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LASER MICROPROBE MASS SPECTROMETRY OF SELECTED COMPOUNDS DIRECTLY FROM NORMAL PHASE HIGH-PERFORMANCE THIN-LAYER PLATES

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SUMMARY

Direct analysis of high-performance thin-layer chromatography (HPTLC) plates by laser microprobe mass spectrometry has been demonstrated. A series of general organics and ionic surfactants were examined as reference materials and after application to normal-phase HPTLC plates. Spectra were generated, containing structurally significant ions and a contribution from the silica chromatography medium. After chromatography in a solvent system, spectra were weak but included sufficient compound-specific ions to permit structural confirmation. The identification of total unknowns by this means alone would at present be speculative but, supplemented by visualisation reagents and known chromatographic reference points, valuable information can be gained.

INTRODUCTION

Current mass spectrometry (MS) technology requires compounds of interest to be removed from the thin-layer plate prior to analysis. This may involve solvent elution or the scraping of the compound, along with the chromatographic support phase, from the plate.

Previous studies of direct analysis of thin-layer plates have incorporated desorption by various heat sources, followed by analysis of the desorbed species by thermal conductivity or flame ionisation detection[1,2], MS[3], or by secondary ion mass spectrometry[4]. The use of lasers simply to desorb[5] or both desorb and ionise species from thin-layer plates[6] has been addressed. On-line mass analysis using both quadrupole and time-of-flight MS has been used.

Laser microprobe MS provides for the energy from a Nd:YAG laser to be directed onto a specimen retained at high vacuum. The ions produced are mass analysed by means of a time-of-flight mass spectrometer. Consequently, full mass spectra may be obtained from specific areas of the specimen.

The facility to examine compounds *in situ* on high-performance thin-layer chromatography (TLC) plates eliminates the possibility of chemically changing species during the elution process or losing trace components during scraping from the plate.

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In addition, the spatial resolution of the laser is such that possible heterogeneity within a located spot on a plate may be examined.

The studies described herein were intended to determine the feasibility of examining HPTLC plates by this means, with reference to compounds selected from the categories of general organics and surfactants encountered in industrial detergent, fuel and oil formulations. Laser ionisation being a relatively recent development, few reference spectra for organic molecules are available. Consequently, laser spectra for those reference compounds chosen were determined prior to application to HPTLC media.

EXPERIMENTAL

Chromatographic procedure

All solvents and visualisation reagents were of analytical reagent grade (BDH, Poole, U.K.). Solvents were redistilled prior to use. Samples analysed were of technical grade from several commercial sources. The chromatography was carried out on pre-cleaned reactivated (120°C) aluminium backed HPTLC plates (Merck, BHD). Samples were applied as 3μl aliquots using the HPTLC spot applicator (Linomatt IV, Camag). The plates were developed in toluene–methanol (4:1).

The solvent front was allowed to migrate 150 mm up the plate, followed by drying in a stream of nitrogen. The spots were visualised using ultraviolet illumination at 254 and 365 nm, followed by iodine vapour visualisation prior to analysis by laser microprobe MS.

Sample presentation

Reference compounds were applied as powders or crystals to double-sided adhesive tape or as thin smears to the surface of aluminium foil. These were mounted onto an aluminium sample holder and retained with a stainless-steel grid which also assisted in residual charge dissipation.

Instrumental

The LIMA-4 laser microprobe (Cambridge Mass Spectrometry, Cambridge, U.K.) optically directs light at 266 nm from a Nd:YAG laser source, onto a specimen retained at ultra-high vacuum. The sample can be moved by means of a stepper-motor driven stage and viewed using an optical microscope–closed-circuit TV system.

Locations on the specimen can be specifically interrogated at high spatial resolution (*ca.* 1 μm). Simultaneous information may be obtained relating to elemental. inorganic and organic components.

Ionised species produced by the laser impact are mass analysed by a time-of-flight mass spectrometer which provides for a simultaneous detection of all ions produced over a large mass range (*ca.* 10 000 daltons) and offers a sensitivity of detection in the order of 10 ppm for selected elemental species.

Specimens were examined in positive and negative ion modes and, in each case, laser power and beam focus were optimised to produce structurally significant mass spectra.

RESULTS AND DISCUSSION

Reference spectra were obtained for several general organic molecules. Fig. 1 shows the spectrum obtained for methoxybenzoic acid under negative ion conditions at optimal laser powers. The spectrum includes an abundant M^- at m/z 152 with an equally intense $[M-OH]^-$ at m/z 135. Structural ions at m/z 107 from the loss of COOH from the molecule and at m/z 93 the $[C_6H_5O]^-$ species were also noted.

The spectrum shown in Fig. 2 is of *p*-aminobenzoic acid, in the positive ion mode. Ions associated with the M^+ at m/z 137, the loss of NH_2 at m/z 121 and the elimination of COOH at m/z 92 were the most significant structure-related ions observed.

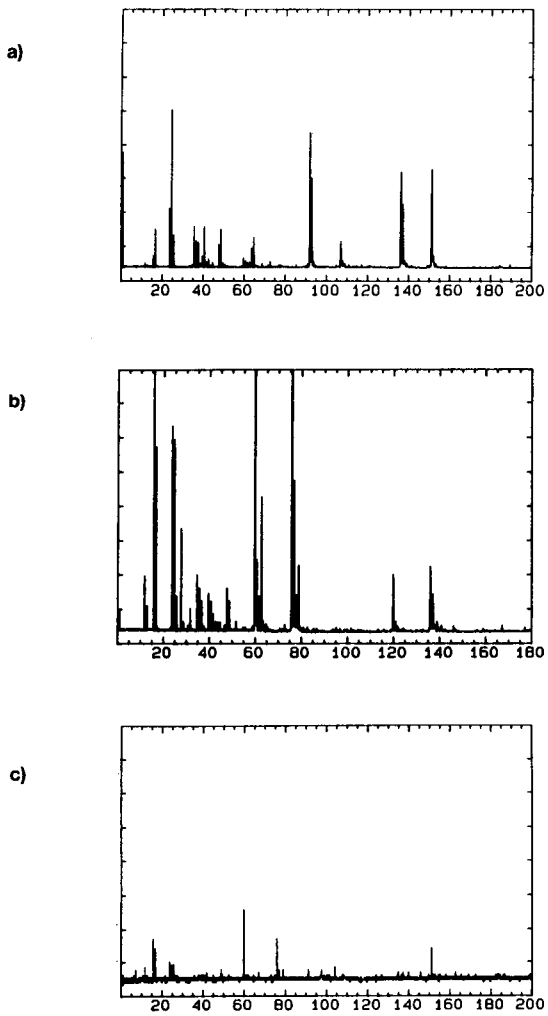

Fig. 1. Laser ionisation mass spectra of methoxy benzoic acid (negative ions). (a) Reference standard, solid; (b) HPTLC plate origin; (c) after chromatography in toluene–methanol (4:1).

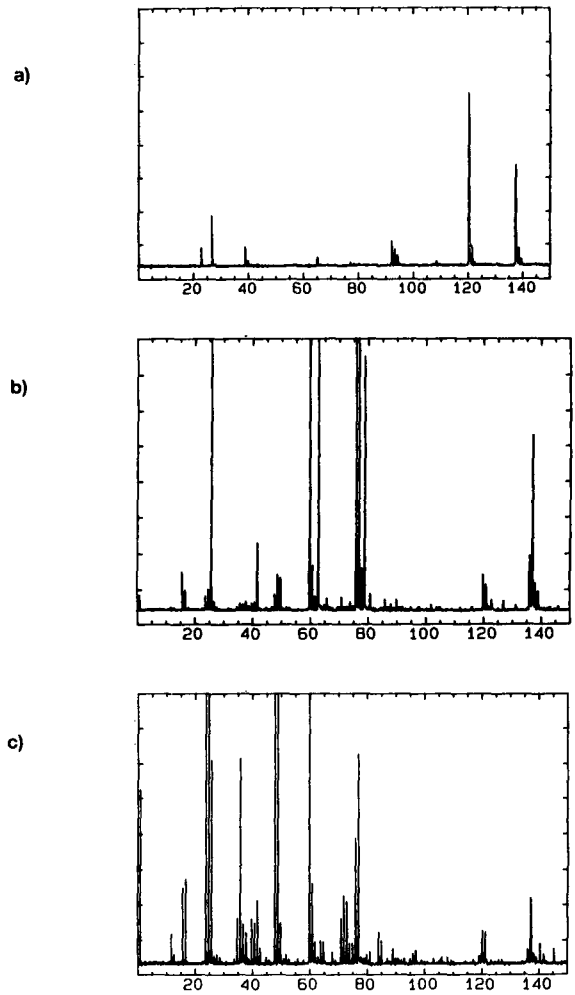

Fig. 2. Laser ionisation mass spectra of *p*-aminobenzoic acid. (a) Reference standard, solid (positive ions); (b) HPTLC plate origin (negative ions); (c) after chromatography in toluene–methanol (4:1) (negative ions).

Cetylpyridinium bromide is a cationic ionic surfactant used in fuels formulations. Its typical laser ionisation spectrum is shown in Fig. 3, with negative ions at *m/z* 380 $[M]^-$, *m/z* 301 $[M-Br]^-$, *m/z* 299 $[M-HBr]^-$, *m/z* 223 $[C_{16}H_{31}]^-$, *m/z* 159 $[C_5H_6NBr]^-$ and *m/z* 79/81 $[Br]^-$ evident.

The anionic surfactants sodium dodecylsulphate and sodium dodecylbenzenesulphonate were similarly examined. Figs. 4 and 5 show the negative ion spectra. Ions corresponding to $[M-Na]^-$ (*m/z* 265), $[NaSO_4]^-$, (*m/z* 119), $[NaSO_3]^-$, (*m/z* 103), $[SO_4]^-$ (*m/z* 96), $[SO_3]^-$ (*m/z* 80), $[SO_2]^-$ (*m/z* 64), $[SO]^-$ (*m/z* 48) and $[S]^-$ *m/z* 32/34 were noted in the former. In the latter, a strong response at *m/z* 184 was noted in a spectrum difficult to rationalise but totally reproducible.

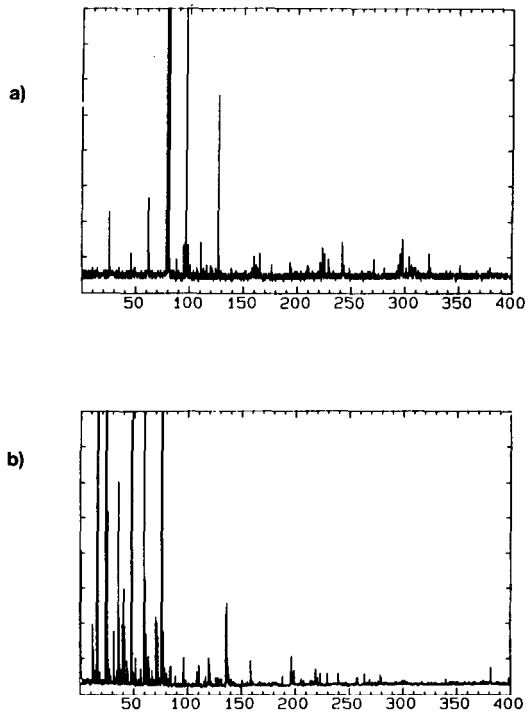

Fig. 3. Laser ionisation mass spectra of cetylpyridinium bromide (negative ions). (a) Reference standard, solid; (b) HPTLC plate origin.

With representative laser ionisation spectra for each compound to be studied available, each selected compound was spotted onto a thin-layer plate and its position of application noted. Sections of plate were removed and examined by laser ionisation mass analysis and spectra compared with those obtained previously.

Figs. 1–5 show the comparable spectra obtained directly by laser ablation and ionisation from normal-phase HPTLC plates. In most cases, the major spectral ions were similar to those obtained from pure reference materials. Generally, however, higher laser powers were required to desorb and ionise equivalent species from the chromatographic matrix.

The influence of the matrix is shown by an examination of Figs. 1 and 2. Methoxybenzoic acid as a solid reference material fragments to yield a strong M^- at m/z 152, with structural ions at m/z 135, 107 and 93, as previously described. When applied to the origin of an HPTLC plate, no molecular ion was evident, the most intense structurally specific ion being from the $[M-OH]^-$ at m/z 135.

p-Aminobenzoic acid yielded a strong positive ion spectrum when examined as a reference material. From the chromatography plate, however, the negative ion spectrum was found to provide more structural information, with prominent M^- and $[M-NH_2]^-$ being noted.

These differences in the qualitative nature of laser ionisation spectra from different matrices demands some caution to be exercised when comparing reference spectra with those from such media as that studied here.

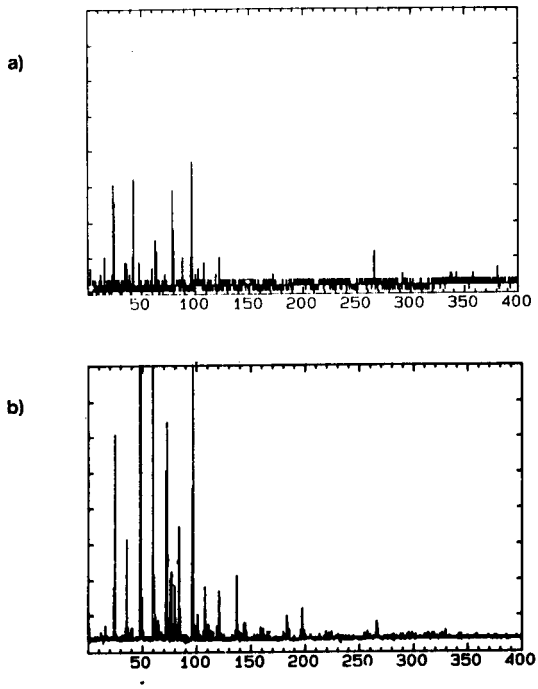

Fig. 4. Laser ionisation mass spectra of sodium dodecylsulphate (negative ions). (a) Reference standard, solid; (b) HPTLC plate origin.

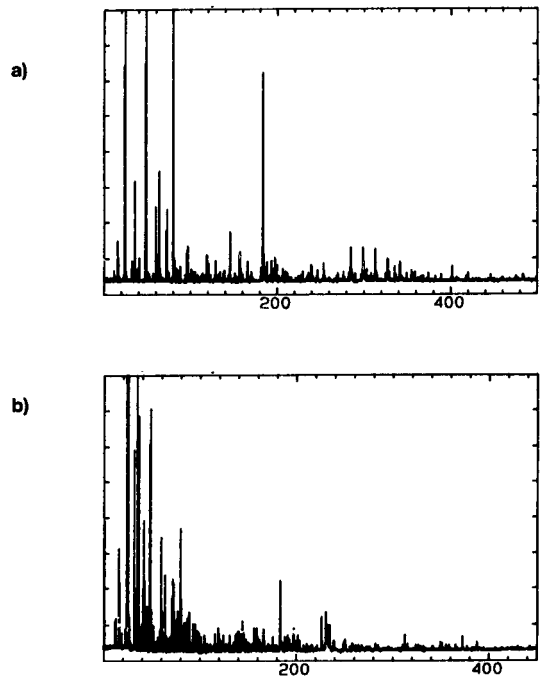

Fig. 5. Laser ionisation mass spectra of sodium dodecylbenzenesulphonate (negative ions). (a) Reference standard, solid; (b) HPTLC plate origin.

Several features of the laser ionisation process may influence spectral quality. Whilst the amount of laser energy incident onto the sample may be nominally similar, the proportion of that power absorbed by the specimen is determined by the chemical nature, morphology and topography of the specimen and matrix. The chromatographic support, being white in colour, tends to absorb heat less efficiently than does a darker medium. Possibly, more of the laser irradiation will be reflected and consequently proportionately less coupled to the sample. In addition, the surface of the HPTLC plate undulates on a micro scale and hence the focussing of the laser beam onto the surface is made more difficult. Also one must consider the effects of striking a matrix composed of potentially ionisable species and possible competitive effects.

The influence of the silica background was apparent in negative ion spectra. Major responses were *m/z* 60 $[SiO_2]^-$, *m/z* 76 $[SiO_3]^-$, *m/z* 72 $[Si–O–Si]^-$, with reduced contributions at *m/z* 88 $[Si_2O_2]^-$, *m/z* 104 $[Si_2O_3]^-$, *m/z* 120 $[Si_2O_4]^-$ and *m/z* 136 $[Si_2O_5]^-$. In some cases, minor silica-derived responses overlapped with major structural ions from the compounds being studied. These contributions were of sufficiently low intensity to be unlikely to compromise sample spectra.

Some of the selected samples were spotted as before onto normal-phase silica and chromatographed in toluene–methanol. Spectra are shown in Figs. 1 and 2 and can be seen to consist of major structural fragments and molecular species as were found in previous experiments.

In essence, the detection of target compounds after chromatography was shown to be feasible. Spectra were, however, weak due to the "smearing" effects of the chromatographic process. More recent studies of regions of HPTLC plates previously visualised with compound-specific reagents have shown the great enhancement of spectral quality to be derived by removing chromatographic phase outside the immediate area of interest. By exposing the aluminium backing around the "spot", the difficulties associated with sample charging can be reduced.

The identification of a total unknown compound by this means alone would be tentative. Additional information, relating to specific visualisation techniques, chromatographic reference points and specimen origin etc would, however, make possible the confirmation of the presence of specified compounds or compound-types. Also, an initial screening of the HPTLC plate by laser microprobe MS for components worthy of further study would be appropriate.

CONCLUSION

Structurally significant mass spectra have been obtained from selected reference compounds separated on HPTLC plates. Direct mass spectral analysis without removal of the component from the plate avoids potential problems of contamination or poor recovery of material by the elution process. Initial studies indicated that spectra were weak but by exposing the metal backing plate around the area of interest, spectral intensity and quality were improved.

The combination of HPTLC separation with laser ionisation and the high sensitivity of detection associated with a time-of-flight mass analyser, has been shown to produce meaningful spectra for low levels of separated components. Used in conjunction with retention and selective chemical visualisation information, the HPTLC–laser microprobe technique is an extremely useful aid to the structural confirmation

of separated components. The spatial resolution of the laser beam may also permit the examination of chemical heterogeneity within the same area of the thin-layer plate.

FURTHER WORK

Further work will include the study of more complex mixtures and establish the potential of the technique for problem-solving on real samples. Initial experiments in this direction are promising and useful information has already been provided. Comparisons with the fast atom bombardment MS–HPTLC technique[7] will be pursued. In addition, some study of means of reducing sample charging after several laser pulses may go some way to improving the spectral intensity, particularly of positive ion species.

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REFERENCES

1 T. Cotgreave and A. Lynes, *J. Chromatogr.*, 30 (1967) 117.
2 H. K. Mangold and K. D. Mukharjee, *J. Chromatogr. Sci.*, 13 (1975) 398.
3 R. Kaiser, *Chem. Br.*, 5 (1967) 54.
4 M. S. Stanley, K. L. Duffin, S. J. Doherty and K. L. Bush, *Anal. Chim. Acta*, 200 (1987) 447.
5 L. Ramaley, M. A. Vaughan and W. D. Jamieson, *Anal. Chem.*, 57 (1985) 353.
6 F. P. Novak and D. M. Hercules, *Anal. Lett.*, 18 (1985) 503.
7 K. J. Bare and H. Read, *Analyst (London)*, 112 (1987) 433.