suffers if the dividing wall is not insulated in some way. This can be done in practice by using two plates separated by a layer of insulation.

Temperature of Heat Supply and Rejection

So far the benefits of thermal coupling have been discussed in terms of the reduced energy required. Let us now consider the temperature at which the heat needs to be supplied and rejected if thermal coupling is used. It is always preferable to add the heat to the reboiler at the lowest temperature possible and to reject heat from the condenser at the highest temperature possible. In the first instance, this allows cheaper hot and cold utilities. In addition, if heat integration of the reboiler and condenser is to be considered, heat integration will also always benefit from lower reboiler temperatures and higher condenser temperatures.

Figure 12 compares a conventional and a thermally coupled arrangement in terms of temperature and enthalpy. In the conventional arrangement there is freedom to choose the pressures of the two columns independently, and thus the temperatures of the two condensers or the two reboilers can be varied independently. In the case of the thermally coupled arrangement no such freedom exists. Although the thermally coupled arrangement requires a smaller heat load than the conventional arrangement, more of the duties are at extreme levels. The smaller duties work to the benefit of utility costs and heat integration but the more extreme levels work against them.

Summary

Thermally coupled distillation columns offer considerable benefits in terms of operating costs. Side-stripper, side-rectifier and fully thermally coupled arrangements such as the Petlyuk column can save typically 30% of the energy consumption compared with sequences of simple columns. The magnitude of the saving depends on the feed composition and relative volatility of the components being separated. The dividing wall column also offers large potential savings in capital cost. Apart from the use of side-stripper arrangements in the petroleum refinery industry there has been reluctance on the part of process designers to exploit the full potential of thermal coupling. Control of thermally coupled arrangements does not present any particularly difficult problems.

See also: **II / Distillation:** Energy Management; Modelling and Simulation; Theory of Distillation.

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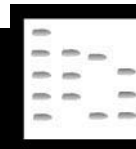
THIN-LAYER CHROMATOGRAPHY – VIBRATION SPECTROSCOPY



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Introduction

The utility of vibrational spectroscopy in chemical structure elucidation of separated thin layer chromatography (TLC) spots has been recognized for many years. Although the traditional method has been infrared spectroscopy (Fourier transform infrared spectrometry (FTIR)) a number of competing techniques now exist including normal Raman scattering (RS), Raman microspectroscopy (Micro-Raman), Fourier transform Raman (FT-Raman) and surface-enhanced Raman scattering (SERS). Since each type of spectra provide essential vibrational profile of analytes from the TLC plate, the different disciplines are natural partners in a general spectroscopic analysis. All methods involve the vibrational energy of the molecule and thus provide molecular and structural information about the separated sample. However, since infrared (IR) absorption, Raman scattering and SERS have different selection rules – what is frequently strong in

a Raman spectrum is weak in an IR spectrum and vice versa. For this reason, a combined IR and Raman system offers the flexibility of working with almost any sample, as well as complete vibrational information from numerous compounds.

Up to now the combination of TLC separation and IR spectroscopy has been approached in roughly two ways. The classical approach for recording IR spectra is to elute the separated TLC zones from the layer onto an IR-transparent pellet or powder (sample transfer TLC-FTIR). Numerous workers have attempted to record diffuse reflectance Fourier transform infrared (DRIFT) spectra directly on TLC plates (*in situ* TLC-FTIR). A small number of research groups have studied the applicability of near-infrared spectroscopy (NIR) in the reflectance mode as an *in situ* detection tool in TLC.

Although IR spectroscopy still yields the largest number of publications in the field of TLC vibrational analysis many serious attempts are now being made to explore the analytic potential of Raman spectroscopy in new and challenging areas of TLC spot identification. The use of Raman scattering eliminates moisture and background absorption problems which may be present in the infrared-based techniques. Therefore, a number of important advantages to this Raman technique exist: (1) most common TLC matrices can be used with little interference, (2) spectra can be taken *in situ* from wet or dry plates, (3) the well-known advantages of NIR excitation ($\lambda_{ex} = 1064$ nm) in FT-Raman (avoidance of fluorescence and photo-induced sample damage), (4) the use of Raman microspectroscopy which allowed unambiguous placement of the laser focus on the TLC plate with spatial resolution of the order of 1 μm.

One of the significant limitations of the application of Raman spectroscopy in the field of TLC chromatogram spot characterization is the lack of sensitivity. An important step forward was made by SERS. Raman scattering intensities from adsorbed substances on nano-metal particles (Ag, Au, Cu) are increased by a factor of 10^5–10^6 compared to those of the nonadsorbed compounds at equal concentration. For TLC this SERS effect is accomplished by spraying chromatograms with colloidal silver solution or silver coating in a vacuum chamber (post-chromatographic SERS activation in TLC). By using SERS microprobe techniques (laser spots down to 1 μm in size) and high performance thinlayer chromatography (HPTLC) plates it is possible to achieve low picogram detection limits for HPTLC spots.

Fourier Transform Infrared Spectroscopy (FTIR)

Sample Transfer TLC-FTIR

The classic approach and most frequently used method for recording infrared spectra of substances separated by TLC or HPTLC involves removal of the sample zone from the plate, usually followed by extracting the analytes via a solvent to an IR-transparent pellet or powder. This technique makes it possible to measure full IR spectra at a reasonable sensitivity using conventional FTIR transmission or DRIFT detection. Commercial accessories are available for a simple and convenient procedure for the transfer of the analyte spots to cups containing IR-transparent substrates. The use of this method has been illustrated by the analysis of dyes, quinones, coal extracts and biochemical substances. Reasonable IR spectra with full spectral features can be obtained from 1 to 0.01 μg of sample per spot. The main reason for using transfer in TLC-FTIR is to avoid the strong absorption bands in the mid-IR (400–4000 cm^{-1}) from the TLC stationary phase. Over the years, numerous other transfer methods for the combination of TLC or HPTLC and FTIR detection have been described in the literature. The normal method involves removal of zones (scraping off), elution of the spot analyte, deposition on the material transparent to IR and the measurement by FTIR. However, the removal of zones from the plate increases the risk of contamination and can result in further reactions. The "wick-stick" technique consists of pressing KBr micro pyramids onto the TLC plate at locations corresponding to the analyte spot. With a development perpendicular to the first development, the analytes are eluted into the pyramids, which are then dried and pressed into pellets. The Eulochrom system involves the elution of analyte zones from silica-gel TLC plates by means of small amounts of methanol, then solvent evaporation and pellet preparation. A simple and convenient procedure in conjunction with TLC sheets with a liquid-permeable support such as the Empore TLC sheet is the transfer of separated spots to a powder layer of potassium bromide. After ordinary TLC development, the sheet is put in a sheet holder chamber and a thin layer of KBr powder is applied on the upper side of the Empore TLC sheet. After this KBr coating, zones are eluted from the sheet by means of a wetted fritted glass unit and thus moved into the powder layer.

Thermal desorption FTIR analysis can be used to avoid the interference from TLC stationary phases with analyte because strong interaction between the analyte and the stationary phase causes significant

band shift. Therefore, vapour-phase spectrum libraries can be used directly for sample identification. This method can be applied to thermally stable substances which can be desorbed from thermally stable TLC stationary phases. The separated TLC spot is scraped off and loaded onto the sample pan of a thermogravimetric analyser (TGA/FTIR). Spectra are easily identified for samples present at a level of 10 μg per TLC zone. A detection limit of 0.8 μg is found for analysis of methyl benzoate.

Over the years, research and practical applications have shown that sample transfer TLC-FTIR technique (offline coupling for TLC and FTIR) can be effective and useful as a reliable TLC spot identification method.

In situ TLC-FTIR

IR spectral identification of a TLC spot by means of sample transfer TLC-FTIR methods is usually time consuming and problematic. Alternatively, IR spectroscopic information about TLC-separated materials can be obtained *in situ* by means of a variety of FTIR techniques. Monitoring the TLC zones by direct DRIFT measurements, transmission spectroscopy, infrared microspectroscopic detection and photoacoustic FTIR (PA-FTIR) enables both qualitative and quantitative characterization of separated spots. To acquire useful IR spectra the *in situ* TLC spot method requires the background measurement of the adsorbent free of any sample. The final IR spectrum is obtained by either ratioing sample and background measurements or subtracting the background spectrum from the sample spectrum. This part of the *in situ* TLC-FTIR detection method is very important for the quality of the resulting analyte IR spectrum. Depending on the nature of the isolated TLC spots and the goal of the analysis, the choice of IR measurement can be either DRIFT or transmission.

Today online coupling of TLC(HPTLC) and DRIFT spectroscopy can be carried out using commercially available equipment. By combination a computer-controlled x-y stage with a specially constructed DRIFT unit and an FTIR spectrometer it is possible to obtain direct IR chromatograms of TLC spots. The chromatograms can be generated both frequency-dependent as spectral windows of a certain range and frequency-independent as a Gram-Schmidt trace. Depending on the infrared absorptivity of the TLC analyte and the distance run in the chromatogram, the limits of identification, the validated detection limits, and the limits of quantification lie between 10 ng and 2.5 μg.

In general, use of automated multiple development (AMD) for TLC separation can improve the online DRIFT identification limit by one-third compared to conventional TLC separation techniques.

The *in situ* FTIR detection of spots on a plate by means of IR transmission measurements requires an IR transparent support, e.g. silica gel coated on AgCl plates. A thin adsorbent coating and IR transparency of the silver chloride support permits enough energy throughput to acquire analyte species at 0.1–10 μg of material. The detection limit of this technique in conjunction with programmed multiple development and special postcoatings can be improved to the nanogram level. This technique is limited to noncommercially available AgCl speciality plates.

The development of microchannel TLC spot identification with diffuse reflectance infrared microspectroscopic detection is also a method for specific practical applications. In this case a zirconia stationary phase is used instead of silica or alumina. Zirconia shows significantly higher reflectivity than silica or alumina resulting in only moderate background interferences. The detection limit for this specially prepared plate is about 1–10 ng.

Photoacoustic FTIR has been suggested as the technique of choice over DRIFT analysis of high IR-absorbing sample matrices. Photoacoustic spectrometry (PAS) is based on the phenomenon that light impinging on the solid TLC plate, can produce an acoustic signal. PAS involves therefore the measurement of oscillating pressure variations of a confined inert gas situated above the TLC plate. In combination with a PAS cell an FTIR spectrometer can yield photoacoustic IR spectra, which can be applied for TLC zone identification purposes. PA-FTIR does not require sample preparation and avoids the effects of light scattering and reflection.

TLC-NIR

Both mid-infrared (mid-IR) and NIR spectroscopy are important techniques for TLC or HPTLC spot analysis because of their sensitivity and versatility. The main difference between mid-IR and NIR is that bands in the mid-IR are primarily due to molecular fundamental vibrations, and absorption in the NIR region, 800–2500 nm, is primarily due to overtone and combination bands of, O–H, N–H, S–H and C–H functionalities. In the NIR region absorption is rather weak and TLC adsorbents such as silica gel have no strong absorption bands in these NIR regions, so background interferences are very small. Also, nearly all analytes of interest absorb in the NIR region.

Direct, *in situ* diffuse transmission FT-NIR microspectroscopic detection of separated HPTLC spots of different kinds of phospholipids give typical results

from which the usefulness of the TLC-NIR method can be evaluated. The limit of detection with a narrow NIR beam of light (0.4 mm^2) is under 1 μg, and the correlation coefficient of the calibration curve is about 0.98 for phospholipid amounts from 1.25 to 10 μg. Detection limits of less than 1 μg of selected sugar samples on developed TLC plates have been demonstrated with the use of NIR detection with a diffuse-transmittance geometry.

Raman Spectroscopy

TLC/Normal RS

In contrast to FTIR spectroscopy, where we have been concerned with the absorption of infrared light, RS depends on the frequency of the laser light scattered by molecules as they undergo rotation and vibration and in this respect it is similar to infrared spectroscopy. Since the selection rules are different, the information obtained from the laser Raman spectrum often complements that obtained from FTIR studies and provides valuable structural information. The intensity of Raman scattering is directly proportional to the laser excitation intensity and to the concentration of the TLC sample. This is important in quantitative studies. Therefore, laser RS can be considered as a tool for *in situ* analysis of TLC or HPTLC spots, since materials such as silica gel matrices give weak Raman spectra and minimal interference with the spectra of the adsorbed species on the TLC plate.

Using an argon-ion laser for visible excitation (488 or 514 nm) and dispersive Raman units, detection limits for different separated nonresonant substances are in the μg region. In the past *in situ* Raman microspectroscopic investigations on different TLC plates have shown that the detection limit by means of this Raman method could be improved up to the ng region. As an example, nonresonant Raman spectroscopy of representative explosive samples separated on silica gel plates have shown that visible conventional macro-sampling RS can be used nondestructively to detect and identify this substance class down to a few micrograms. The same investigations by means of visible Raman microprobe spectroscopy (scanning laser diameter of 8 μm) gave improved detection of these explosive substances (the detection limit of TNT is 0.5 μg).

TLC/Resonance RS

As the laser exciting frequency in the Raman experiment approaches an allowed electronic transition in the molecule being investigated, those normal modes that are vibronically active in the electronic transition exhibit a pronounced enhancement in their Raman intensities. Most examples of resonance-enhanced RS involve the enhancement of totally symmetric modes. The resonant Raman effect can enhance Raman intensities by factors of the order of 10^5. This means that corresponding lower concentrations of scattering molecules on the TLC spot can be used, therefore improving the detection limit. An example of the enhanced intensity from the resonance Raman effect is the investigation of silica gel TLC zones of metalloporphyrins. Using 514.5 nm excitation and an optical multichannel analyser, nanogram levels of the strong absorbing analytes (Ni-uroporphyrin, Ni-protoporphyrin) in the visible spectral range have been obtained.

TLC/FT Raman

The past few years have seen pronounced growth in the use of dispersive and interferometric RS in the field of TLC spot identification, largely attributable to increased awareness of the technique's potential as well as the new methodology and hardware. These developments include, in particular, near infrared Fourier transform Raman spectroscopy (NIR FT-Raman). Since the development of NIR lasers, both fluorescence and photodecomposition problems have been reduced or avoided in most cases. In particular, the Nd:YAG lasers emitting at 1064 nm have proven advantageous due to their long wavelength λ, high power, and stable intensity. Absorption of the TLC plates from the silica coating and the glass substrate are avoided and the full Raman spectral range may be collected without interference. Commercially available X-Y-Z stages for TLC plates can be placed directly in FT-Raman instruments and the measurement of the TLC spot taken *in situ*. One further advantage arises from the use of newly developed HPTLC plates for Raman spectroscopy (Merck, HPTLC aluminium sheet Si 60 F254s Raman). The potential and benefit of this *in situ* hyphenation of HPTLC and Raman spectroscopy in its broad spectral range, no interference by the silica matrix, identical spectra with standard Raman spectra, high sensitivity of detection and about 10-fold intensity of signal/noise compared to conventional HPTLC plates.

The feasibility of measuring the Raman spectra of chlorinated hydrocarbon pesticides on TLC adsorbents was initially studied using high concentrations (about 100 μg cm^{-2}) of separated pesticides on normal silica gel TLC plates. In the case of N-heterocyclic pesticides the detection limit on normal HPTLC plates could be reduced to low μ-gram region. As an example, **Figure 1** shows the FT-Raman investigations of the MPP(1-methy-4-phenylpyridi-

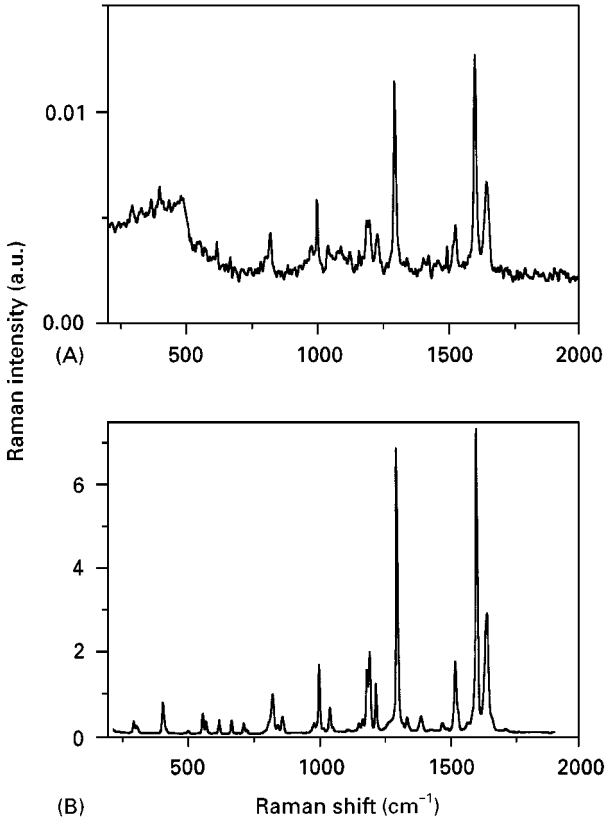

Figure 1 FT-Raman spectra of the pesticide 1-methy-4-phenylpyridiniumiodine (MPP). (A) 500 ng of MPP spotted on a HPTLC silica gel 60 plate and (B) as the pure crystalline powder. Bruker: NIR FT-Raman spectrometer RFS 100; $\lambda_{ex} = 1064$ nm, laser power 630 mW, 4 cm^{-1} resolution.

niumiodine) pesticide on a silica gel HPTLC plate and the corresponding pure material. In comparing the two spectra, no significant band shifts were observed but all bands in the spectrum of the HPTLC spot were broader than in the spectra of the polycrystalline pure substance.

In some basic experiments, the feasibility was demonstrated of obtaining artifact- and fluorescence-free spectra by *in situ* FT-Raman spectroscopy of paracetamol, fluorene and rhodamine B on silica gel TLC plates. For the strong Raman scatterer fluorene, the detection limit was found to be 500 ng for a 3 mm diameter TLC spot.

Several pharmaceutical test compounds have also been investigated and together with the use of FT-Raman spectral libraries for identification of TLC spectra and different search algorithms have been compared.

At present further work is in progress to investigate factors which could improve the *in situ* spot analysis of TLC, HPTLC and Raman-HPTLC plates by means of FT-Raman spectroscopy.

SERS Spectroscopy

The discovery that Raman vibrational signals from molecules adsorbed on nanometer scale metal particle structures are enhanced by 10^6 to 10^9 has caused extraordinary interest and excitement. This Raman technique, known as SERS offers new possibilities as a spectroscopic probe in the field of TLC separation science. It was shown that excellent Raman spectra could be obtained for low nanogram to picogram amounts of nonresonant and fluorescent substances on filter paper, paper chromatographic supports, and TLC or HPTLC plates using SERS spectroscopy.

The Raman scattering cross-section of an adsorbed molecule on nanometal structures can be further increased by utilizing a laser excitation frequency which is in resonance with an electronic transition in that molecule. This molecular resonance Raman scattering and the SERS effect can combine to give surface-enhanced resonance Raman scattering (SERRS) so that the limit of detection is further increased. Therefore, by detecting resonant molecules on TLC (HPTLC) plates in conjunction with the SERRS effect, very low concentrations have been achieved in many fields of research. Another striking feature of SERRS spectroscopy is that the fluorescence of the analyte on TLC plates can be completely quenched by the presence of a nanometal surface. Therefore, the SERRS quenching effect generates a high-quality surface Raman spectrum.

For TLC (HPTLC) this SERS or SERRS effect is accomplished by spraying chromatograms with colloidal silver solutions (reduction of $AgNO_3$ with $NaBH_4$ or citrate). In order to further improve the sensitivity of the TLC-SERS method experiments have been carried out in the field of well-defined vacuum-deposited silver films onto the separated and developed TLC plates. The TLC plates were mounted on a holder inside a vacuum chamber where silver is thermally evaporated onto the plate. The evaporation rate and the silver thickness are controlled in order to find out the most intense SERS signals. Such SERS-activated TLC plates are stable for many weeks and can therefore be considered as an 'analytical diskette' for Raman spectroscopy. In addition to these post-activation methods, two other possibilities for SERS activation can be identified: (1) the simultaneous activation of the plate with spotting of the sample, e.g. by dissolving the sample in Ag colloidal solution, (2) pre-activation of TLC plates via *in situ* decomposition of silver carboxylates.

In order to reveal the optimal conditions in TLC-SERS spectroscopy, atomic force microscopy (AFM) was applied to investigate the surface morphology of the Ag labelled TLC substance spots (colloid

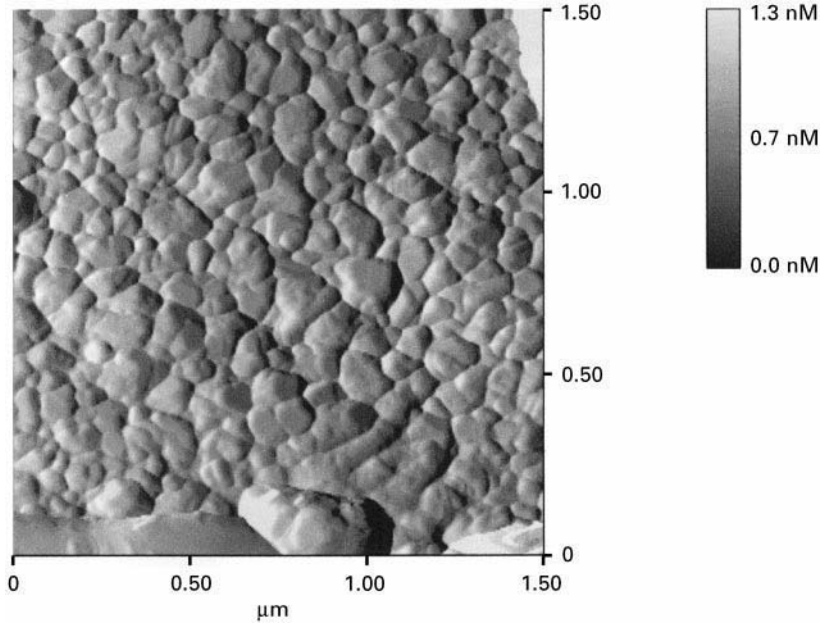

Figure 2 (See Colour Plate 120) AFM photograph of a silver-coated HPTLC silica gel KG60 plate (post-overlayer SERS activation). NanoScope III; tapping mode.

TLC-SERS and overlayer TLC-SERS). **Figure 2** shows an example of the AFM picture of a post-activated silver-coated HPTLC silica gel plate in the µm scale (post-overlayer SERS activation).

TLC/VIS-SERS

The first report on the combination of planar chromatography and SERS with colourless (non-resonance Raman scatterer) analyte spots was the direct analysis of HPTLC spots of nucleic purine derivatives in 1987. After separation and drying, HPTLC plates were sprayed to wetness with colloidal silver solution by a spray atomizer. The chromatogram zones were analysed at room temperature by a computer-controlled double beam monochromator and the excitation wavelength was the 514.5 nm line of an argon ion laser. Limits of detection were estimated to be less than 5 ng/spot. Comparison of these HPTLC/VIS-SERS spectra with normal Raman spectra of molecules in aqueous solution reveals significant differences: (1) the relative band intensities are changed due to vibration-dependent surface-enhanced scattering mechanism, (2) band shifts can occur (e.g. shift of ring breathing mode of adenine from 724 to 736 cm^{-1}), (3) band broadenings were observed in the HPTLC/VIS-SERS spectra.

In clinical chemistry, immunoassay methods in conjunction with TLC play an important role in the routine determination of active substances in body fluids. For instance it is possible to separate theophylline without difficulty from its positional isomers and from other xanthine derivatives. *In situ* identification of these compounds using online HPTLC/VIS-SERS can be performed in the ng region.

Examples of TLC/VIS-SERS measurements from many research groups have been selected to illustrate the high sensitivity, molecular specificity, accuracy, easy SERS sample preparation, and the significant manifold application.

The result of all these investigations is that the TLC/VIS-SERS detection appears to depend on different factors which must be considered before any normal application. Close approach to, or direct contact with, silver colloids of investigated analytes are a prerequisite of Raman scattering enhancement. Furthermore the size, shape, dimension and electrical charge density of the silver colloids are very important. Control and optimization of all these parameters would increase the feasibility of TLC/VIS-SERS *in situ* detection to a maximum number of chemical compounds separated on TLC(HPTLC) plates.

TLC/SERRS

The surface-enhanced resonance Raman effect in the field of chromatogram spot identification, first reported in 1984 by Tran (see Further Reading), has led to the study of a variety of separated dye molecules with different chromatographic techniques. Three structurally similar dyes, crystal violet, malachite green and basic fuchsin were chosen in order to show the potential of SERRS for the direct identifica-

tion of chromatogram spots separated by means of paper chromatography. The detailed vibration spectra allowed identification and the limit of detection was 2 ng cm^{-2}.

After this work, different types of dyes, TLC (HPTLC) plates, the role of the supporting matrix and sol preparation protocols were investigated and examined for their influence on the SERRS signal. The investigations of all these effects and the construction of a remote sensing Raman spectrometer to investigate the model compound pararosaniline resulted in the maximum SERRS intensity yielding a detection limit of about 108 femtomol (33 pg) of this pararosaniline dye.

Another very interesting application is the incorporation of SERRS as a detector for a liquid chromatograhpy (LC)-coupled TLC system. In this SERRS/LC/TLC system, effluent from the LC system was deposited onto the TLC plate and an activated Ag sol was added to the plate. Optical fibres carried the 514.5 laser light to the TLC plate and the scattering radiation to the Raman spectrometer. The dye molecule, pararosaniline acetate, was found to give a linear SERRS signal over the concentration of 1×10^{-5} to 1×10^{-7} M range and the limit of detection was 750 fmol.

The measurements of other highly fluorescent molecules such as acridine orange, rhodamine, Glu-P2 as well as N-containing PAHs separated on HPTLC plates have shown that the sensitivity is so high that *in situ* vibrational investigations are possible with low picogram amounts of material.

Today, it is clear that TLC/SERRS spectroscopy can be widely applied to obtaining structural information about very small amounts of coloured substances (dyes, pigments) separated by TLC or HPTLC.

TLC/Micro-SERS

Following the rule that 'an optimized Raman scattering sample is a micro sample' Raman microspectrometers have been designed for investigation of small particles in the μm range. Although there is earlier work in the coupling of a microscope and a Raman spectrometer, by far the largest amount of work has been carried out in the past 10 years. The new generation of Raman microprobe spectrometers comprise modern monochromators with notch filter systems, charged coupled device (CCD) detectors and confocal optics. This type of laser confocal Raman microspectrometer permits the acquisition of Raman spectra from TLC(HPTLC) spots down to 1 μm in size and allows the unambiguous placement of the laser focus at the chromatogram with a spatial resolution of 1 μm.

In the first study on HPTLC/Micro-SERS, a Raman microspectrometer consisting of an argon ion laser, a microscope, a triple monochromator and a multichannel detector was used. With this system it was possible to acquire VIS-SERS spectra of silica gel HPTLC spots of DNA bases and dibenzofuran at the low picogram level. Considering the fact that the laser focus is about 1 μm, the irradiated spot mass is only a few fermtograms. Combining this microsurface-enhanced Raman scattering and HPTLC has also enabled *in situ* analysis of chromatogram spots of cationic surfactants in amounts down to subnanogram levels.

Micro-Raman equipment and the SERRS effect have been used for selective detection of structurally similar aminotriphenylmethane dyes separated by HPTLC. *In situ* SERRS spectra were recorded after the application of aqueous Lee-Meisel hydrosol solution (reduction of silver nitrate with sodium citrate) to the analyte spot. The limits of identification of the dyes are of the order of 5 ng (applied amount).

Recently the optical detection and spectroscopy of single molecules and single nanoparticles was achieved with the use of SERRS spectroscopy for rhodamine 6G adsorbed on selected silver nanoparticles. This was the first application of Raman spectroscopy in the field of 'probing single molecules by means of Raman vibration spectroscopy'.

As a result of the dramatic improvement of the sensitivity in the coupling of TLC and SERRS microspectroscopy, it is possible to probe low numbers of molecules on TLC plates. **Figure 3** shows the high sensitivity of TLC/Micro-SERRS spectroscopy by demonstrating the identification of rhodamine 6G on a HPTLC plate at a concentration as low as 1 μL of 10^{-10} M solution applied on the plate. Considering the fact that the 488 nm laser spot is only 1 μm in diameter, the number of R6G molecules in the analysed area are substantial between 400 and 600.

Figure 4 shows the topography of a selected area from the silver colloidal SERS activated silica gel (KG60) HPTLC spot of R6G. This AFM picture clearly shows the Ag nanoparticle adsorbed on the silica gel particles of the HPTLC plate. Depending on the colloid aggregation on the substance/silica zone, the diameter of the Ag particles is in the region between 10 and 60 nm.

According to the short range sensitivity of SERRS (about 1 nm from the surface) the spectra obtained from the HPTLC spot are attributed to molecules which have a direct contact to the surface of the colloids.

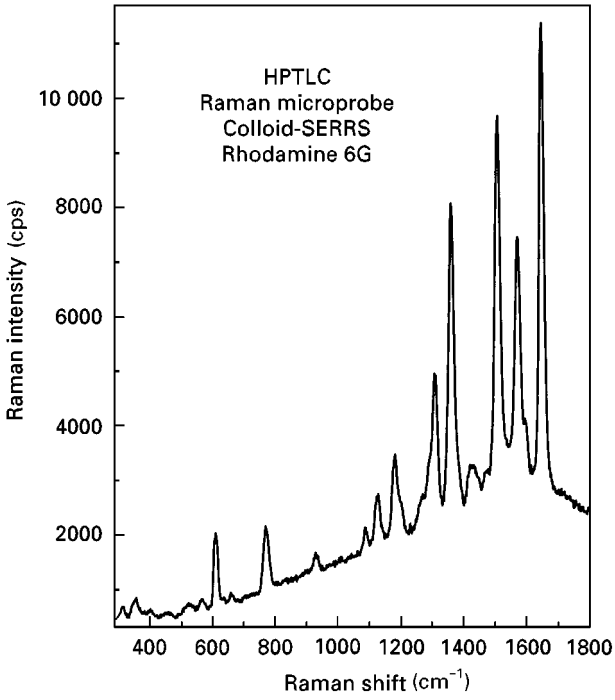

Figure 3 HPTLC/SERRS microprobe analysis of rhodamine 6G. 1 μL of 10^{-10} M R6G applied on the silica gel (KG60) HPTLC plate. 1 μm selected with the focused laser beam. Laser excitation line, 488 nm; laser power at the spot, 8 mW; integration time, 3 s; number of reads, 80. Number of molecules in the scattering volume 400–600 R6G molecules.

TLC/FT-SERS

Some years ago, NIR FT-SERS spectroscopy was suggested as an alternative experimental method to conventional dispersive Raman spectroscopy in TLC/SERS. Because of the NIR excitation at 1064 nm and the fluorescence-quenching effect of the SERS effect, TLC plates containing indicator could be used without any problem. In the first TLC/FT-SERS experiments, good-quality FT-SERS spectra of a spot of 40 ng rhodamine B was recorded after spraying with a silver colloid solution. These preliminary results have shown that the SERS effect also works on TLC plate spots in the NIR spectral range. In general, it was also found that the enhancement factor at 1064 nm excitation was increased by one or two orders of magnitude as compared with the 514.5 laser excitation.

Direct analysis of sub-femtogram quantities of carotenoids on different types of TLC plates has been made possible by associating FT-Raman micro spectroscopy with the SERS effect. In this HPTLC/micro-FT-SERS experiment, detection of 10^{-5} M crocetin corresponded to a deposited mass of 1.65×10^{-8} g, thus in the 8 μm laser spot an analysed mass of 0.02 fg. These postactivation SERS investigations have also shown that SERS intensity is not dramatically influenced from the TLC layer thickness, particle size distribution, mean particle size and presence or absence of a fluorescence indicator.

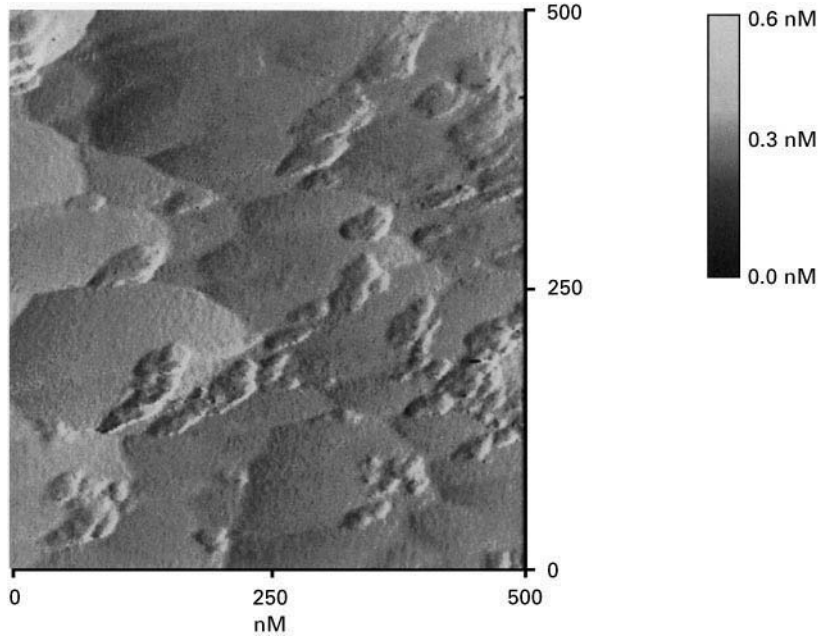

Figure 4 (See Colour Plate 121) AFM (tapping mode) surface plot of a silver colloidal SERRS activated silica gel plate (KG60, Merck). A selected X = Y = 500 nm spot area of a 1 μL of 10^{-10} M rhodamine 6G spotted on the plate. The silver hydrosol marker has a diameter of between 10 and 60 nm.

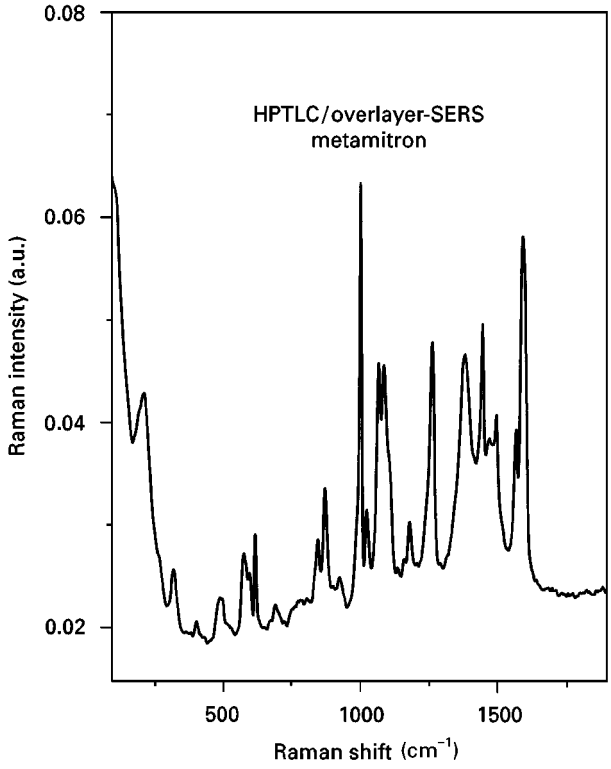

Figure 5 HPTLC/FT-SERS analysis of metamitron on a KG60 plate with a silver-coated layer of 20 nm thickness. 1 μL of 10^{-5} M metamitron spotted on the KG60 plate. Bruker: NIR FT-Raman spectrometer RFS 100 S; $\lambda_{ex} = 1064$ nm, laser power 400 mW, 4 cm^{-1} resolution.

The possibility of TLC/FT-SERS pre-activation of TLC plates has been demonstrated for heterocyclic and aromatic species (pyrene, anthracene fluoranthene, biphenyle and benzoquinoline). The pyrolysis of pre-deposited silver oxalate forms FT-SERS system based on TLC plate. This thermal decomposition of silver oxalate represents a new, simple method of pre-activation in TLC/FT-SERS spectroscopy. The disadvantage is that R_F values on these pre-activated TLC plates are strongly influenced by the Ag clusters on the plate. Therefore, in this case one has new separation conditions on the TLC plate.

The most useful and effective method for TLC/FT-SERS spectroscopy is the postchromatographic silver coating of the TLC plate inside a vacuum evaporator (overlayer TLC/FT-SERS). As an example to demonstrate this new technique, a HPTLC/FT-SERS spectrum of metamitron (1 μL of 10^{-5} M metamitron spotted on HPTLC-KG60 plate) which was coated with a 20 nm thick layer of silver, is shown in **Figure 5**. Therefore this overlayer TLC/FT-SERS spectroscopy is very suitable for the investigation of separated pesticide spots on TLC plates.

As a consequence of their advantages the combination of TLC/FT-SERS techniques with silver coating of the TLC plate inside a vacuum evaporator will extend the use of surface-enhanced Raman spectroscopy to a much greater range of samples than examined so far.

Conclusion

The examples of the coupling of TLC and vibration spectroscopy in the separation technique reviewed in this article have been selected to illustrate the sensitivity, molecular specificity of separated substance zones, accuracy, ease of sample preparation, and the many significant applications of FT-IR, Raman analysis and SERS spectroscopy for substances in the adsorbed state on TLC (HPTLC) plates. The sensitivity and rich spectral information that FT-IR, Raman and SERS provide have spurred the rapid development of technology for using vibration spectroscopy as an analytical detection method of separated TLC spots. With the extremely high enhancement of the Raman scattering signals on SERS-activated TLC plates it is possible to identify *in situ* separated TLC spots at the pico- and femtogram level. Further work is in progress to investigate factors which could improve the utility of vibrational spectroscopy in chemical structure elucidation of separated TLC zones.

See Colour Plates 120, 121.

See also: **II/Chromatography: Thin-Layer (Planar):** Modes of Development: Conventional. **III/Dyes:** Thin-Layer (Planar) Chromatography. **Pesticides:** Thin-Layer (Planar) Chromatography. **Pigments:** Thin-Layer (Planar) Chromatography.

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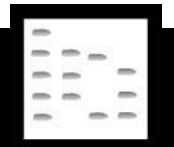
TOBACCO VOLATILES: GAS CHROMATOGRAPHY



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Introduction

The leaves of tobacco plants, *Nicotiana tabacum*, have been shown by Tso and Stedman to consist of a wide array of organic and inorganic compounds. For general classification, the components of the leaf can be described as volatile, semivolatile and nonvolatile. The nature of the compounds making up the components of the leaf range from nonpolar constituents such as hydrocarbons to very polar compounds such as carboxylic acids and amines. The structural identity of these components has been determined in many laboratories, including those of DeMole and Lloyd, through the use of classical wet chemical separation techniques coupled with end determinations involving, in some cases, further separations by gas chromatography (GC). In some cases, headspace volatiles have been trapped on Tenax or charcoal then either thermally or solvent desorbed. Supercritical carbon dioxide has also been used for extraction. In some of the earlier work, the separations were performed on packed glass GC columns and somewhat later on coated glass columns.

With the advent of multidimensional GC (MDGC), the labour-intensive sample preparation techniques associated with wet chemical separations of selected fractions were for the most part eliminated. With the MDGC approach, a concentrated solution of a tobacco essential oil in a volatile organic solvent can be directly injected on to a fused silica capillary precolumn, followed by heart-cutting on to another column. The phase of the pre-column of the MDGC system essentially serves as a replacement for the wet chemical separation approach. In this way, as many as 80 new compounds were tentatively identified in a flue-cured tobacco essential oil. Detection and identification of the volatile components of the essential oils were possible through the use of mass selective detectors and matrix isolation Fourier transform infrared spectroscopy.

Another approach for the lower molecular weight volatiles employed automated purge-and-trap (P & T)-GC with flame ionization (FID) and mass selective detection (MSD). A relatively nonpolar DB-5 ((5% phenyl)methylpolysiloxane) column was employed for the separation of the volatiles. In further advances on the P & T method, the influences of ionic strength and pH on the yield of volatile materials have been reported. By employing a cryofocusing approach coupled with a DB-1701 ((14% cyanopropyl-phenyl)methylpolysiloxane) fused silica column of intermediate polarity, and an internal standard, semiquantitative analytical data were provided on the volatiles from a variety of natural products.

Through the use of multiple fused silica GC columns of different bonded phases, a universal commonality describing the nature of the volatile materials from a wide range of natural products has been developed. The types and profiles of volatile low molecular weight compounds found in the headspace above tobacco leaves has been shown to bear remarkable similarities to the profiles from a diverse array of heat-treated natural products such as coffee, peanuts and soybeans. The common thread linking all of the products has been ascribed to the presence of specific reactions between amino acids and other nitrogen sources with sugar molecules to yield volatile components such as pyrazines and aldehydes. For details of the work outlined in this section, see the papers by Coleman *et al.* in the Further Reading section.