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# Effect of the preparation conditions on the surface-enhanced Raman-spectrometric identification of thin-layer-chromatographic spots

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## Abstract

In situ surface enhanced Raman spectroscopic (SERS) analyses of thin layer chromatographic (TLC) spots of *p*-dimethyl-amino-benzylidene-rhodanine have been carried out using Nd-YAG and He-Ne lasers. With both lasers the enhancement factor was influenced by the analyte to metal (silver or gold) ratio as well. Since the enhancement factor with the Nd-YAG laser showed a stochastic distribution below 5 µg/TLC spot concentration, the NIR-SERS method can be used for in situ qualitative analyses, only. With the He-Ne laser, however, SERS spectra could be recorded in the pmol region using a concentrated silver sol. In the 1–5 µg/TLC spot region a linear relationship has been found between the SERS signal and the concentration for at least six different Raman bands. Therefore, an in situ qualitative and quantitative determination can be carried out in a single step. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Thin-layer chromatography; FT-Raman spectrometry; Raman microscopy; Surface enhancement

## 1. Introduction

Thin-layer chromatography (TLC) is a powerful separation technique, since the efficiency of separation can be increased with the change of several parameters. Generally, the detection of the components after separation is carried out by colour reagents or, more recently, by a UV lamp on TLC layers preliminarily activated by fluorescent dyes (e.g. F254). These methods, however, are not suitable for the identification of unknown analytes. Detection of TLC spots by Fourier transform infrared

(FTIR) spectroscopy is widely discussed in the literature [1–3]. These efforts are aimed at the identification of analytes of overlapping chromatographic spots via recording the full vibrational spectra of the partially separated compounds. In certain cases in situ quantitative analyses are also feasible [3]. In most of the cases, however, the in situ IR detection of TLC spots is difficult due to the spectral disturbance of the TLC layer itself.

Since the Raman scattering of the stationary phase is negligible or can be compensated, the disturbing effect of the layer can practically be ignored [4]. Under normal conditions the intensity of the Raman scattering is inherently low. With the use of the surface enhanced technique, however, the signal

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intensity can be increased with several orders of magnitude. The application of surface enhanced Raman spectroscopy (SERS) in the study of chromatographic systems is very promising, especially for post-separation detection [5–7].

The SERS effect can only be observed on special surfaces (e.g. etched Ag, Au, Cu surfaces or in the presence of Ag, Au, Cu metal sols). Therefore, the SERS spectrum of an analyte can only be recorded if the TLC spot is treated with a suitable metal sol or vapour. Both the post or pretreatment of the TLC layer as well as the simultaneous application of a metal sol were already reported [6,8,9]. The SERS technique is a potential tool in the hands of the chromatographer if all the parameters influencing the Raman signal can be kept under strict control and the activation procedure is simple.

In the present study, the applicability of SERS for the in situ quantitative analysis of TLC spots is demonstrated via the example of *p*-dimethyl-aminobenzylidene-rhodanine (rhodanine). It is demonstrated that with the proper selection of the excitation laser and the metal to analyte ratio convenient analyses can be carried out without disturbing the process of chromatographic separation.

## 2. Experimental

Aliquots of 5  $\mu\text{l}$ , containing 1, 2, 5 and 10  $\mu\text{g}$  rhodanine (Reanal, Budapest) in tetrahydrofuran solutions, were prepared onto a Kieselgel 60 F254 (Merck, 0.2 mm) type stationary phase. The chromatograms were developed using an acetic acid (15% v/v)–water eluent mixture. The development of the chromatograms was necessary to establish real separation conditions. After drying, the TLC spots were activated in two different ways depending on the type of the excitation laser used.

### 2.1. Sample preparation for NIR–SERS tests

Evaporation of silver and/or gold onto the TLC surface was performed by means of a Leica-SPC 020C type vacuum evaporator. The maximum SERS effect was recorded with 50 mm electrode distance, 20 mA current and 120 s evaporation time. Under these conditions the size of the evaporated metal

particles was in the 20–80 nm range. SERS spectra were recorded by means of a Bio-Rad dedicated FT-Raman spectrometer (Nd-YAG laser, 500 mW laser power, 180° optics, 4  $\text{cm}^{-1}$  resolution and 300–800 scans).

### 2.2. Sample preparation for Raman microprobe spectroscopy

A silver sol was prepared following the method of Lee and Meisel [10], which was then concentrated ten times by centrifugation (4000 r.p.m., 30 min.). Portions of the sol, 3  $\times$  5  $\mu\text{l}$ , were applied to the spots by a microsyringe. The spots were dried by hot air between the subsequent additions of the sol. SERS spectra were recorded by means of a Renishaw 1000 type Raman microscope system connected to an Olympus BHSM microscope having 10 $\times$ , 20 $\times$  and 50 $\times$  objectives, including a monochromator, a filter system and a charge-coupled device (CCD). Raman spectra were excited by a Spectra Physics model 127 He-Ne laser (633 nm) and recorded at a resolution of 2  $\text{cm}^{-1}$ .

## 3. Results and discussion

The molecular structure of rhodanine used as a model compound is given in Fig. 1. Rhodanine is connected to the silica gel type stationary phase via hydrogen bonds established through the C=O, C=S and N–H groups. Therefore, the bands of the aromatic benzene and thiazolidine rings can be expected to appear in the Raman spectra [11–13].

Fig. 2 shows that in the FT-Raman spectrum of the 5  $\mu\text{g}$  TLC spot (spectrum b) only the ring stretching band at 1584  $\text{cm}^{-1}$  can be used for analytical purposes. (With the He-Ne laser the same

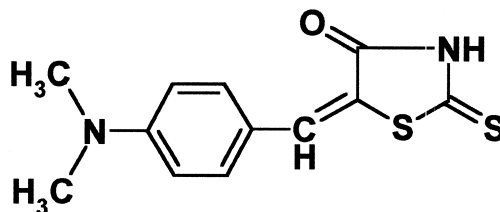


Fig. 1. Molecular structure of Rhodanine.

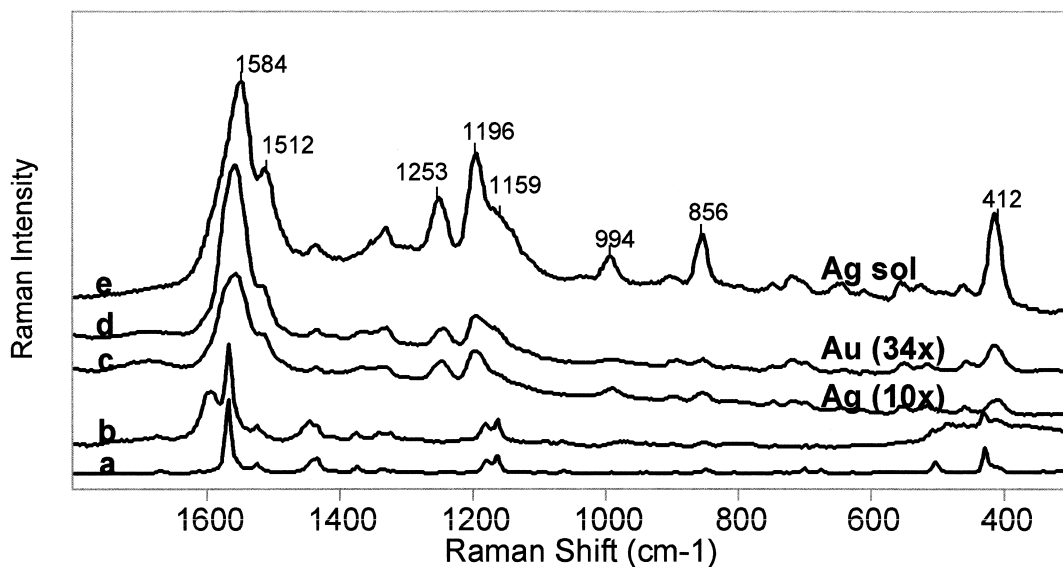


Fig. 2. Raman spectra of rhodanine: (a) powder, FT-Raman with Nd-YAG laser; (b) on TLC plate, FT-Raman with Nd-YAG laser; (c) SERS on TLC plate with Nd-YAG laser and silver vapour; (d) SERS on TLC plate with Nd-YAG laser and gold vapour; (e) SERS on TLC with He-Ne laser and silver sol.

spectral information can be obtained with less favourable signal to noise ratio and a shifted baseline as a result of fluorescence). With the evaporation of silver and gold onto the TLC spot, spectra c and d were recorded, respectively. The signal intensity increased 10 times with the evaporation of silver and 34 times with the use of gold vapour. It should be noted, however, that no SERS effect was obtained with the silver sol which, after Matejka et al. [6], can be due to the influence of the TLC layer on the morphology of the silver particles. This observation is in harmony with the theoretical consideration that with low energy lasers a higher enhancement factor can be anticipated using gold or copper [12]. In addition to the laser wavelength and the quality of the metal used, the roughness of the surface and the dielectric properties of the analyte can influence enhancement in a complex way. According to our experience, the analyte to metal ratio also has an important role in the development of the enhancement factor.

Fig. 3 shows the enhancement factor calculated for the  $1584\text{ cm}^{-1}$  rhodanine band from three parallel measurements as a function of the analyte load using gold vapour and a Ne-YAG laser. The enhancement

factor shows a stochastic distribution below the  $5\text{ }\mu\text{g/TLC spot load}$ , then it goes through a maximum at cca.  $5\text{--}6\text{ }\mu\text{g analyte/TLC spot concentration}$ . It can be seen that reproducible results can be obtained over the  $5\text{ }\mu\text{g/TLC spot load}$ . Another difficulty is the rapid decrease of the signal when the Ne-YAG laser is used. (After 50 min there is practically no effect with silver vapour; with gold the signal is decreased by cca. 50%). If it is possible to accumulate a sufficient number of spectra (to improve the signal to noise ratio) in approximately 10 min after applying the metal, some 5–10% of the signal intensity is lost only. In this way a detection limit of cca.  $30\text{ ng/mm}^2$  could be achieved for rhodanine. Considering the size of the TLC spot and the Raman cross sectional area (the analytical information is obtained from an area of cca.  $1\text{ mm}^2$ ), this is equivalent with a total analyte load of  $2\text{ }\mu\text{g/TLC spot}$ .

The best quality SERS spectra were obtained with the use of  $3\times 5\text{ }\mu\text{l}$  concentrated silver sol and He-Ne laser. Due to the faster agglomeration, the concentrated sol ( $\sim 10^{-2}\text{ M}$ ) can only be used with safety for approximately two weeks after preparation. The disturbing effects caused by fluorescence can be

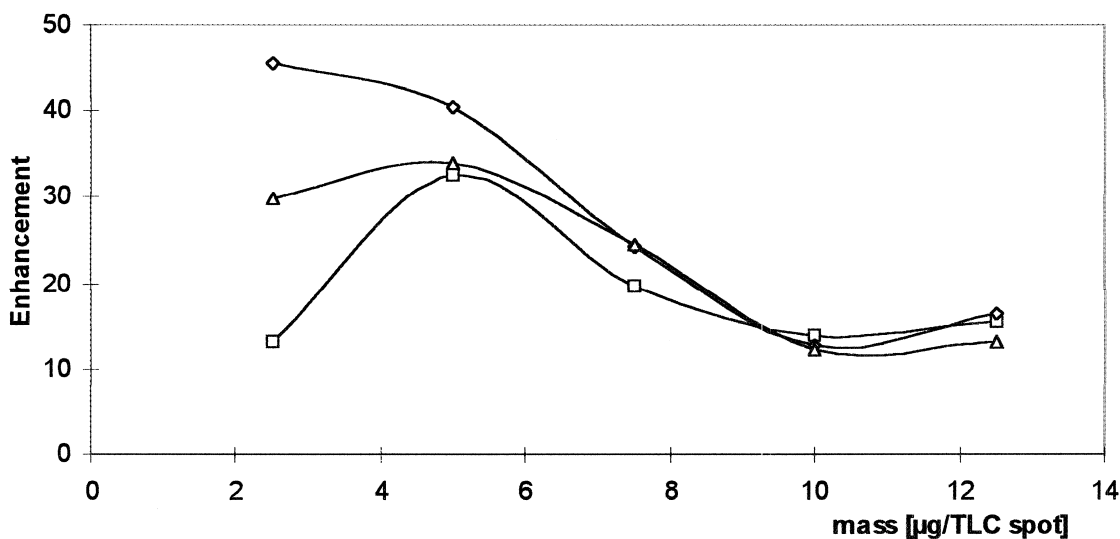


Fig. 3. The enhancement factor as a function of the analyte load on the TLC layer (three parallels).

reduced to a minimum with the use of an optimal amount of metal on the surface (e.g. with a cross sectional area of  $1 \mu\text{m}^2$  using the He-Ne laser, spectrum e in Fig. 2 represents a  $10^{-5}$  ng sample when the analyte load is  $1 \mu\text{g}/\text{TLC spot}$ ). While with the Ne-YAG laser, significant increase in band intensity was obtained in the  $1600\text{--}1520 \text{ cm}^{-1}$  and the  $1400\text{--}1100 \text{ cm}^{-1}$  regions only, at least eight intense bands could be recorded with the He-Ne laser. Since rhodanine is connected to the stationary phase through polar groups, the bands belonging to the aromatic and the thiazolidine rings showed the highest increase in intensity. The change in intensity of the bands as a function of time is given in Fig. 4.

It can be seen that after approximately 130 min the enhancement becomes practically constant. It can also be experienced that in the  $1\text{--}5 \mu\text{g}/\text{TLC spot}$  range, the enhancement shows increasing tendency, while in the  $5\text{--}10 \mu\text{g}/\text{TLC spot}$  range – in accordance with Fig. 3 – a decrease is observed. In addition, the band ratios also change with time (see Fig. 4), therefore, the analyte concentration versus band intensity function shall be considered at identical time intervals. Since the enhancement factor shows little change in the  $120\text{--}190$  min range, the concentration dependence of the signal intensity was considered 135 min after applying the silver sol.

Six out of the eight most intense bands of the

SERS spectra show very good correlation between Raman intensity and concentration (see Fig. 5). The worst fit was obtained for the  $1512 \text{ cm}^{-1}$  ( $r^2=0.977$ ) and the  $1584 \text{ cm}^{-1}$  ( $r^2=0.976$ ) bands not shown in Fig. 5. Due to the low cross sectional area of the excitation laser beam, the analytical signal is collected from an area of less than  $\sim 0.8 \mu\text{m}$  diameter. In view of this fact, the in situ SERS analysis of TLC spots of lower loads can be very promising. Considering the fact that with Raman intensity values of  $250\text{--}500$ , a spectrum with reasonably good signal to noise ratio can be obtained, a detection limit of  $0.1 \text{ nM}/\text{TLC spot}$  (cca  $0.03 \mu\text{g}/\text{TLC spot}$ ) could be achieved for rhodanine on Kieselgel 60 ( $0.2 \text{ mm}$ ). Since the Raman cross sectional area is always significantly lower than the spot area, the detection limit can be increased with the increase of the local concentration (i.e. with the reduced spread of the TLC spots).

#### 4. Conclusion

Considering the average load of a typical TLC layer and the change of the enhancement factor with time, the NIR-SERS spectroscopy can be recommended for the detection of  $2\text{--}6 \mu\text{g}/\text{TLC spot}$  analyte quantities. Although the detection limit is

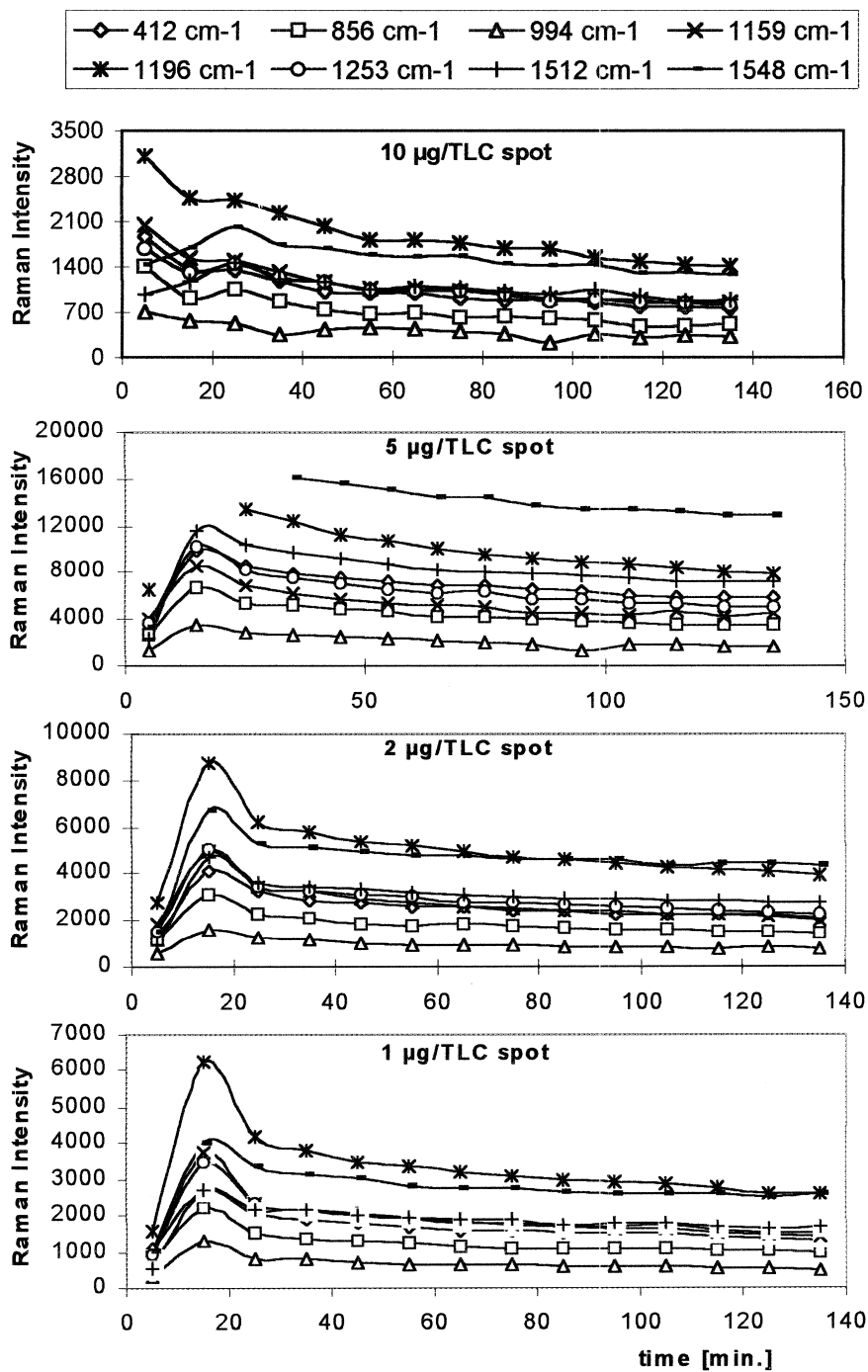


Fig. 4. The intensity change of the six most intense bands of rhodanine as a function of time.

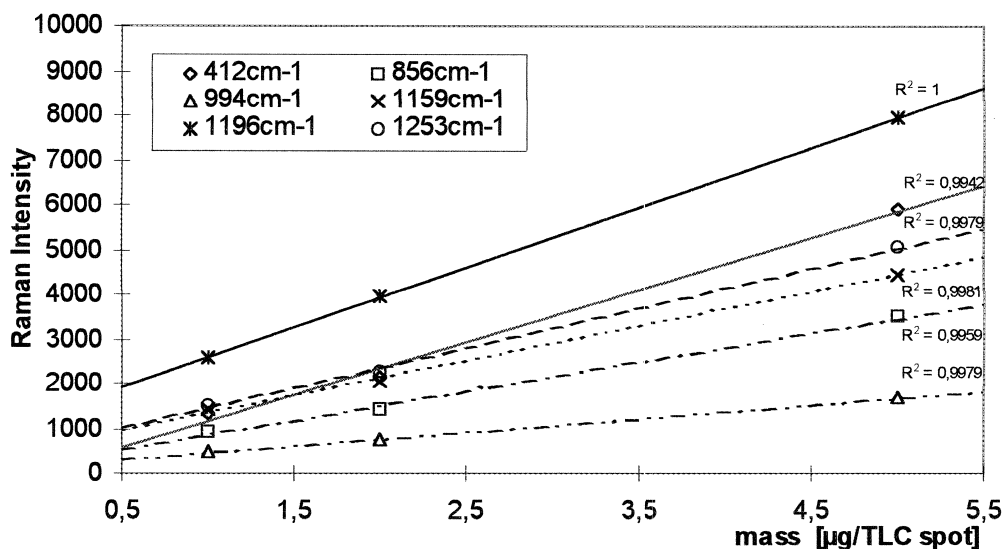


Fig. 5. Raman intensity versus analyte mass curves for six selected bands.

similar to those of the fluorescence or colour reagent techniques, the possibility to record the entire vibrational spectrum is of great advantage. With the use of the He-Ne laser, the detection limit can be reduced by two orders of magnitude. It is a further advantage that with the He-Ne laser more bands appear in the SERS spectra, thereby material identification and quantitation can be carried out in a more convenient way.

The SERS technique can expect a widespread application if the reproducibility of microscopic surfaces can be achieved. When the SERS method is to be used for both identification and quantitative determination, it is necessary to select the right surface treatment method and analyte load.

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