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POTENTIALITY OF THE COUPLING OF COLUMN LIQUID CHROMATOGRAPHY AND FIELD DESORPTION MASS SPECTROMETRY

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SUMMARY

The first use of column liquid chromatography in combination with field desorption mass spectrometry is exemplified by the separation and identification of the components of a steroid mixture extracted from rat serum. There are two main reasons for the high-molecular-ion intensities obtained: (1) the smaller transferred energy in the field ionization process compared with other ionization modes increases the chance of detecting the intact molecular ions; and (2) the samples are not introduced via the commonly used direct introduction system for evaporation, but are applied to the field ion emitter from a solution using the emitter dipping technique. Hence ionization and desorption of the adsorbed molecules can be performed with minimum thermal stress.

Potential applications for the coupling of liquid chromatography and field desorption mass spectrometry are discussed, especially in relation to the handling of the sample and the limits of detection.

INTRODUCTION

Since the first application of field desorption mass spectrometry (FD-MS) to an organic compound[1], there has been a rapid development in the technique for preparing chemically and thermally resistant field ion emitters of high sensitivity[2–4]. In view of recent progress in high-pressure liquid chromatography (LC), coupling of both analytical methods might now be feasible and would constitute an ideal combination with particular relevance for the detection and identification of thermally unstable compounds. In contrast to other mass spectrometric methods, low- and high-resolution FD-MS has demonstrated the possibility of determining the molecular weight of submicrogram amounts of underivatized substances of extremely high polarity such as sugars[1], amino acids[5], peptides[6], salts[2,7], sulphonic acids[8], nucleosides, nucleotides[9] and metabolites of drugs[10] or pesticides[11–13]. The interfacing of a column liquid chromatograph with the FD mass spectrometer could be particularly useful when applied to small amounts of such polar substances to which the widely used gas chromatography–mass spectrometry coupling is not directly applicable.

EXPERIMENTAL

The LC separation was performed on a commercially available chromatograph (Siemens S200 P, supplied by Siemens, Karlsruhe, G.F.R.) with a spectrophotometric detector (Zeiss PM4 CHR, Zeiss, Oberkochen, G.F.R.). The column length was 30 cm and the I.D. 2 mm. The column packing consisted of Spherosil XOA 400 (Pechiney Saint Gobain) with a particle distribution from 4–8 μm. The non-polar part of a ternary mixture of methylene chloride, ethanol and water served as the mobile phase with a flow-rate of 1.14 ml/min (ref. 14), the sample volume always being 20 μl. A 2-μg amount of prednisolone was used as the internal standard; the plate height for it was about 0.09 mm (number of theoretical plates *ca.* 3500). The pressure drop was 80 bar. The low resolution FD-MS data were obtained with a Varian MAT CH4 mass spectrometer equipped with a micro-manipulator for optimal adjustment of the emitter[9], and 10-μm high-temperature activated tungsten wire emitters were used. The spectrum shown later in Fig. 3 was obtained by magnetic scanning and electric detection from samples concentrated to 0.001 *M* and a volume of the dissolved sample of about 2 μl. The high-resolution FD mass spectra were recorded photographically on a modified CEC21-110B instrument using vacuum-evaporated AgBr photoplates (Ionomet, Burlington, Mass., U.S.A.). About $5 \cdot 10^{-8}$ g of the solid probe had to be deposited from a concentrated solution in order to obtain the high-resolution data. The higher sample consumption is due to the lower transmission of this double-focussing machine.

RESULTS AND DISCUSSION

From the beginning of our FD experiments, it was clear that the main problem would be the adsorption of a sufficient amount of the sample on to the field ion emitter. This is particularly difficult because the concentrations yielded by LC are in the region of 0.00001 *M* and less. Investigations with FD-MS have been limited so far by the fact that a minimum amount of sample solution is needed into which the emitter has to be dipped. In our measurements, a volume of *ca.* 2 μl proved to be sufficient. A 10-μm tungsten wire emitter with a length of about 4 mm and a strong activation yielding graphitized micro-needles of about 30 μm length is now conventionally used. The maximum volume of the solution adsorbed on the emitter surface can be estimated to be $1–2 \cdot 10^{-6}$ ml. In recent experiments exploring the sensitivity of the FD method with available spectrometers (Varian CH3/CH4), reliable and reproducible spectra were obtained with different substances of various chemical and physical properties when a concentration of 0.001 *M* was maintained. In some instances, however, 0.0001 *M* solutions gave detectable, but faint, ion current intensities. From these data, it can be inferred that the handling of the sample and the emitter dipping technique require 1–2 μg of sample dissolved in 1–2 μl of solvent. Although the chemical nature of the solvent strongly influences the FD mass spectrum[2], all common solvents can be used and a pH range produced by 0.1 *N* HCl to 0.1 *N* KOH can be utilized. The decrease in the sensitivity of the emitter caused by corrosive substances can be compensated for in some instances by a short (10 min) re-activation in the activation cell[3].

For one FD measurement, the amount of sample adsorbed can be only in the

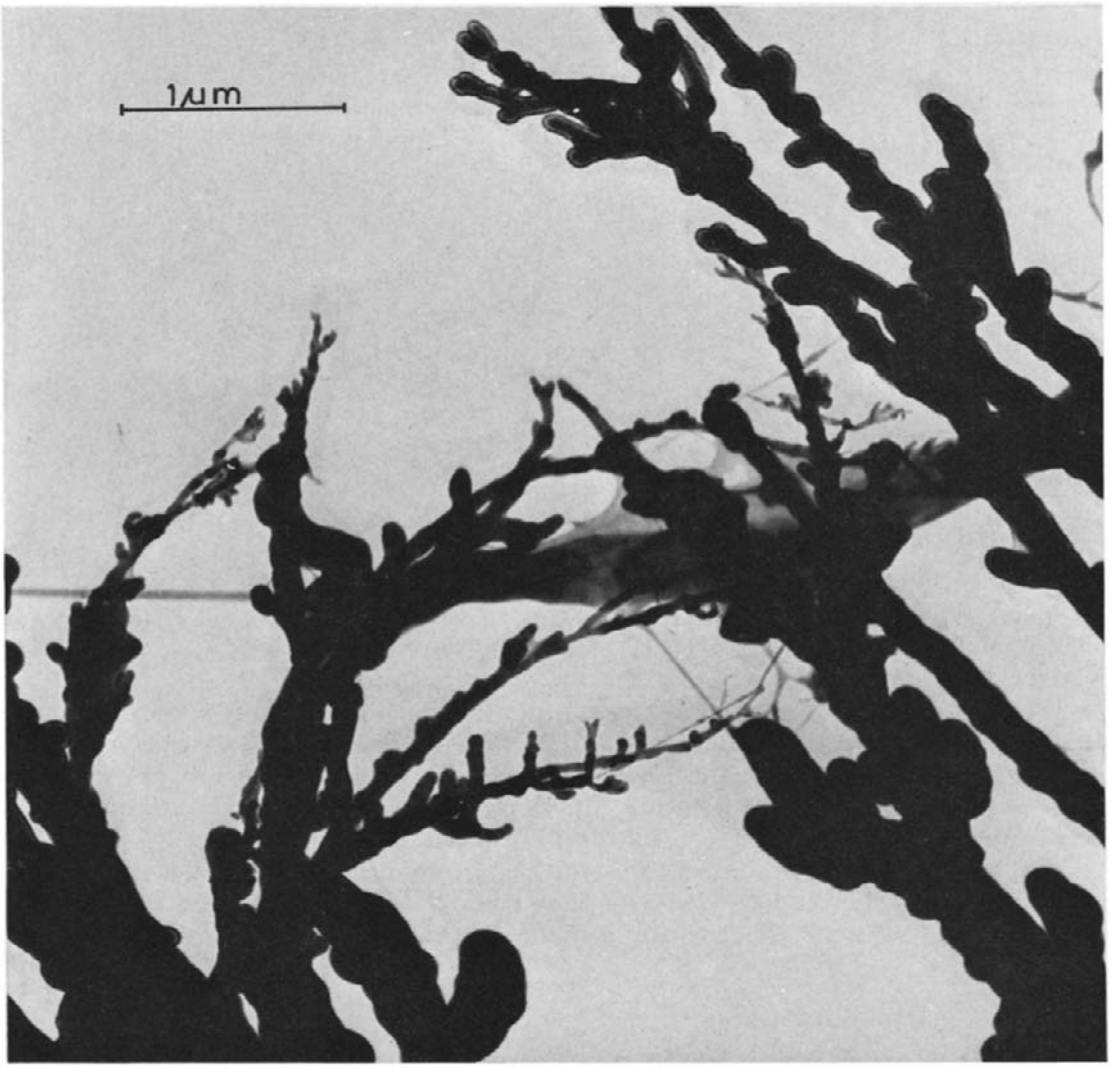

Fig. 1. Some of the micro-needles of a high-temperature activated emitter after dipping into a 0.001 *M* solution of a sample and evaporation of the solvent in the high vacuum of the ion source. (Electron micrograph by U. Nitschke, Bonn, G.F.R.).

nanogram range. Therefore, several repeated FD mass spectra can be obtained from the amount of sample mentioned above. The resulting spectra reveal the molecular ions and, when the appropriate parameters, *e.g.*, field strength and emitter temperature, are varied, interesting structural information becomes available[15]. Moreover, as virtually the whole of the sample amount remains, it can be investigated by means of other tests that might be especially relevant for the biochemical analysis of samples from biological sources.

Fig. 1 shows how a sample from a 0.001 *M* solution adheres to the surface of the micro-needles. The sample often forms a kind of "cobweb" network when the

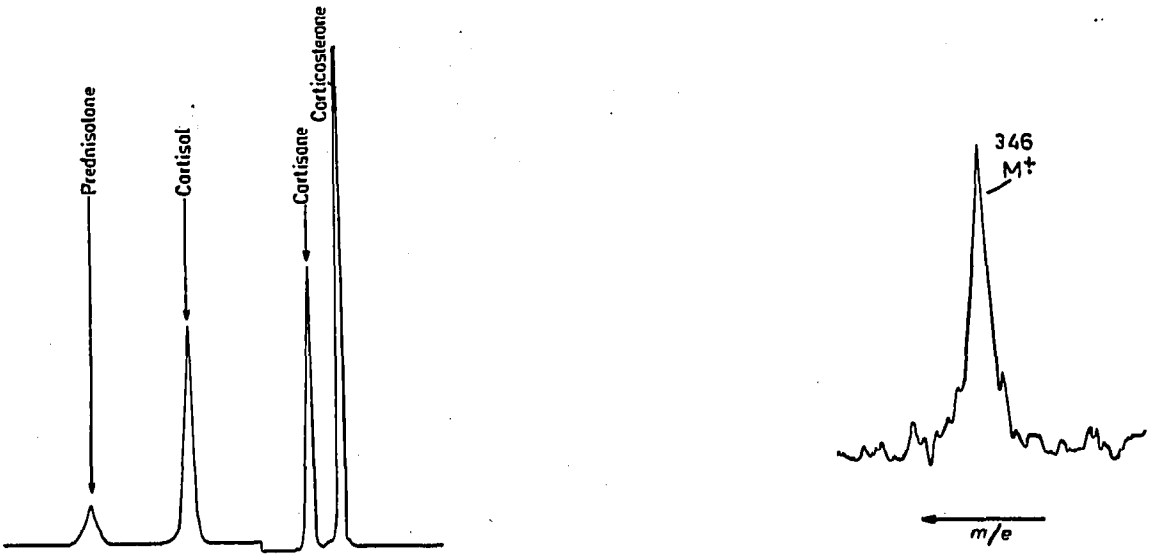

Fig. 2. Column liquid chromatogram of a steroid mixture from rat serum[16].

Fig. 3. Molecular group of corticosterone identified by low-resolution FD-MS in a collected sample (1 ml, concentration about 0.00001 *M*) from the chromatogram shown in Fig. 2.

solvent evaporates after introducing the emitter into the high vacuum of the ion source. Under the conditions of field desorption, *e.g.*, a high voltage of 10 kV applied across a gap of 2 mm between the field ion emitter and the counter electrode and the FD-emitter at the best anode temperature[5], the sample molecules diffuse to the tips of the micro-needles, where they are ionized and desorbed.

A first experiment demonstrates the results that can be obtained by the off-line coupling of LC and FD-MS. The four eluents of the chromatogram in Fig. 2 were collected, and after concentration of the 1–1.5 ml volumes down to 1–2 μl in a

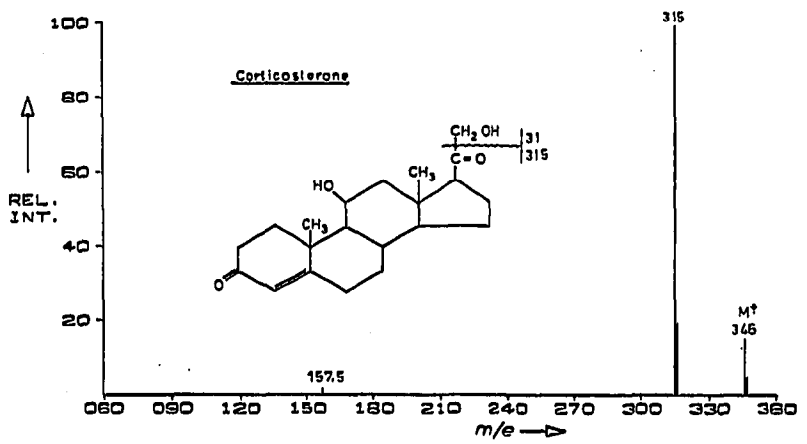

Fig. 4. High-resolution FD-MS of corticosterone. CEC21-110B mass spectrometer, photographic detection. Resolution 20,000 (10% valley definition).

vacuum desiccator, the wire emitter was submerged in the solution. The walls of the sample container were carefully cleaned twice with pure solvent. By scanning the range of the molecular ion of corticosterone at *m/e* 346, the peak shown in Fig. 3 was recorded. The peaks at *m/e* 345 and 347 are not completely resolved owing to the low resolution of the simplified instrumental arrangement.

The high-resolution FD-MS of corticosterone shown in Fig. 4 was recorded photographically with the double-focussing spectrometer and contains a readily detectable molecular ion at *m/e* 346. The base peak of the spectrum results from a loss of CH_2OH, indicating a cleavage between C_{20} and C_{21}. The residual fragment generates a doubly charged ion at *m/e* 157.5. The high-resolution FD-MS of prednisolone (Fig. 5) gives analogous information. Again, only few fragments are recorded, which were attributable to the loss of the side-chain ($C_2O_2H_3$). The FD-MS of progesterone (Fig. 6) confirms these findings. The base peak of the spectrum is the molecular ion, a general feature in field desorption, and the fragments are of lower intensities, again

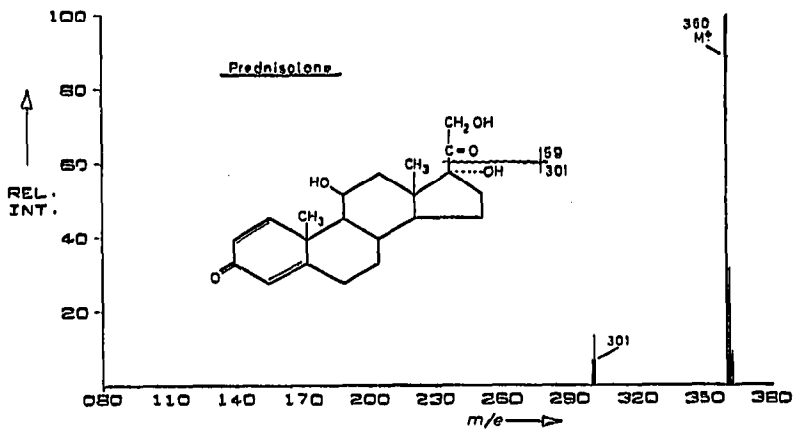

Fig. 5. High-resolution FD-MS of prednisolone.

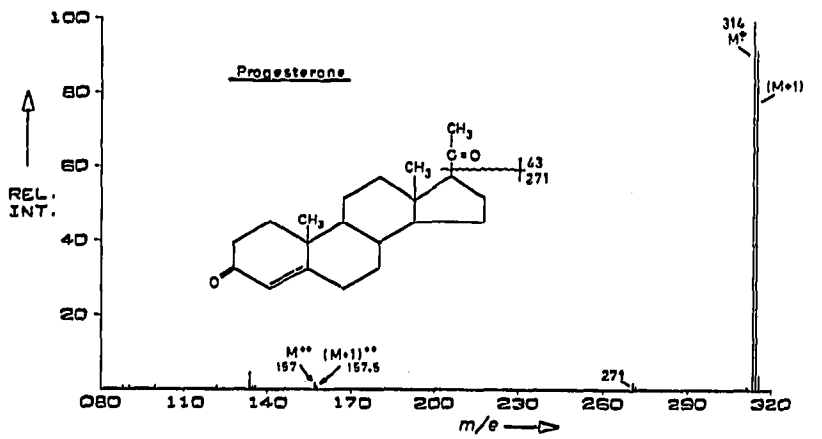

Fig. 6. High-resolution FD-MS of progesterone.

derived from the loss of the side-chain. The doubly charged molecular ion $(M)^{2+}$ and quasimolecular ion $(M+H)^{2+}$ are remarkable (see also Ref. 15).

The next stage in our investigations will involve an attempt to identify an unknown chemical species hidden in the peak of a liquid chromatogram, and later perhaps the sophisticated technical problem of an on-line coupling will be attacked.

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