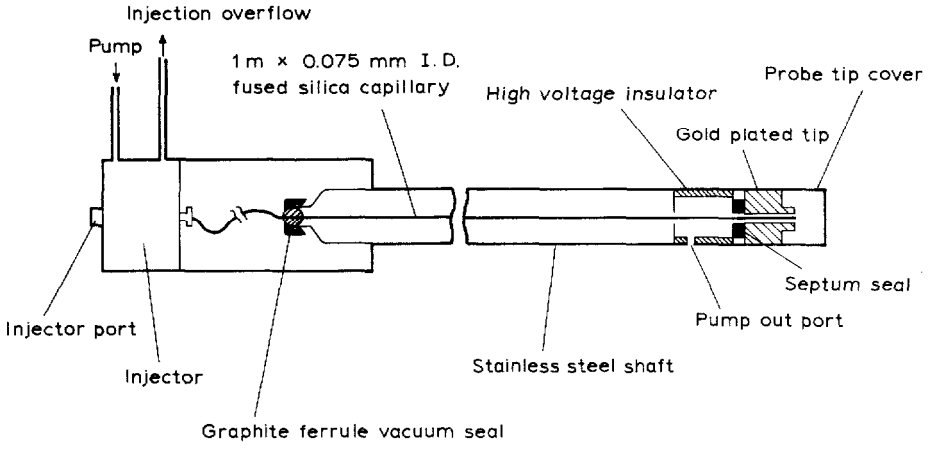

Fig. 1. Schematic diagram of the continuous-flow FAB probe.

EXPERIMENTAL

A commercially available (Kratos Analytical) continuous-flow FAB probe[3] (Fig. 1) comprising a hollow shaft which ends in an angled tip through which a 0.3-mm hole is drilled was used throughout these studies. This allows a flow of 2–10 μl/min to be introduced into the FAB source. A length of 0.075 mm I.D. fused-silica capillary was passed through the shaft to end flush with the angled tip. This capillary may be connected in one of several ways to the liquid supply: (a) via a standard LC injection port for the direct injection of successive aliquots, (b) as a sampling capillary which is introduced directly into a reaction mixture, (c) as one arm of a splitting tee attached to the exit of a higher flow-rate LC column, (d) as a short extension attached to the exit of a slurry packed microbore LC column which operates at flow-rates compatible with the pumping efficiency of the mass spectrometer.

An MS50FSTC (Kratos Analytical) mass spectrometer was used in conjunction with the continuous-flow FAB probe. The instrument was not specially modified except that the FAB source was fitted with a heater and temperature sensor, and generally maintained at 40–60°C. Gentle heat is necessary in order to maintain a constant evaporation rate of the aqueous or aqueous–organic solvents continually flowing into the source. Samples were introduced in either water–glycerol (95:5) containing 0.1% trifluoroacetic acid (solvent A), or water–acetonitrile–glycerol (17:78:5) (solvent B) at a flow-rate of 4 μl/min. Constant flow-rates were achieved with a Brownlee MPLC Micropump.

The mass spectrometer was operated at either 4 or 8 kV accelerating voltage, and scanned at 3 s per mass decade from 2000 to 100 u under data system (DS90) control.

The mass spectrometer was equipped with a FAB gun (Ion Tech) which was operated at 8 kV with xenon gas.

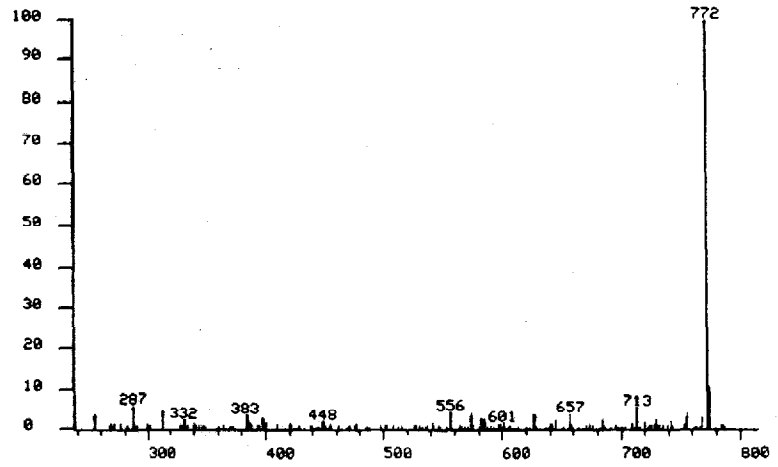

Fig. 2. Continuous-flow FAB spectrum of the heptapeptide kemptide (mol. wt. 771) showing a protonated molecular ion at *m/z* 772.

RESULTS

A wide range of peptides has been successfully analysed using the continuous-flow FAB technique. The heptapeptide kemptide (Leu-Arg-Arg-Ala-Ser-Leu-Gly; mol. wt. 771) (Fig. 2) introduced in solvent A illustrates the low level of background noise achievable with this method, due to the much reduced level of matrix

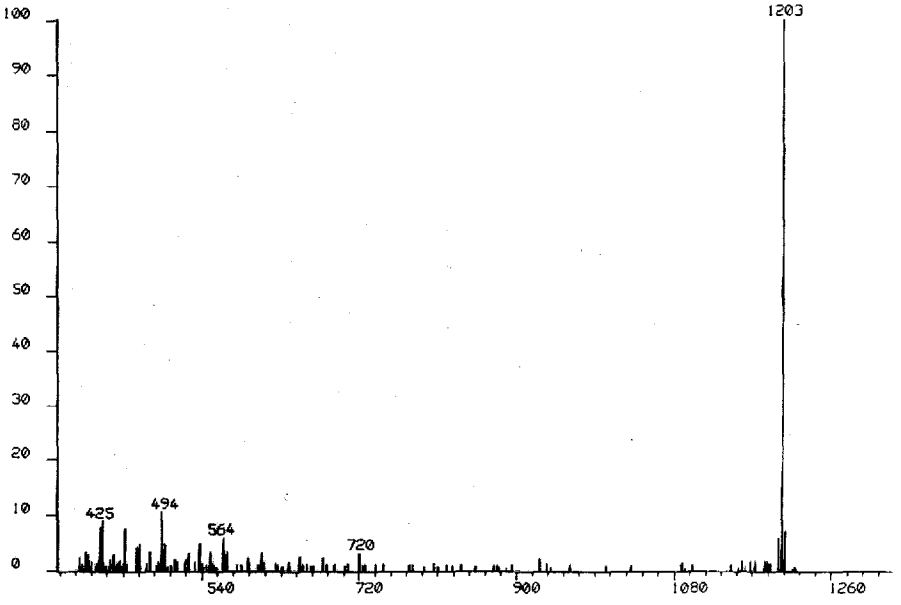

Fig. 3. Continuous-flow FAB spectrum of the cyclic peptide cyclosporin A (mol. wt. 1202) showing a protonated molecular ion at *m/z* 1203.

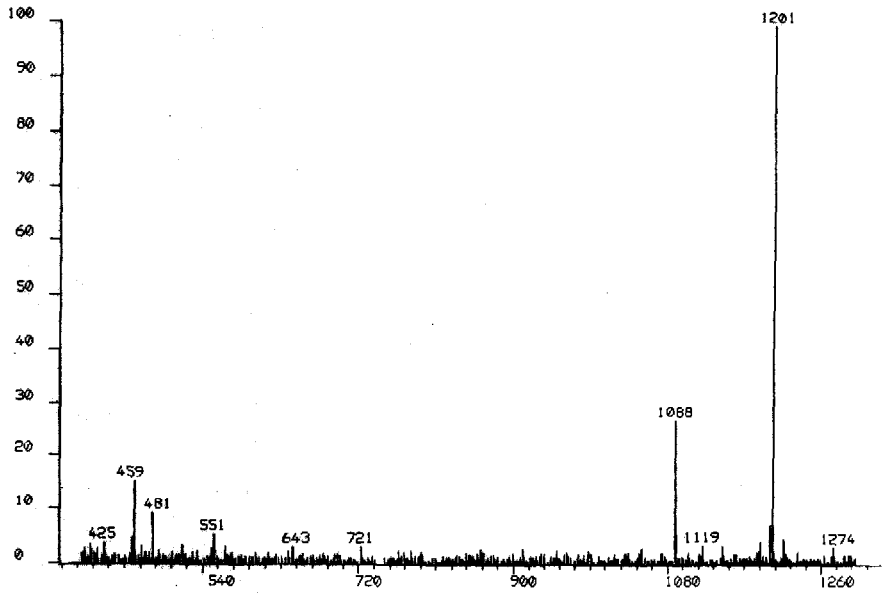

Fig. 4. Continuous-flow FAB spectrum of the cyclic peptide cyclosporin A (mol. wt. 1202) obtained with negative ion operation showing $(M-H)^-$ at m/z 1201.

required compared with conventional FAB. A protonated molecular ion at m/z 772 confirms the molecular weight.

The continuous flow FAB mass spectrum of the cyclic peptide cyclosporin A (mol. wt. 1202) shows a protonated molecular ion at m/z 1203 (Fig. 3). In comparison, the same sample was analysed in the negative ion mode of operation (Fig. 4), producing a strong ion at m/z 1201 corresponding to $(M-H)^-$. Thus continuous flow FAB can be used to produce molecular weight information in either positive or negative ion operation.

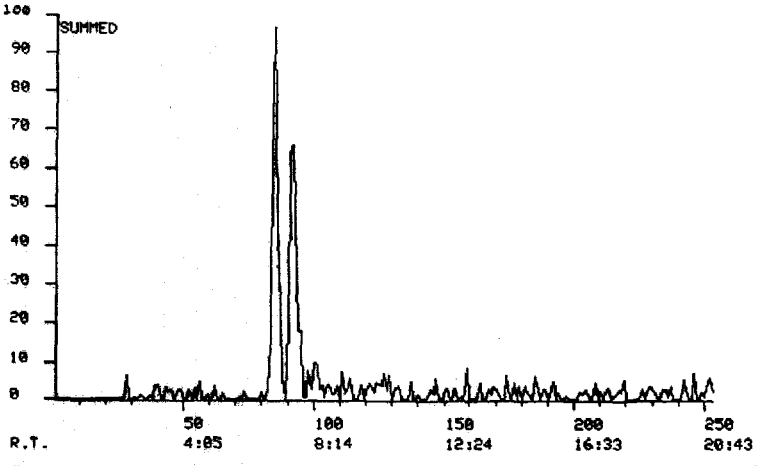

Fig. 5. Continuous-flow FAB-LC–MS chromatogram showing the separation of dansylated alanine and dansylated phenylalanine using an ODS LC column (1 mm × 15 cm I.D.) with solvent B.

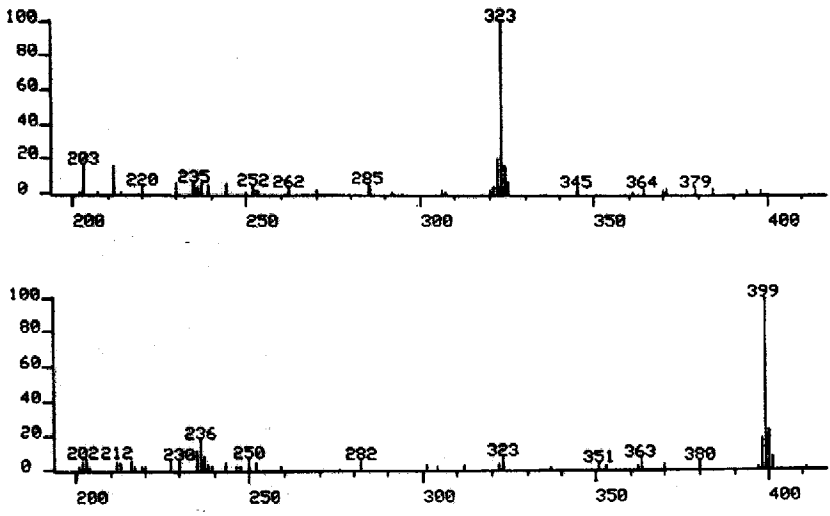

Fig. 6. Continuous-flow FAB-LC–MS spectra of separated dansylated alanine (mol. wt. 322) and dansylated phenylalanine (mol. wt. 398) showing protonated molecular ions at *m/z* 323 and 399, respectively.

The sharp response obtained from samples injected in relatively small volumes by Caprioli *et al.*[3] indicated that the technique may be suitable for LC–MS. A standard ODS LC column (15 cm × 1 mm I.D.) was used to test the probe's performance as an LC–MS interface. A flow-rate of 0.5 ml/min of solvent B through the column was maintained, and the flow split after the column by use of a tee piece containing two fused-silica capillary outlets. The split ratio was set to 99:1 by adjustment of the relative lengths of the silica capillaries, with 5 μl flowing directly through the continuous-flow probe into the FAB source. For this analysis, it was found to be convenient to add glycerol directly to the eluent as it had little effect on the elution strength of this system. Alternatively post-column addition of the matrix could have been carried out. A small percentage of matrix in the liquid phase is required to ensure steady evaporation of the solvent in the ion source.

A mixture of dansylated alanine (mol. wt. 322) and dansylated phenylalanine (mol. wt. 398) was separated using this system, and the FAB-LC–MS chromatogram is shown in Fig. 5. Spectra of the two components are presented in Fig. 6, with both exhibiting protonated molecular ions. Chromatographic resolution has been maintained by comparison with HPLC–UV detection, using the same sample, column and eluent.

Another important application of the continuous-flow FAB probe is that of constant reaction monitoring in areas such as the enzymatic degradation of peptides. In such cases, the composition of the reaction mixture may change over a relatively short period of time, and information can be lost if the reaction is only monitored periodically. By dipping the end of the fused-silica capillary from the continuous-flow FAB probe into a reaction mixture, a flow of solution into the FAB source is achieved. A 1-m length of silica will typically provide a flow-rate of 4–5 μl/min.

The carboxypeptidase Y degradation of the peptide substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met; mol. wt. 1347) is one example of this. For this

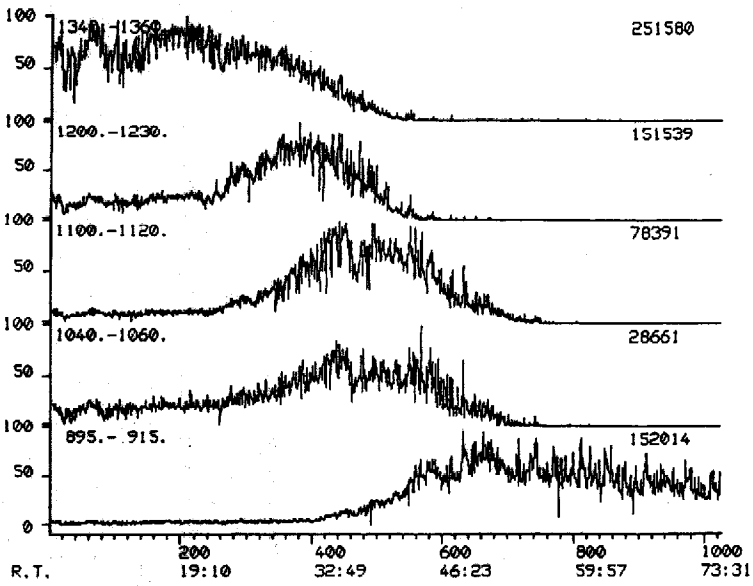

Fig. 7. Enzymatic degradation of substance P showing the decline of the protonated molecular ion (*m/z* 1348) and the appearance and disappearance of ions at *m/z* 1218, 1105, 1048 and 901, corresponding to loss of methionine, leucine, glycine and phenylalanine, respectively.

experiment, a sample vial containing the peptide and enzyme in Tris 7 buffer was maintained at 25°C. The fused-silica capillary from the continuous-flow FAB probe was coupled with a tee piece to two identical lengths of fused-silica capillary (15 cm each). One length was placed in the enzymatic reaction mixture, and the other in a vessel containing solvent A. Equal volumes from each vessel flowed into the probe. A series of mass chromatograms is shown (Fig. 7), illustrating the decline of the protonated molecular ion (*m/z* 1348) and the appearance and subsequent disappearance of ions occuring at *m/z* 1218, 1105, 1048 and 901. These ions correspond to the first four sequential losses of amino acids from the carboxy terminus of substance P *i.e.* loss of methionine followed by leucine, glycine and phenylalanine. In such a way, peptides can be sequenced using the continuous-flow FAB probe, thus avoiding the need for isolation procedures.

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