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Short communication

Coupling of high-performance liquid chromatography with Raman spectrometry

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Abstract

Raman spectrometry has been tested as a method for detection in HPLC. Using a laboratory-made cell similar to classical UV cells, toluene and naphthalene have been analyzed as solutions in acetonitrile–water (75:25 or 95:5 v/v) mixtures. Using a monochannel detection, a detection limit of about 480 mg/l (9.6 μg for the 20-μl injection loop) for toluene has been found and the possibility to distinguish between co-eluted toluene and naphthalene and to make a quantitative analysis has been proved.

Keywords: Raman spectrometry; Toluene; Naphthalene

1. Introduction

HPLC (high-performance liquid chromatography) is widely used in analytical chemistry as a technique for the separation and analysis of various chemical mixtures. Several detection techniques are at present available for HPLC, the most frequently used being UV absorption and refraction index measurements [1–4] because of their simplicity of use and the reasonable cost of the corresponding apparatus. In order to get structural information on the eluted compounds, NMR (nuclear magnetic resonance), mass or infrared absorption spectrometric techniques are also used [5–14], each of them has its own advantages and disadvantages.

Raman diffusion, like infrared absorption, corresponds to vibrational transitions and can help to identify the chemical species, but unlike infrared spectroscopy, most solvents used for HPLC have weak Raman spectra and this is particularly true for water. Thus, the whole frequency range can usually be investigated accurately. Moreover, it is a non-destructive method applicable to all kinds of mixtures, organic and inorganic solutions, and no sampling is needed. Various studies made in this laboratory have shown its applicability to remote in situ, real time, on-line and multi-site analyses [15], increasing the potential of the method. Because of the weakness of the Raman effect, the coupling of HPLC and Raman techniques has been done using the resonance Raman or the SERS effect (surface enhanced Raman spectroscopy) [16–18]. Since these techniques are not of universal use as numerous compounds present good Raman spectra without showing either resonance Raman or SERS effects, it

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appears necessary to optimize the performances of the HPLC–classical Raman coupling first.

In this paper, some performances of a laboratory-made Raman detection cell using classical Raman effect coupled to a HPLC unit are described.

2. Experimental

2.1. The Raman cell

The cell used (Fig. 1) is very similar to the principle UV cells used for HPLC. It is made from a stainless cylinder drilled along its axis to make an inner chamber 10 mm long and 1 mm in diameter. Two quartz windows close this chamber, enabling the laser excitation radiation to be transmitted and the Raman emitted radiations to be collected, while two small perforations perpendicular to the chamber, one at each end, permit a continuous flow of the mobile phase. The analyzed volume is about 7.8 μl. The major improvement of this cell, compared to conventional UV cells lies in the polishing quality of the inner surface of the measuring chamber which is made down to 20 nm, and is greatly superior to the traditional mirror reflective limit ($\lambda/4$) for the 514.5 nm radiations used in the present Raman measurements.

2.2. The Raman spectrometer

The Raman spectra and the chromatograms were obtained using a monochannel triple monochromator DILOR RTI 30 spectrometer. The excitation radiation (1 W at 514.5 nm) was obtained with a Spectra-Physics 2000 ionized argon laser. The collection of the Raman radiations towards the spectrometer was made through an entrance slit 18 mm high and 0.9 mm wide giving a resolution of about 8 cm^{-1} (using a 6× optics).

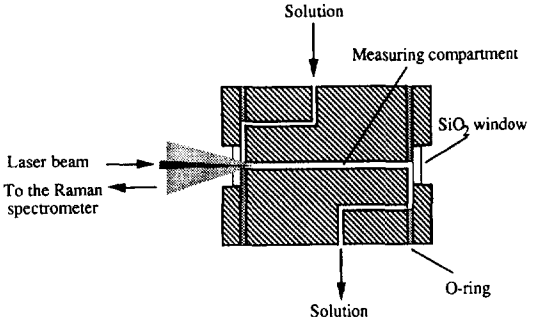

Fig. 1. Diagram of the HPLC Raman cell.

2.3. The chromatographic unit

The Vademecum Waters 510 pump was fixed to a Merck column Lichrosphere 100 RP (particle size: 5 μm; reversed phase; stationary phase: ODS; length and internal diameter of the column: 250 mm and 4 mm) equipped with a Merck Lichrocart 4-4 pre-column.

2.4. Chemicals

HPLC grade toluene, naphthalene, and acetonitrile, from Fluka, were used without further purification. Ultrapure water obtained by triple osmosis was used for preparing the solvent. All the samples were prepared by successive dilutions, starting from a 10 g/l mother solution.

2.5. Analytical procedure

In order to test the feasibility of this coupling, a classical chromatographic case was chosen: measuring toluene with an acetonitrile–water (75:25, v/v) mixture as eluent. Since a monochannel detection spectrometer was used, the signal had to be recorded, as a function of time, at a characteristic frequency of toluene, chosen at the intense Raman peak at 1002 cm^{-1} of the aromatic ring. All chromatograms were recorded for a flow-rate of 1 ml/min (16.7 μl/s), with an integration of the signal and a reading every 5 s. The injection is made through a loop of 20 μl.

3. Results

3.1. Tests for feasibility

The sensitivity of the mounting was first checked using a solution directly injected into the measuring chamber, without elution. A detection limit of about 2 ppm (2×10^{-5} M) of toluene in the acetonitrile –water (75:25, v/v) solvent was thus obtained.

Comparing the signal with the one obtained using the classical setting with standard capillary tubing of about 1 mm diameter, placed perpendicular to both the laser and the entrance slit, the new Raman cell gave a signal approximately 10^3 times stronger. Coupling the cell with the HPLC apparatus, several different solutions of toluene were analyzed in the same acetonitrile–water (75:25, v/v) solvent as for elution. The chromatograms obtained, as shown in Fig. 2, clearly present a peak at 75 s due to the perturbations in the flow corresponding to the introduction of the sample in the column, and another peak, at 275 s whose intensity increases with the concentration of toluene. According to these spectra, the actual detection limit can be estimated at about 480 mg/l toluene in the 20 μl of injected solution (assuming that a peak is observable when the signal-to-noise ratio is bigger than 3). This corresponds to 9.6 μg of toluene injected, i.e. 10.4×10^{-8} mol.

In order to study the repeatability of the measurements, the following experiment was repeated 10 times: 24 μg of toluene are injected into the cell and the intensity of the peak at 275 s is measured on the chromatogram. The mean square variation found was 8%. This relatively high variation is due to various causes such as electronic and optic stray noises, fluctuations of the laser power and of the flow-rate. It is also worth noting that the alignment of the laser with the axis of the cell is not perfect in these first experiments. The optical setting needs to be more optimized in order to obtain a perfect introduction of the laser beam into the cell, resulting in a better collection of the Raman diffusion.

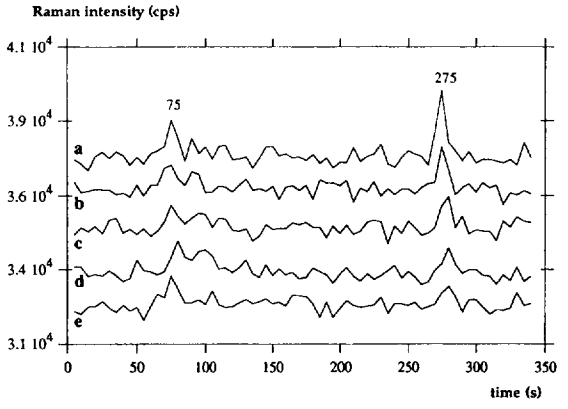

Fig. 2. Chromatograms obtained by the elution of solutions of toluene in the acetonitrile–water solvent (75:25, v/v) (a: 19.2 μg; b: 14.4 μg; c: 9.6 μg; d: 7.2 μg; e: 4.8 μg).

Finally, to check the linearity of the measurements, chromatograms of toluene were recorded with the amounts of toluene injected varying from 4.8 to 19.2 μg, comprising seven series of experimental prints, each point representing the average of five consecutive measurements of the same solution. A plot of the intensity of the peak at 275 s as a function of the amounts of toluene injected effectively does give a straight line with a regression coefficient of 0.995.

3.2. Selectivity test: identification of two co-eluted products

The major interest of Raman diffusion detection, compared to UV absorption detection, is the possibility of both detecting and making the spectroscopic analysis of the eluted product. Thus, when two or more products are co-eluted, it becomes possible to distinguish them from one another by studying the Raman spectrum recorded during the chromatographic analysis. This possibility is demonstrated with toluene–naphthalene mixtures taken as an example.

Toluene effectively presents a strong characteristic peak at 1002 cm^{-1}, easy to distinguish from that of naphthalene at 759 cm^{-1}. These peaks, being sufficiently distinct from that of the solvent, were chosen to be used for detection of the corresponding products (Fig. 3). Chromatograms corresponding to the elution of toluene (with the monochannel detector set at 1002 cm^{-1}) and to the elution of naphthalene (at 759 cm^{-1}), using the acetonitrile–water (95:5, v/v) mixture as solvent show that these two products present the same elution time (within 5 s) (Fig. 4) and should thus be co-eluted by this solvent when they are mixed. To test the selectivity of Raman detection, measurements were made on three different mixtures of toluene and naphthalene in the same conditions. For each mixture, the chromatograms were recorded twice, one with detection at 1002 cm^{-1} and one with the detection at 759 cm^{-1}. The results are presented in Fig. 5. For both detection settings, the variation of the intensity of the toluene and of naphthalene peaks is in good agreement with the corresponding variations of the concentration of these two products in the three analyzed mixtures.

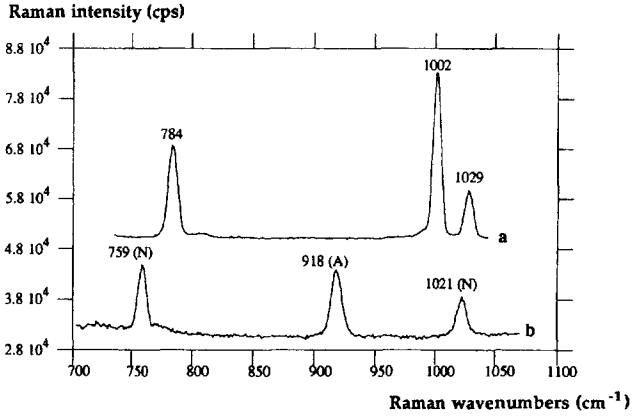

Fig. 3. Raman spectra of pure toluene (a) and of 4 g/l solution of naphthalene in acetonitrile–water (95:5, v/v) solvent (b). (N: naphthalene peak; A: acetonitrile peak).

4. Conclusion

This preliminary study shows that the direct coupling of a Raman spectrometer, used as a detector, with a HPLC column, poses no major technical problem and the performances, even when using a non-dedicated Raman device, are good. The identification of species which are co-eluted in HPLC yet show distinct Raman peaks is thus possible and potentially interesting. Because it increases the interaction between the excitation laser beam and the sample in the detection chamber, the new Raman cell for HPLC shows an increase of three orders of magnitude compared with the classical Raman position where the capillary tubing is perpendicular to the incident laser beam and to the detection direction. The increase of the chromatographic peaks width due to the cell is comparable to that of a cell for UV detection. This cell can be connected at the end of a HPLC column for Raman measurements in a continuous mode. Classical buffer salts like phosphate, citrate, acetate and borate and ion-pair reagents like ammonium quaternary, alkyl sulfates or sulfonates which have been added in the eluent possess distinct and discrete Raman peaks. It is then

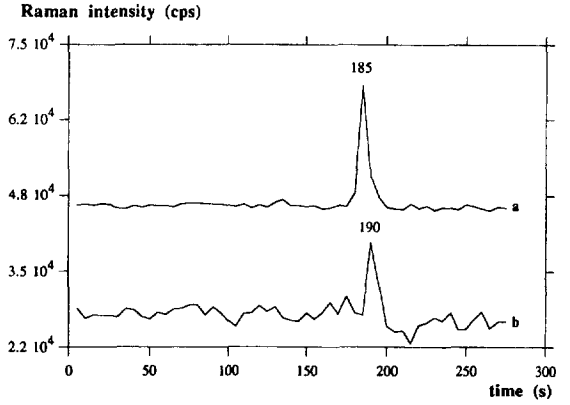

Fig. 4. Chromatograms of 17.3 mg toluene (a) and of 80 μg naphthalene (b) eluted by the same acetonitrile–water (95:5, v/v) solvent.

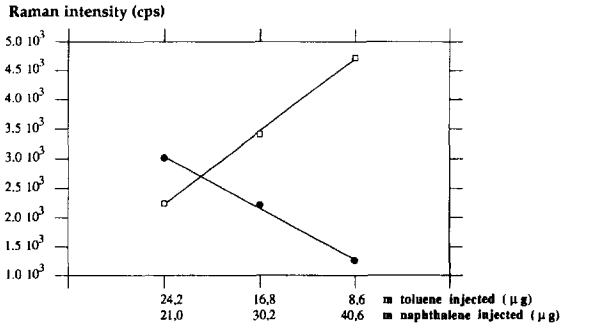

Fig. 5. Test of Raman detection selectivity: Results of the Raman HPLC analysis of 3 different mixtures of toluene and naphthalene in the same acetonitrile–water (95:5, v/v) solvent. Solid circles represent the peak intensity of toluene at 1002 cm^{-1}. Open squares represent the peak intensity of naphthalene at 759 cm^{-1}.

possible to choose the right buffer salt for the Raman use in the case where peaks of the analyte and the buffer salt coincide. The main drawback of the method at present is still its relatively low sensitivity. Typically, the classical limit of detection of toluene for UV–HPLC is 1 mg/l (to be compared with our results: 480 mg/l), while toluene is not detectable by IR spectrometry coupled with HPLC. Thanks to the latest improvements of Raman spectrometry, and to the fact that the related Raman techniques, resonance Raman spectroscopy and the SERS as they amplify the signal by a factor of 10^3–10^6, greatly increase the detection limits in some specific cases, the hyphenated Raman–HPLC technique appears to be useful in a certain number of HPLC analytical cases. In addition, the Raman effect is highly selective and also gives structural information on the chemical compounds of the analyzed mixtures. This is why it seems good to us to explore this alternative.

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