In Situ Raman Spectroscopic Analysis of Thin Layer Chromatograms

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Raman spectra of hexamethylenetetramine and several substituted benzophenones have been studied *in situ* on thin layer chromatography plates. Success depends upon the nature of the substrate (Kieselgel HR was the best found), the eluant, the retention factor, fluorescence of both plate and sample, and the scattering efficiency of the sample itself. It is concluded that the method is viable within the restrictions discussed, but that an instrument with rapid spectrum accumulation facilities would be necessary for routine analytical application.

THE high sensitivity of the laser-Raman technique allows spectra to be obtained by use of microgram quantities of sample. One of its more impressive achievements is the demonstration that it is possible to record Raman spectra of vapours adsorbed on high-area substrates, *e.g.*, pyridine on silica, alumina, rutile, *etc.*[1] Since similar substrate loadings are involved in thin layer chromatography (t.l.c.) we have attempted to extend the Raman technique to this area and investigate the feasibility of distinguishing mixtures of compounds eluted on t.l.c. plates. The ability to make *in situ* analysis of t.l.c. spots would be a great improvement upon the normal, time-consuming and therefore expensive, procedure of physical removal of the 'spot' followed by extraction and identifiction by physical methods. Since the focused laser beam strikes a minute area of plate this technique also allows, in principle, examination of partially overlapped eluted spots. We do not know of any previous publication on this topic.

EXPERIMENTAL

The plates used were based upon microscope slides. Strips of Sellotape were placed lengthwise along each side and a slurry of substrate spread to the thickness of the tape with a glass rod. They were dried at 70 °C for 1 h. Raman spectra were recorded on a Coderg PHO spectrometer with either 488·0 or 632·8 nm excitation. The usual choice, where it was not dictated by sample considerations, was 488·0 nm radiation since the intensity of scattered Raman light increases with ν_0^4 and the photomultiplier response is considerably better with blue than red light.

Optimum running conditions were investigated by varying the nature of the substrate, its thickness, the exciting frequency (either focused or defocused), and the geometry of the plate with respect to the laser beam. The thinnest plates yielded the best results, and the best signal intensity combined with lowest background was obtained with the laser beam striking the plate at glancing incidence. 90° Scattering collection was used.

Microlitre quantities of organic compounds in suitable solvents were spotted quantitatively on the plates, beginning with relatively high concentrations, reducing to those normal in analytical t.l.c. work. To obtain spectra of eluted spots preliminary experiments were carried out with silica gel H with fluorescent additive so that the position of the eluted spots could be determined in the usual way. Eluants were chosen so as to give the least diffuse spots and the retention factor determined so that the positions of eluted spots could be predicted for undeveloped plates without fluorescent additive.

Where necessary simple spectrum accumulation was employed: the amplifier output was fed into a Beckman FS300 A/D converter and recorded with a Facit 4070 paper-tape punch. The A/D converter and spectrometer were controlled by an automatic device which also allowed sample interval and range to be varied. Spectra were computer-averaged and plotted by use of a programme written by Mr. I. R. Gardner.

RESULTS

Substrates.—Three basic types of substrate were tested. Supreme, a grade of china clay manufactured by English China Clays showed very intense fluorescence which swamps any Raman signal and was therefore rejected. Rutile yields strong Raman lines up to *ca.* 1100 cm^{-1}, although it could be useful if only the higher-frequency region were of

[1] P. J. Hendra and E. J. Loader, *Nature*, 1967, **216**, 789; 1968, **217**, 637.

interest. Two high-grade silica gels, Kieselgel H and HR, were free from strong Raman lines above 650 cm^{-1} and showed relatively little intrinsic fluorescence (fluorescent additive was not used). Some plates with binder were also tested. The binder contributes a few weak lines, the organic type more than $CaSO_4$, but either would be acceptable for this type of work. Although the spectrum of Kieselgel is largely featureless (when it is treated as a sample), under the forcing conditions necessary to study eluted spots many weak bands are seen which interfere with detection of the sample emission. Similarly the weak background fluorescence from the gel becomes significant under high gain, giving rise to poor signal : noise ratios. We have found a few instances in which fluorescence emission from an eluted sample plate is apparently greater than that of sample and plate taken separately, suggesting some interaction. For the experiments described below Kieselgel H (Merck) without fluorescent additive was used.

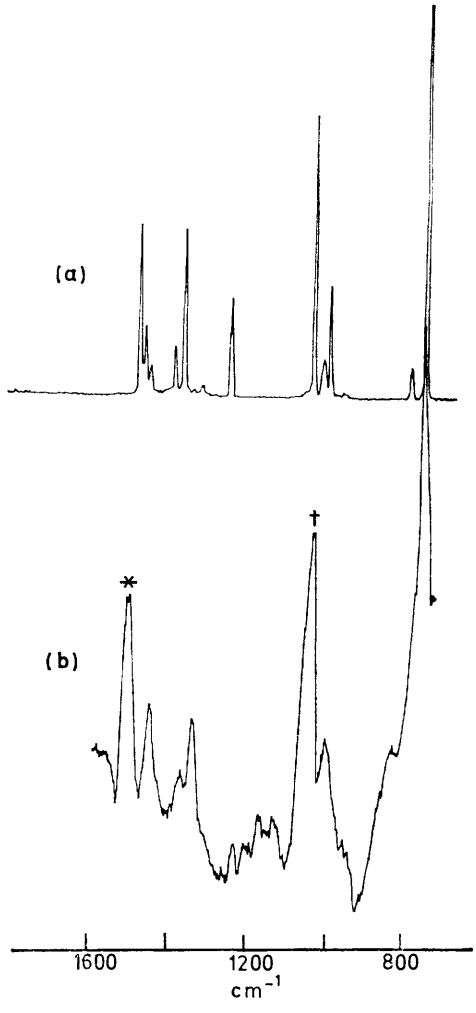

FIGURE 1 (a) Raman spectrum of crystalline hexamethylenetetramine, spectral slit width 2 cm^{-1}, scan speed 25 cm^{-1} min^{-1}, time constant 0·65 s, 488·0 nm excitation, 40 mW at sample. (b) 4 µl 5% chloroform solution of hexamethylenetetramine on t.l.c. plate, spot not eluted. Spectral slit width 6 cm^{-1}, scan speed 25 cm^{-1} min^{-1}, time constant 5·4 s, 488·0 nm excitation, 120 mW at sample; * = Band due to plate, † = Band partly due to plate

Spectra of T.l.c. Plates.—Initial work was done with hexamethylenetetramine as this was found to be a good Raman scatterer with no fluorescence, yielding a spectrum with both strong and weak bands [Figure 1(a)]. The spectrum of Figure 1(b) was obtained from a non-eluted spot from 4 µl of a 5% solution in chloroform. Six of the main bands of hexamethylenetetramine are readily identified on the plate spectrum. The strong line at 1045 cm^{-1} has been overlaid by a broad band at 1060 cm^{-1} due to the substrate, while bands below *ca.* 700 cm^{-1} are hidden by background emission from substrate.

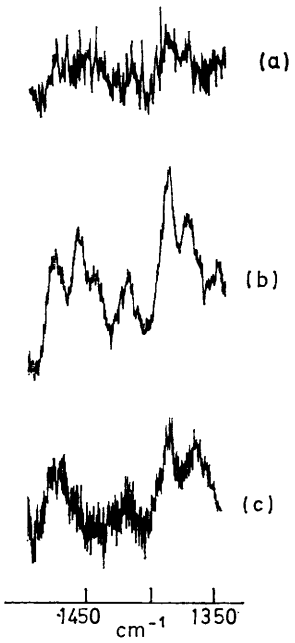

FIGURE 2 Raman spectra of hexamethylenetetramine on t.l.c. plate. (a) Spot of Figure 1(b) eluted. Spectral slit width 4 cm^{-1}, scan speed 50 cm^{-1} min^{-1}, time constant 0·65 s, 488·0 nm excitation, 70 mW at sample. (b) Average of 49 spectra of (a), 4 sample points per cm^{-1}. (c) Average of 49 background spectra of plate treated as in (a) but without sample

On elution with methanol the spot spread considerably, thereby greatly reducing the surface concentration of hexamethylenetetramine: only the strongest Raman line (781 cm^{-1}) could then be detected. By use of spectrum accumulation (49 runs) approximately sevenfold improvement in signal : noise ratio was obtained (Figure 2). In the region studied (1345—1495 cm^{-1}) four of the five sample lines could then be identified, only the weakest (1431 cm^{-1}) being unobserved.

Further experiments were then performed with a series of related substituted benzophenones, representing a synthetic problem in which several similar products might be present. We discuss one of these (A) as typical (see Figure 3). A drop of 5% chloroform solution with an estimated content of 136 µg of the benzophenone (A) was placed on the plate: this yielded an excellent spectrum showing all but the weakest bands in the Raman spectrum of solid benzophenone (A). After elution with benzene (chosen because it gave a small retention factor and a less diffuse spot than other solvents) the spectrum of Figure 3(b) was obtained and was of sufficient intensity that spectrum accumulation was not required. Bands due to the benzophenone (A) were

readily identified by comparison with the spectrum of the blank plate [Figure 3(c)] which had been subjected to parallel treatment with solvents. Some of the bands of the benzophenone (A) on the t.l.c. plate occurred at slightly different

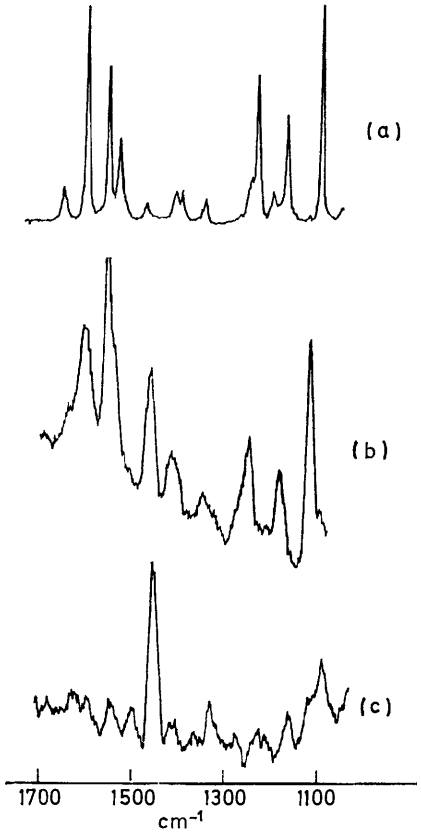

FIGURE 3 Raman spectra of the benzophenone (A). (a) As crystalline powder. Spectral slit width 2 cm^{-1}, scan speed 25 cm^{-1} min^{-1}, time constant 0·65 s, 488·0 nm excitation, 25 mW at sample. (b) 2·7 μl 5% chloroform solution of the benzophenone (A) on t.l.c. plate eluted with benzene. Spectral slit width 6 cm^{-1}, scan speed 25 cm^{-1} min^{-1}, time constant 5·4 s, 488·0 nm excitation, 40 mW at sample. (c) Blank t.l.c. plate treated as sample plate in (b) and recorded under same conditions

positions from those in the neat material, and would give information on the nature of binding to the surface if the initial interpretation of the spectrum of the benzophenone (A) were first established.

A drop of 5% chloroform solution of a 1 : 1 mixture of hexamethylenetetramine and the benzophenone (A) was spotted and eluted with benzene. The benzophenone yielded a spectrum similar to that of Figure 3(b), although only *ca.* 60 μg of sample were initially spotted on the plate.

DISCUSSION

The feasibility of studying t.l.c. plates routinely by Raman spectroscopy depends upon several factors. (i) We admit that our knowledge of t.l.c. technique is primitive and that better results could be achieved by experts. However, it is clear that the quality of spectrum obtained is strongly dependent upon surface concentration of sample in the eluted spot, and that this is strongly affected by the eluant and the retention factor. Thus hexamethylenetetramine always gave rather diffuse eluted spots and had a much higher retention factor than the benzophenone (A) which yielded rather concentrated spots. The consequences of this are seen in the spectra and the need for spectrum accumulation in the case of the amine. (ii) Both substrates are good Raman scatterers, though they were not selected for this reason. We have recorded Raman spectra of many types of organic compound, and believe that most of them could be detected under t.l.c. conditions. Most of the compounds which we have studied show several strong Raman lines of diagnostic value.

There will always be some compounds for which Raman spectroscopy is difficult or impossible; *e.g.*, those which fluoresce, photodecompose, or have broad electronic absorption bands that re-absorb Raman emission. (iii) Further study should be devoted to production of a form of silica gel, or other substrate, free from fluorescence emission: this would allow considerable increase in sensitivity of the method. (iv) Our spectrum accumulation equipment is rather basic, but use of rapid-scan Raman equipment such as the Coderg 'Iteraman' instrument would give better results, and with careful attention to t.l.c. technique would lead to a practicable *in situ* analysis of t.l.c. plates by the Raman technique. Development of a non-fluorescent substrate would confer further advantages.

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