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MICRO-LIQUID CHROMATOGRAPHY WITH DIODE ARRAY DETECTION

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SUMMARY

Commercial diode array detectors can be adapted to micro-liquid chromatography (LC) by replacing their detector cell with a miniaturized detector cell. In appropriate conditions, micro-LC can then generate an useful UV spectrum with 10 times less sample than needed with conventional LC. Some aspects of detector cell miniaturization are discussed. Results with two commercial diode array detectors, modified with a miniaturized detector cell, are presented.

INTRODUCTION

Micro-LC is liquid chromatography on packed fused-silica (or other) capillary columns with an internal diameter (I.D.) below 500 μm[1–3]. It has many advantages over "conventional" LC using mostly 4.6 mm I.D. columns[4]. One of these is better mass sensitivity. It is important to exploit this in conjunction with a diode array detector (DAD). With most commercial DAD instrumentation this is not possible because the detector cells are not adapted to micro-LC dimensions. In the present paper we report on cell modification for two commercial DAD instruments to allow micro-LC-DAD. Examples of chromatographic results are presented.

EXPERIMENTAL

The chromatographs with DAD used in the present study were an HP-1090 (Hewlett-Packard, Palo Alto, CA, U.S.A.) and an LKB-Diode Array Rapid Spectral Detector (LKB-Produkter, Bromma, Sweden). The columns were 4.6 mm I.D. Lichroma tubing for the "conventional" size LC, and polyimide-coated fused-silica capillaries of 320 μm I.D. (RSL, Eke, Belgium) for micro-LC. The packing material was in all cases 5-μm ROSiL-C_{18}-D (an octadecylated spherical silica gel from RSL). The miniaturized detector cells were developed at our laboratory and are now available from RSL.

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RESULTS AND DISCUSSION

Detector cell modification for micro-LC

Yang[5] has described how an empty extension of a fused-silica column, after the packed bed, can be used for UV detection in miniaturized LC. The protecting polyimide is removed over a small distance from the fused-silica capillary and detection is achieved by placing this part of the column in the light path of an UV detector. Yang calls this technique "on column" detection. The detector cell, which is part of the column, has a tubular shape. An easier way to achieve the same result is to build a separate detector block in which a piece of fused-silica capillary, free of polyimide over a few mm, is permanently mounted. In this case too the detector cell has a tubular shape. UV detectors with cassette-like detector flow-cells (like the Varian 2050, and similar Jasco, Wescan and Uvicon detectors, or the earlier fixed wavelength Varian Aerograph UV detector), can thus easily be modified for micro-LC. Exchange of the original cassette for a micro-LC cell cassette is readily achieved. Connection of the actual LC column via a 50 or 100 μm I.D. fused-silica capillary to this detector cassette is realized with a short piece of suitable PTFE tube or with "glass or fused silica column connectors".

Due to the tubular shape of these adapted detector flow-cells, they are very sensitive ro refractive index changes[6] (Fig. 1). The flow-cell acts as a dispersive medium. This increases the number of parameters that determine the eventual amount of light incident on the photosensitive device. Some of these parameters can be controlled in a suitable substitute detector cell block (internal and external diameter of the flow-cell; slit width; position and width of the entrance slit to the photo-sensitive device; alignment of the light beam, flow-cell and photo cell). Other parameters are much more difficult or cannot be controlled in/by an add-on substitute detector cell (convergence and divergence in the incident light beam; positioning of the refraction grid). Some of these points and the construction of a suitable add-on detector cell for micro-LC have been discussed by us[6].

Micro-LC on-column detection with a photo diode array detector was suggested by Yang in 1981[5]. Micro cells for multichannel UV detectors were discussed first (as happened so many times in micro-LC development) by Ishii *et al.*[7]. In that study, parallel (Z type) and cross flow-cells (on-column detection) are compared. The cells

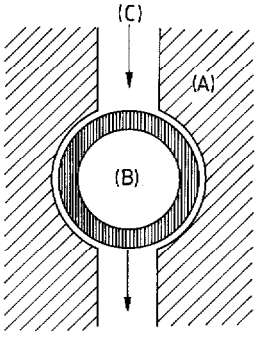

Fig. 1. Miniaturized cell design. Two piece block (A) with fused-silica capillary column (B) of 320 μm I.D. with spacers determining the slit width of 200 μm (C).

were developed specifically for the Micro Gate Photodiode Array Detector of Union Giken. Few technical details about the micro cell construction are available. Differences in the relative peak heights for a series of polycyclic aromatic hydrocarbons when changing from parallel to cross flow-cells are primarily ascribed to extra-column dead volume band broadening for earlier peaks with the parallel cell. A closer look at the chromatograms shows that this cannot be the explanation. If extra-column dead volume were mainly involved, the peak height change in question would follow a simple pattern, predictable for each compound and repeating itself in all the chromatograms shown. This is not the case. Some peaks (compounds) are more affected than others. The variations seem thus to be compound (spectrum) dependent. We have observed the same phenomenon with our cells. Furthermore, large hypsochromic or bathochromic shifts (10 nm or more) can sometimes be observed in the spectra by switching from one to another micro cell. This could result from imperfect alignment of the detector parts switching the light beam to higher or lower diodes in the array. This phenomenon may also account for variable peak height changes. The design of micro cells thus deserves closer and further attention.

Whatever the reasons for chromatigraphic peak intensity or wavelength changes in function of micro cell design, in order to obtain useful UV spectra strict adherence to the above mentioned principles has to be observed. Otherwise, wavelength-dependent refractive index effects lead to distorted UV spectra, and perhaps to different sensitivities when chromatograms are recorded at a single wavelength. Most essential is the slit width. Reducing the slit width however reduces the sensitivity. A compromise has to be adopted. Fig. 2a shows such a distorted UV spectrum for pyrene, obtained with a too wide 400-μm entrance slit of a modified flow-cell for the

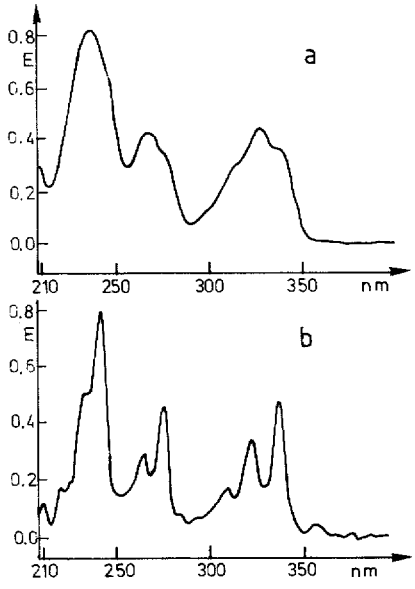

Fig. 2. UV spectra of pyrene solutions placed in micro cells: (a) with an unsatisfactory modified cell (HP-1090 diode array), (b) with an improved cell modification (HP-1090 diode array).

DAD of the HP-1090 liquid chromatograph. An improved cell design with a narrower 200-μm entrance slit (for a 320 μm I.D. detector tube as in Fig. 1), with very strict alignment of the light source, detector cell and photocell, produced an optimized flow-cell as described recently[6]. This leads to an UV spectrum for pyrene as shown in Fig. 2b, also obtained with the HP-1090 DAD. Such a spectrum is useful, but close comparison with a spectrum obtained with a larger cell shows minor differences, as discussed above.

A similar improved sub-microlitre detection cell can be placed in the LKB diode array detector in the same way as the conventional flow-cell. By simply changing the cassette, the detectors can either be used for conventional or for micro-LC. Fig. 3a shows the reversed-phase chromatogram of a mixture of five polyaromatic compounds on a conventional column. Fig. 4 shows the UV spectrum of the fourth peak (pyrene) obtained with the conventional 5-μl, 5-mm pathlength flow-cell (LKB-DAD). Fig. 3b shows the same separation on a micro-LC column with a micro detector cell in the LKB instrument, and Fig. 5 shows the spectrum for the fourth peak under these micro-LC conditions. A first conclusion is that micro-LC and the two DAD instruments mentioned, equipped with a micro cell, can give a useful UV spectrum via diode array detection.

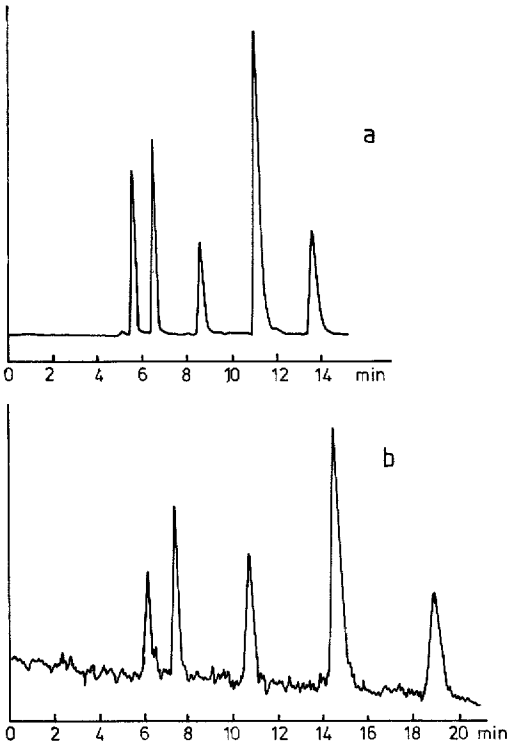

Fig. 3. Reversed-phase test chromatograms for (a) "conventional" and (b) micro-LC. (a) Column: 5 μm ROSiL-C_{18}-D (25 cm × 0.46 cm). Solvent: acetonitrile–water (75:25). Detection: 255 nm. Solvent flow-rate: 1 ml/min. Volume injected: 6.4 μl. (b) Column: 5 μm ROSiL-C_{18}-D (260 mm × 0.32 mm). Solvent: acetonitrile–water (72:25). Detection: 255 nm. Solvent flow-rate: 4.1 μl/min. Volume injected: 200 nl.

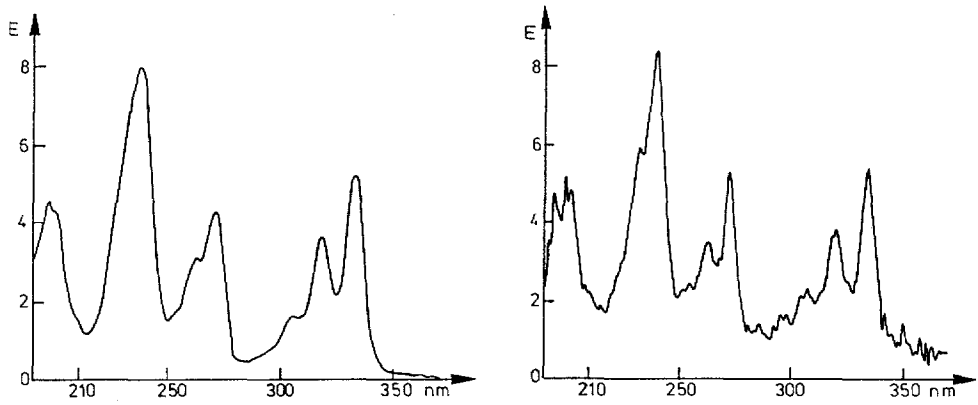

Fig. 4. UV spectrum of the fourth peak (pyrene) of Fig. 3a (conventional column, LKB diode array).

Fig. 5. UV spectrum of the fourth peak (pyrene) of Fig. 3b (micro-LC column with modified micro detector cell cassette LKB diode array).

Sensitivity of conventional versus micro-LC and DAD detection

Another important point is how large the smallest sample has to be that can just generate a useful spectrum. The dimensions of the columns under discussion are 250 mm × 4.6 mm and 260 mm × 0.32 mm. The volume ratio for the two columns is therefore 197, and the expected "elution dilution" or chromatographic dispersion is in favour of the micro-LC column by the same factor of 197. For the same concentration of the sample solutions and adapting the sample size to the column surface area ratio (in this case 206), the sensitivity of detection for the micro-LC system is reduced by the same factor. Under these conditions, the observed sensitivity will be determined mainly by the length of the detector cell. For the detector cells under discussion this favours conventional LC by a factor of about 15–30. Fortunately, the capacity of

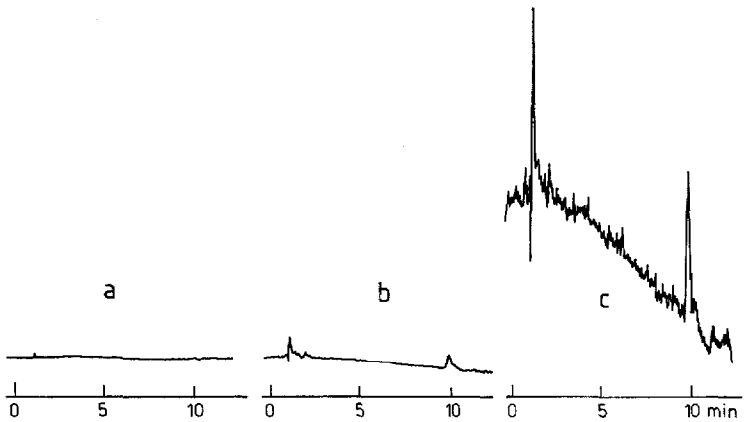

Fig. 6. Chromatograms of 10 ng pyrene under the conventional conditions of Fig. 3a, except that only 60 nl were injected. Unmodified HP-1090 diode array detector. (a) Normal attenuation setting; (b) 10 times more sensitive; (c) 100 times more sensitive.

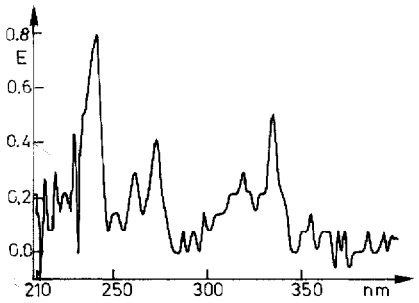

Fig. 7. UV spectrum for 10 ng pyrene obtained from the chromatogram in Fig. 6.

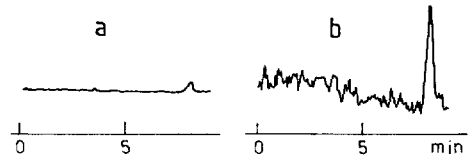

Fig. 8. Chromatograms of 1 ng pyrene under the micro-LC conditions of Fig. 3b, except that only 60 nl were injected. HP-1090 diode array detector with modified detector cell. (a) Normal attenuation setting; (b) 10 times more sensitive.

silica gel phases is larger than required by usual analytical samples. Therefore the samples injected on the micro-LC system can be larger than those that calculation would indicate. Still, under "normal" conditions, conventional LC is more sensitive than micro-LC because of the detection aspect just mentioned and because the amount of sample injected on the micro-LC column (if the same sample concentrations are used on both systems) is so much smaller. Therefore the chromatogram of Fig. 2b had to be recorded with a more sensitive attenuation setting as is seen by the noise ratio.

When the concentrations injected are different, but the injected mass of compound is the same, then the better mass sensitivity of the micro-LC system becomes apparent. This can be important when the sample size available is restricted. For our HP-1090 DAD system, the limit that can still generate an useful UV spectrum was estimated at about 10 ng[8]. Fig. 6 shows chromatograms produced on the HP-1090 for 10 ng pyrene with the column of Fig. 3a and with various attenuation settings. The signal-to-noise ratio is about 8. Fig. 7 shows the UV spectrum. The pyrene spectrum can just be recognized. The detection limit under these conditions is therefore indeed about 10 ng. Similar results for 1 ng on the micro-LC column are shown in Figs. 8 and 9. The signal-to-noise ratio is about 6. The UV spectrum is now even better than with the conventional system with 10 ng of sample.

The HP-1090 DAD, provided with a micro detector cell and with a micro-LC

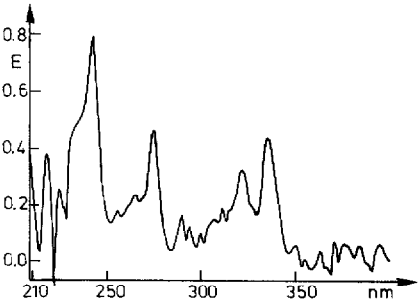

Fig. 9. UV spectrum for 1 ng pyrene obtained from the chromatogram in Fig. 8.

column, can thus generate a useful UV spectrum with about 1 ng of material. The newest instruments are about 10 times better[8]. Probably the micro cell construction can also be improved, so that still lower figures may be reached.

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