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Note

Characterization of nanogram levels of metalloporphyrins with thin-layer chromatography–resonance Raman spectroscopy

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Several useful techniques exist for detecting compounds adsorbed on various solid supports and matrices. Of these the UV–VIS[1,2] and luminescent[3–6] methods are the most popular and straightforward. However, in many cases more extensive structural information is needed to evaluate thoroughly a substance. Vibrational spectra, in particular, contain the type of information needed to characterize a compound. Ideally one would like a technique that provides the information of vibrational spectra and that also has the sensitivity of the aforementioned UV or luminescence methods. Until recently this combination was impossible. For example, it is generally difficult to obtain quality infrared spectra from low levels of compounds adsorbed on cellulose or silica gel based matrices. Although diffuse reflectance infrared Fourier transform (DRIFT) spectrometry can be used, it is relatively insensitive, costly and time consuming[7]. The use of Raman scattering eliminates problems due to moisture and background adsorption which plague the infrared based techniques. However, early attempts to use Raman spectrometry as a detection method for thin-layer chromatography (TLC) suffered from poor sensitivity, sample heating and decomposition[8,9]. Hence the technique was not widely utilized.

Recently, an important advance in methodology was made by Tran[10,11]. It was shown that excellent Raman spectra could be obtained for nanogram to subnanogram levels of several dyes adsorbed on filter paper or paper chromatographic supports by using surface enhanced Raman scattering (SERS). The technique involved impregnating the cellulose matrix with a colloidal silver sol and taking a Raman spectra of the wet spot[10,11]. Both the sensitivity and vibrational information obtained with this technique were impressive.

In this work, resonance Raman spectroscopy in conjunction with TLC is used to separate, detect and characterize trace levels of porphyrins. The Raman spectra obtained from various planar surfaces are compared to traditional solution spectra. There are a number of important advantages to this technique. For example, most common TLC matrices can be used with little interference. Spectra can be taken from wet or dry plates and are not appreciably affected by most impurities or sampling methods. There is no need to prepare a stabilized colloid and treat the plate with the

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preparation. One disadvantage of this technique is that fluorescence can be a major source of interference for some compounds. However, using micellar mediated resonance Raman spectroscopy[12], this problem can be circumvented.

EXPERIMENTAL

All porphyrins were obtained from Porphyrin Products and used as received. Silica gel TLC plates (K5) were obtained from Whatman. Spectroscopically pure solvents were used in all experiments. The continuous wave (CW) spectra were recorded using a Coherent Innova Argon ion laser and a SPEX Triplemate monochromator with 1200 gratings/mm. A PAR (Model 1420) optical multichannel analyzer with grated intensifier was used for signal detection. The optical multichannel analyzer was controlled with the PAR Model 1215 optical multichannel analyzer controller. A backscattering geometry and an unfocused laser beam were used for the samples deposited on TLC plates. A 90° geometry was used for the solution spectra. The concentration of porphyrin solutions was 100 μM. The amount of material spotted on the TLC plate was varied between 50 ng and 20 μg. The sensitivity was very dependent on spot dispersion. The laser power for the TLC spectra was 200 mW while that for the solution spectra was 400 mW. The background (presumably light reflected from the TLC plate) was diminished considerably if the plate was skewed 3–10° from perpendicular to the laser beam. Further experimental details are given with each figure.

RESULTS AND DISCUSSIONS

Porphyrin and metalloporphyrin research is an important and rapidly expanding field[13,14]. Studies involving vibrational spectra are essential in the characterization and understanding of these molecules[15,16]. Consequently TLC–resonance Raman spectroscopy was developed to evaluate the purity and to characterize trace levels of these and other compounds. Fig. 1 shows the TLC chromatograms for nickel uroporphyrin (NiUroP) and nickel protoporphyrin dimethyl ester (NiPPDME). Chromatographic analysis revealed the presence of three to four compounds in all of the supposedly pure metallouroporphyrin samples. A single spot was found for

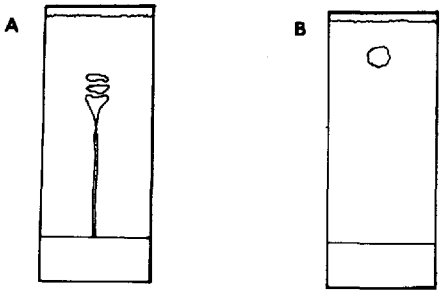

Fig. 1. TLC plates of: (A) nickel uroporphyrin developed with ethylene glycol–isopropanol (6:4, v/v) and (B) nickel protoporphyrin dimethyl ester developed with toluene–ethyl acetate (6:4, v/v). Silica gel plates were used in both cases.

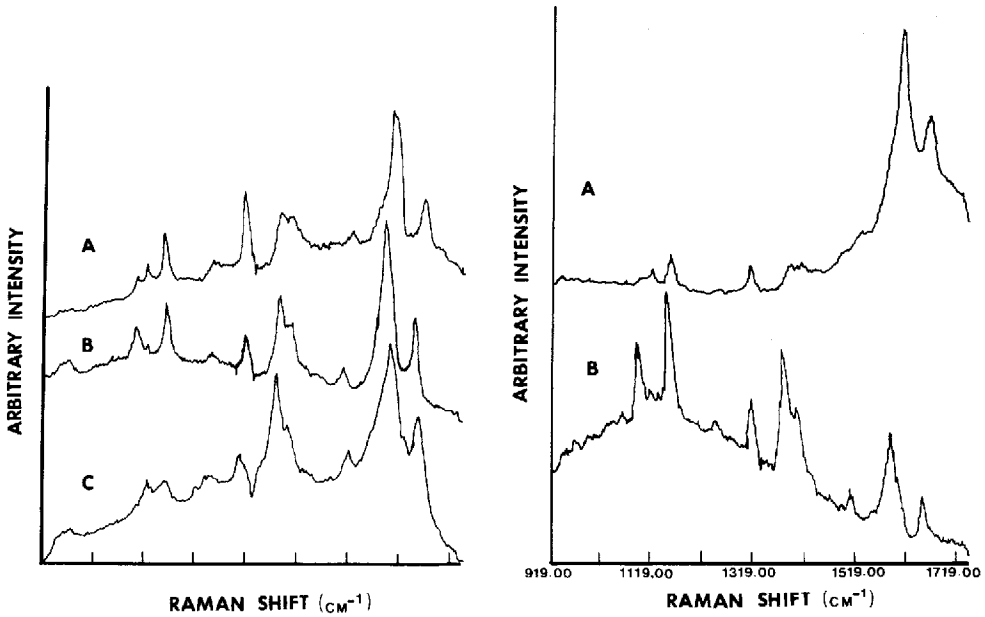

Fig. 2. Resonance Raman spectra of: (A) nickel uroporphyrin, (B) copper uroporphyrin, and (C) nickel protoporphyrin dimethyl ester taken from dried silica gel TLC plates. Excitation wavelength was 514.5 nm. The Raman shift scale is identical to that in Fig. 3.

Fig. 3. Solution resonance Raman spectra of: (A) nickel uroporphyrin and (B) nickel protoporphyrin dimethyl ester. The concentration of metalloporphyrin in both solutions was 100 μM. Excitation wavelength was 514.5 nm.

NiPPDME. Resonance Raman analysis was done on all TLC samples and on solutions made from the same unpurified samples. Typical resonance Raman spectra taken from the TLC plates are shown in Fig. 2 and the corresponding solution spectra for NiUroP and NiPPDME are shown in Fig. 3. A number of things are evident from this data. First, the quality of the resonance Raman spectra obtained from the surface of chromatographic media often is as good or better than that obtained from analogous solution samples. This is true even though considerably less sample is needed for the surface technique. Also, there are significant changes in the relative peak intensities and small shifts (< 5 cm^{-1}) in the peak positions. Note for example, the ν_2 bands between 1582 and 1603 cm^{-1}. This could be indicative of changes in certain vibrational modes of freedom in the adsorbed *versus* solution state. Interestingly there were no significant differences, within the experimental limits of this equipment, in the resonance Raman spectra of the various spots obtained from the TLC of the uroporphyrins (Fig. 1A). Vibrational modes in the high-frequency region (> 1000 cm^{-1}) involve in-plane motion of the conjugated bonds of the porphyrin macrocycle[17] and are largely insensitive to the ordering of peripheral substituents. Thus, the different TLC spots most likely represent isomers of the uroporphyrins which occur as a result of differences in the ordering of these substituents. As was also noted by Tran[10], significantly increasing the laser power and focusing the laser beam increases the chance of local heating effects and photodecomposition of the sample.

It is apparent that the combination of planar chromatographic methods with specialized Raman detection is a potentially powerful technique for the evaluation and characterization of trace levels of organic compounds. The future possibility of a hyphenated micellar liquid chromatography–resonance Raman spectroscopic technique is even more intriguing, as this method would not suffer from the solvent background problems currently plaguing liquid chromatography–Fourier transform infrared spectroscopy. Experiments which will lead to the development of this technique are now in progress in our laboratory.

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