

This article was downloaded by:

On: 17 January 2011

Access details: *Access Details: Free Access*

Publisher *Taylor & Francis*

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713640455>

Direct Determination of Isomeric Polycyclic Aromatic Hydrocarbons in Environmental Samples by Conventional and Laser Excited Shpol'Skii Spectroscopy

I. S. Kozin^a; C. Gooijer^a; N. H. Velthorst^a; J. Harmsen^b; R. Wieggers^b

^a Department of General and Analytical Chemistry, Free University, Amsterdam, HV Amsterdam, The Netherlands ^b The Winand Staring Centre (SC-DLO), AC Wageningen, The Netherlands

To cite this Article Kozin, I. S. , Gooijer, C. , Velthorst, N. H. , Harmsen, J. and Wieggers, R.(1995) 'Direct Determination of Isomeric Polycyclic Aromatic Hydrocarbons in Environmental Samples by Conventional and Laser Excited Shpol'Skii Spectroscopy', *International Journal of Environmental Analytical Chemistry*, 61: 4, 285 – 297

To link to this Article: DOI: 10.1080/03067319508027244

URL: <http://dx.doi.org/10.1080/03067319508027244>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

DIRECT DETERMINATION OF ISOMERIC POLYCYCLIC AROMATIC HYDROCARBONS IN ENVIRONMENTAL SAMPLES BY CONVENTIONAL AND LASER EXCITED SHPOL'SKII SPECTROSCOPY

I. S. KOZIN*, C. GOOIJER and N. H. VELTHORST

*Department of General and Analytical Chemistry, Free University, Amsterdam, De
Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

J. HARMSSEN and R. WIEGGERS

*The Winand Staring Centre (SC-DLO), Marijkeweg 11/22, P.O. Box 125, NL-6700 AC
Wageningen, The Netherlands*

(Received, 10 January 1995; in final form, 13 February 1995)

Various modes of Shpol'skii spectroscopy have been employed to directly determine a number of isomeric four to six-membered ring polycyclic aromatic hydrocarbons (PAHs) in crude extracts of soil and sediment samples with different levels of pollution. The high selectivity of conventional Shpol'skii spectroscopy (lamp excitation–fluorescence and phosphorescence detection) enabled besides several priority PAHs, unambiguous identification of individual monomethylpyrenes, five- and six-membered ring PAH isomers. For a 10 μ l sample volume, the absolute limit of detection varied from 0.2 picomole for benzo[e]pyrene (phosphorescence measurements) to 3 femtomole for perylene (lamp excitation/fluorescence detection). Application of laser excited Shpol'skii spectroscopy (LESS) with time-resolved fluorescence detection allowed direct ultra-selective determination of the most potent known to date carcinogen—dibenzo[a,l]pyrene (DB[a,l]P). For this compound, a low 2 femtomole limit of detection (15 percent repeatability) was achieved. DB[a,l]P contents were in the range of tens to hundreds ng/g for different samples.

KEY WORDS: Polycyclic aromatic hydrocarbons, dibenzo[a,l]pyrene, Shpol'skii spectroscopy, environmental samples.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) as ubiquitous environmental contaminants exhibiting mutagenic and carcinogenic properties are regularly monitored by applying commonly used chromatographic methods (e.g.: HPLC, GC, GC-MS). As a rule, these techniques provide accurate determination of different PAHs, listed as priority pollutants, in various complex matrices. Most recently, significant progress has been made in the analysis of PAHs by utilizing particle beam mass-spectrometry (PBMS)

* Corresponding author.

coupled to liquid chromatographic separation (LC)^{1,2}. For example, employing a LC-PBMS system, enhanced possibilities for identification of structural PAH isomers including high molecular weight compounds have been reported³. However, since crude extracts of environmental samples are usually composed of a variety of closely related chemical species, unambiguous identification of isomeric PAH compounds may be hampered as a result of co-elution, 'matrix' effects, and similarities in mass-spectroscopic data. In this respect, identification of minor constituents of extracts is especially difficult, since both high selectivity and sensitivity of the analysis are needed. Apparently, due to the aforementioned problems, there is still a lack of data on certain geochemical markers and highly toxic pollutants present in the environment. For example, unambiguous identification of the most potent carcinogenic compound, dibenzo[a,l]pyrene (DB[a,l]P)⁴, in complex environmental samples (e.g.: soils and sediments) by means of chromatographic separation has not been reported yet.

In principle, there is an alternative analytical approach to solve the problem, namely the Shpol'skii spectroscopy, a high resolution low temperature molecular luminescence technique, which is capable of selective and sensitive identification of individual PAH isomers in a direct way. Under the Shpol'skii conditions, the influence of solution inhomogeneity on luminescence spectra of rigid planar luminophores (most of the PAHs) can be strongly reduced by isolating the guest molecules of analytes in an appropriate host matrix (usually, *n*-alkanes) under low (helium) temperature. Consequently, vibrationally resolved emission spectra, favorable for fingerprint identification, can be observed. The technique is not restricted to the straightforward recording of fluorescence spectra. As will be shown below, application of phosphorescence measurements, laser excitation, and time-resolved detection leads to extra selectivity of the analysis. The fundamentals and analytical aspects of Shpol'skii spectroscopy have been extensively described elsewhere⁵⁻⁷.

In the last decade the applicability of conventional Shpol'skii spectroscopy utilizing lamp excitation and fluorescence detection was demonstrated for the analysis of parent priority PAHs in crude extracts of environmental samples^{8,9}. This conventional mode was also used for identification of a number of PAH derivatives in narrow chromatographic fractions obtained after HPLC separation¹⁰. Laser excited Shpol'skii spectroscopy (LESS) was employed for selective identification of nitrogen-, oxygen-, and sulfur-containing polycyclic aromatic compounds (PACs)¹¹ and PAH metabolites¹². Nevertheless, the analytical advantages of Shpol'skii spectroscopy are still scarcely used in chemical analysis. In this paper, it will be demonstrated that by utilizing various experimental modes of Shpol'skii spectroscopy, a number of isomeric parent and alkylated PAHs can be determined directly in crude extracts of environmental samples.

EXPERIMENTAL

Chemicals

Standard PAHs and perdeuterated perylene were available from the chemical stock at the Department of General and Analytical Chemistry, Free University, Amsterdam. The purity of the compounds used was checked by recording their fluorescence emission and excitation spectra, which were completely identical to those available from the literature⁶.

n-Octane was purchased from Janssen Chimica (Geel, Belgium). Methanol and n-hexane were 'Baker analyzed' grade (J. T. Baker B. V., Deventer, The Netherlands). The purity of the solvents was spectroscopically tested, and they were used without further purification.

Sampling and sample treatment

Five samples of surface layers of soils and sediments with a different level of pollution were collected at several sites in the Netherlands. Sampling locations and samples characteristics are given in Table 1.

Non dried samples were extracted with acetone-petroleum ether (3:1, v/v) under vigorous agitation for 10 min. at room temperature, following the standardized procedure denoted as NEN 5771, which is common for Dutch environmental institutions for determination of PAHs in sediments. Acetone was subsequently removed with an excessive application of water, so that the extracts were petroleum ether solutions. For the PAH analysis, initial brownish extracts were simply diluted (usually 10-fold) with n-octane or n-hexane.

Shpol'skii spectroscopy

In the conventional Shpol'skii spectroscopy mode, for fluorescence excitation-emission measurements, a 450 W xenon arc lamp and a Bausch & Lomb (Rochester, NY)

Table 1 Characteristics of samples analysed for PAH contents by conventional (lamp) and laser excited Shpol'skii spectroscopy.

<i>Sample No.</i>	<i>Location in The Netherlands</i>	<i>Total PAH content,* mg/kg (dry weight)</i>	<i>Organic matter content (%)</i>	<i>Sample description</i>
SC1	Klompenwaard	10	12.9	Soil from the river Waal floodplain (Rhine sediment)
SC3	Geulhaven (Rotterdam)	10	4.3	Sediment from the Rotterdam harbour, after 4 years of landfarming
SC4	Zierikzee	25	5.3	Sediment from Zierikzee harbour, after 4 years of landfarming
SC5	Wemeldinge	50	6.7	Sediment from the harbour of Wemeldinge
SC6	Petrol-harbour (Amsterdam)	500	11.8	Sediment from 'Petrol-harbour', Amsterdam

* - estimations made on the basis of analytical data obtained for samples collected from the same areas (HPLC/fluorescence measurements for 16 PAHs listed by US Environmental Protection Agency).

monochromator (bandpass 2 to 5 nm) were used. To gain additional selectivity, optimum excitation wavelengths were applied for detection of each compound. For laser excited Shpol'skii spectroscopy (LESS) a tunable excitation range of 408–418 nm was obtained by frequency mixing of the output of the oxazine170 dye laser (Quantel) pumped by a Quantel frequency doubled Nd:YAG laser, with the fundamental 1064 nm output. The laser pulse rate was 10 Hz.

Four 10 μ l samples could be cooled simultaneously down to 26 K by a CTI Cryogenics (Waltham, MA) closed-cycle helium refrigerator. The emission signal was collected at a 20° angle from the excitation beam by a positive lens, focused on the entrance slit of a Jobin-Yvon (Longjumeau, France) HR1000 monochromator (0.08 nm spectral resolution), and detected by an EG & G (Princeton, NJ) Model 1421 B blue-enhanced intensified linear photodiode array equipped with a Model 1463 detector controller. Spectral data were processed by a Model 1460 OMA III console. Triggering for the gated fluorescence detection was provided by an EG& G pulse generator with a fixed gate of 40 ns. For quantitative determination, an internal standard procedure utilizing perylene *d12* was applied. The repeatability of the measurements was in the order of 10 to 15 % for different PAHs (three replicates in each case).

RESULTS AND DISCUSSION

In this section, first, conventional Shpol'skii fluorescence spectroscopy in a lamp excitation/fluorescence detection regime will be demonstrated to be capable of identification of a number of closely related compounds. Subsequently, it will be shown, that application of lamp excitation/phosphorescence detection leads to a significant increase of the analytical potential of the technique. Finally, the exceptionally high selectivity of laser excited Shpol'skii spectroscopy utilizing time-resolved fluorescence detection will be exemplified by the direct determination of dibenzopyrene isomers.

Lamp excitation/fluorescence detection

Shpol'skii spectroscopy with lamp excitation/fluorescence detection can be considered as a convenient analytical tool for selective determination of individual PAHs with closely related molecular structure in crude extracts of environmental samples. Under Shpol'skii conditions both emission and the long wavelength part of excitation spectra of analytes reveal highly-resolved quasi-linear structure. Therefore, a simple selection of appropriate optimum excitation wavelengths for each compound is frequently sufficient to provide their unambiguous identification by comparing subsequently recorded fluorescence spectra to the relevant data on reference analogues. In this respect, a set of quasi-linear fluorescence bands, which is highly-specific for each compound ('fingerprint'), serves as a necessary basis for positive identification. The examples depicted in Figures 1 and 2 demonstrate that different isomeric five- and six-membered ring parent PAHs can be easily determined in crude sediment extracts by employing this technique. In this case, the spectra obtained were compared with those available from the spectral library⁶.

Under lamp excitation, however, in order to gain required selectivity, the spectral shape of low temperature excitation spectra should be taken into account. As indicated in the foregoing paragraph, it should be realized that not the full excitation spectra are highly-resolved. This is an important point to consider, when the selectivity achievable with lamp excitation is concerned. According to the Heisenberg principle, higher

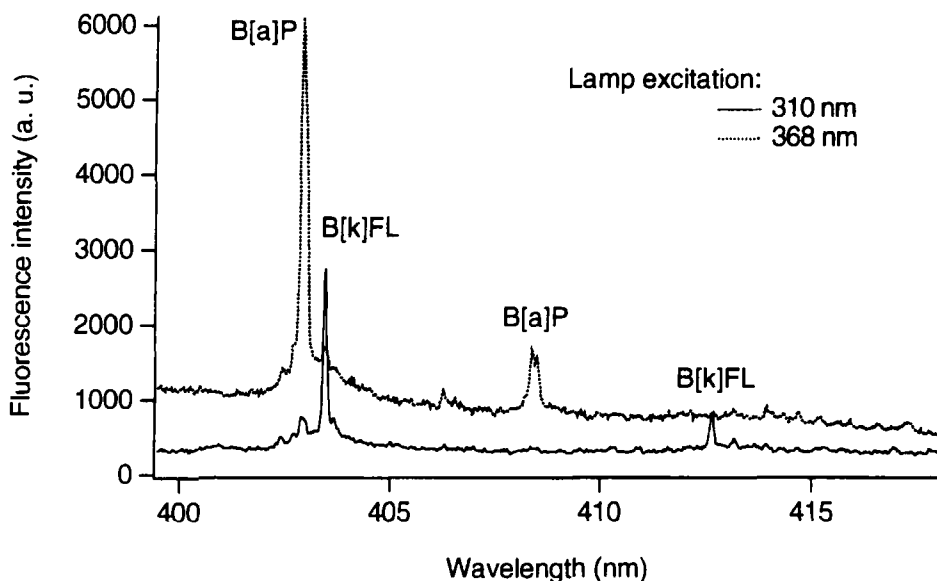


Figure 1 Shpol'skii fluorescence spectra of a crude sediment extract (SC5 sample) Lamp excitation, n-octane, T = 26K.

electronic states usually have much shorter lifetimes. Consequently, in an absorption spectrum the linewidth of vibronic transitions to the lowest excited singlet state (longer wavelength region) as a rule is much narrower than that of transitions to higher energy levels (short wavelength region). Hence, important from an analytical point of view, involving longer wavelength absorption bands for fluorescence excitation may result in considerable improvement in selectivity. In general, such experimental parameters can be used both for laser and conventional (lamp excited) fluorescence measurements. In case of lamp excitation, a relatively narrow excitation beam (1–2 nm) can be selected by employing appropriate optical slits of the excitation monochromator. The influence of scatter processes can be successfully reduced by means of proper optical alignment and relevant cut-off filters installed in front of the entrance slit of the emission monochromator.

On the basis of such an approach, applying four excitation wavelengths subsequently shifted from 339 to 348 nm for the fluorescence measurements, parent pyrene and monomethylpyrene isomers were determined in crude n-octane extracts of soil and sediment samples. Positive identification of these compounds was achieved either by matching with the library spectra⁶, or with the independently recorded spectra of the reference compounds (see Figures 3, 4 and 5).

Besides monomethylpyrenes, some other PAH derivatives could be observed in the same extracts. However, since the adequate reference PAHs and (or) spectral data were not available, only a tentative identification of such compounds was made by recording their low temperature excitation spectra. For example, Shpol'skii fluorescence spectrum of a compound tentatively identified as ethyl-substituted pyrene is depicted in Figure 5. The overall shape of the excitation spectrum of this compound was rather similar to that red-shifted of parent pyrene. On the other hand, since no match was found with the Shpol'skii spectra of mono- or dimethyl-substituted pyrenes (a longer red-shifted 0–0 transition of the unidentified compound was observed), it was assumed that the

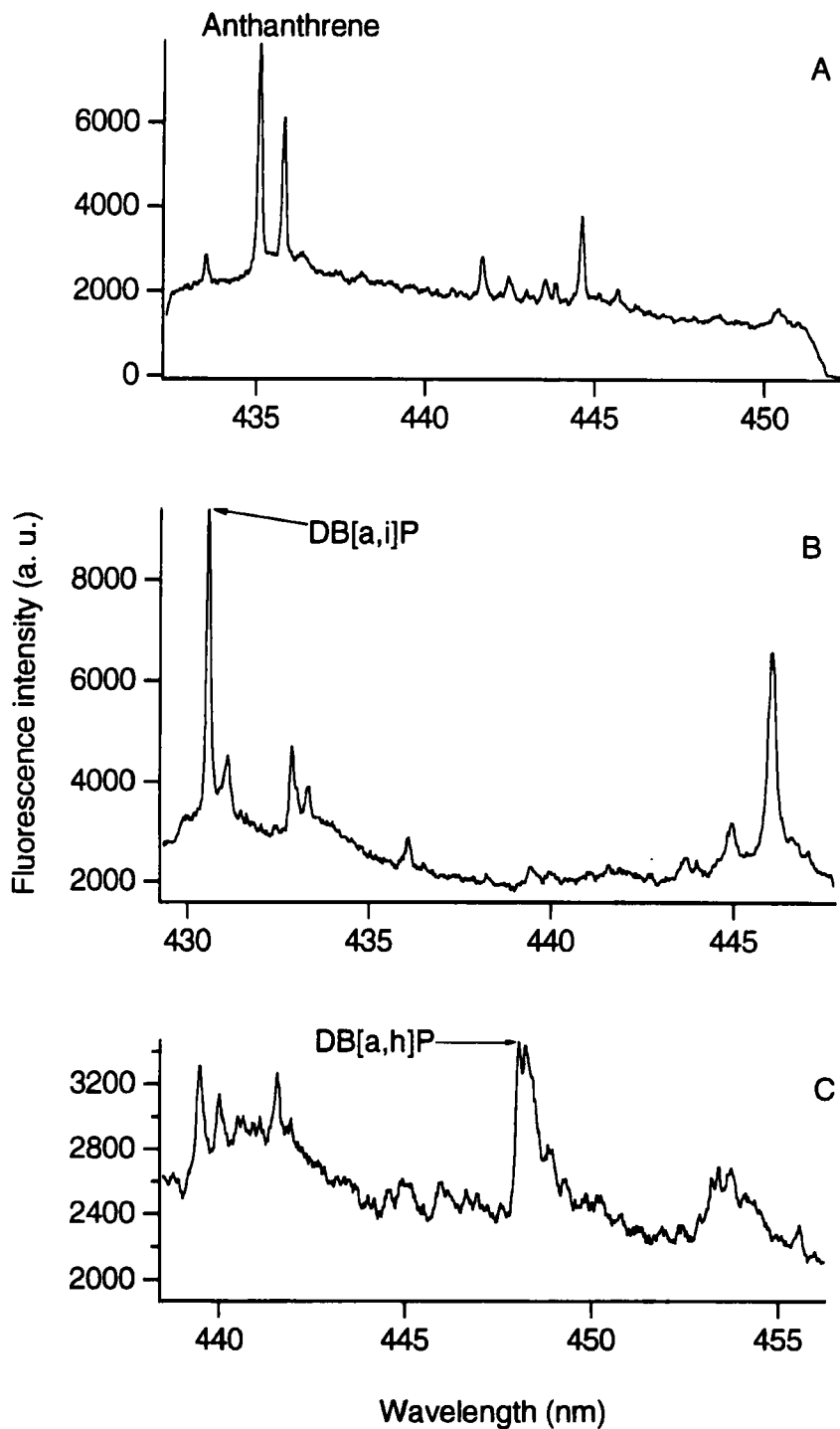


Figure 2 Shpol'skii fluorescence spectra of a crude soil extract (SC1 sample). Lamp excitation: A – 310 nm, n-octane; B – 398 nm, n-hexane; C – 312 nm, n-hexane, T = 26K.

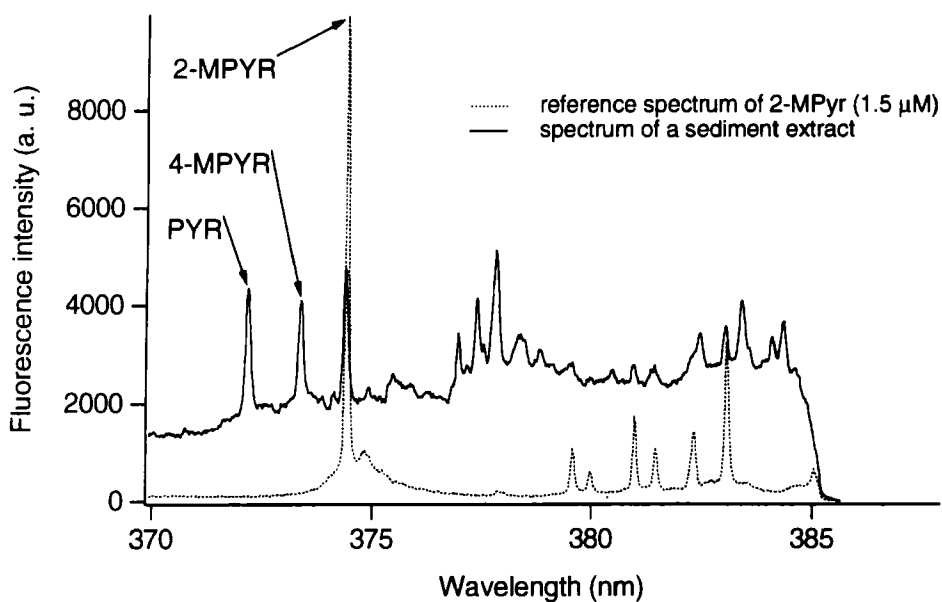


Figure 3 Identification of 2-methylpyrene in a crude sediment extract (SC6 sample). Lamp excitation at 342 nm, n-octane. T = 26K; (note, that the fluorescence bands of pyrene and 4-methylpyrene were identified by matching with the reference spectra [6]).

compound could very well be ethyl-substituted pyrene. The shape of 0–0 transition not affected by spectral broadening caused by polar substituents (see Figure 5), also supported our conclusion. Further confirmation, however, by comparing with the reference analogue is still needed.

In the conventional Shpol'skii fluorescence mode the absolute limit of detection achieved for a number of compounds (e.g.: B[a]P, B[k]FL, Perylene,) was as low as 3 femtomole. PAH contents determined by applying lamp excitation/fluorescence detection are listed in Table 2.

Lamp excitation/phosphorescence detection

In the analysis of major and minor PAH constituents of crude extracts' by means of Shpol'skii spectroscopy with lamp excitation/fluorescence detection, spectral overlap cannot always completely be prevented. In this case, it can be tried to tackle the problem of identification by using phosphorescence detection. Various PAHs exhibit significant phosphorescence complementary to fluorescence emission. An example of selective identification of benzo[e]pyrene in a polluted sediment sample is given in Figure 6. For this compound utilizing phosphorescence detection, a relatively low limit of detection (0.2 picomole for 10 μ l sample volume) was achieved, which is low enough to enable its direct detection in the sample at hand.

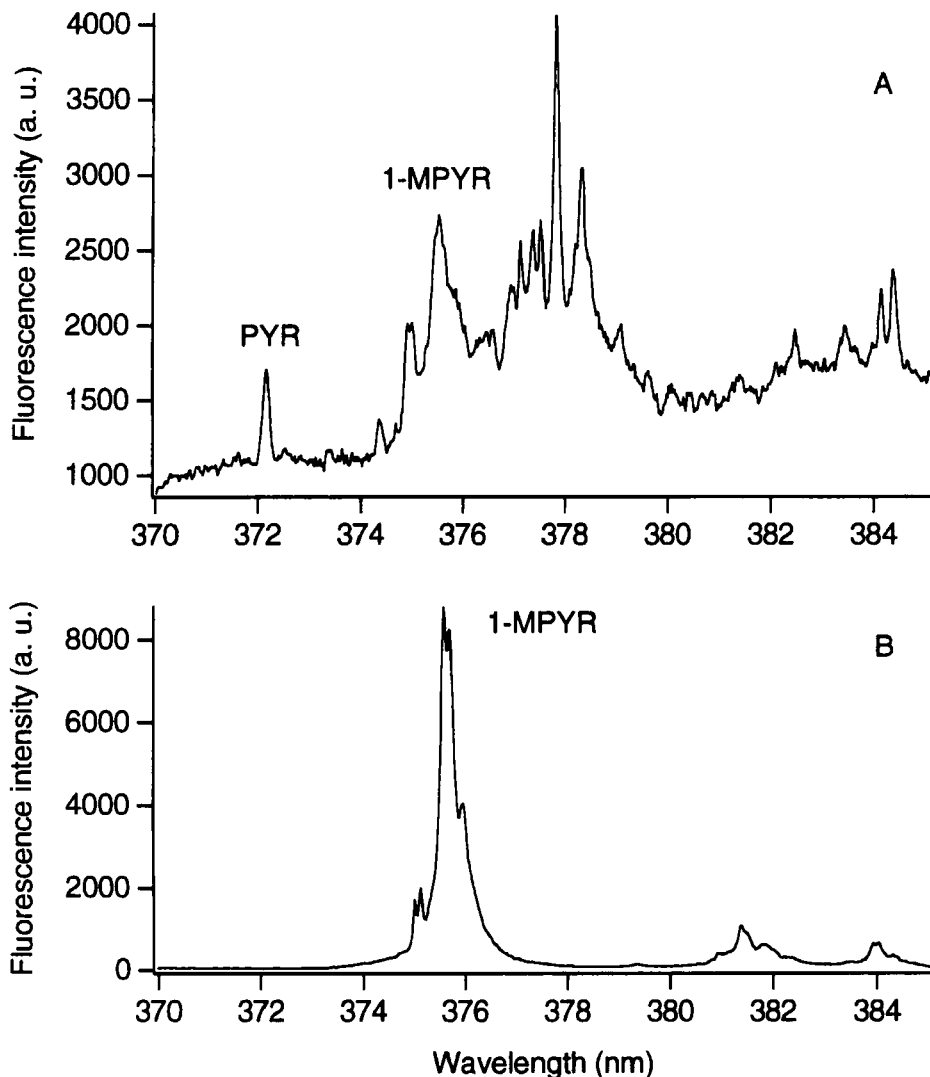


Figure 4 Shpol'skii spectra of 1-methylpyrene: A – crude extract of a sediment sample (SC6), B – standard n-octane solution ($1.3 \cdot 10^{-6}$ M). Lamp excitation at 346 nm, $T = 26\text{K}$.

Laser excitation/time-resolved fluorescence detection

When identification of a particular individual PAH compound present at low concentrations in complex extracts of environmental samples containing many other PAHs in much larger amounts is required, extra selectivity and sensitivity of the analysis is strongly needed. These can be gained by employing a laser as a monochromatic excitation source. Tuning the laser excitation wavelength results in highly efficient optical selection of analytes (narrow absorption bands close to 0–0 transition region should be involved for excitation to achieve optimum selectivity), and the high excitation power of a laser beam provides exceptionally sensitive measurements. Since in some

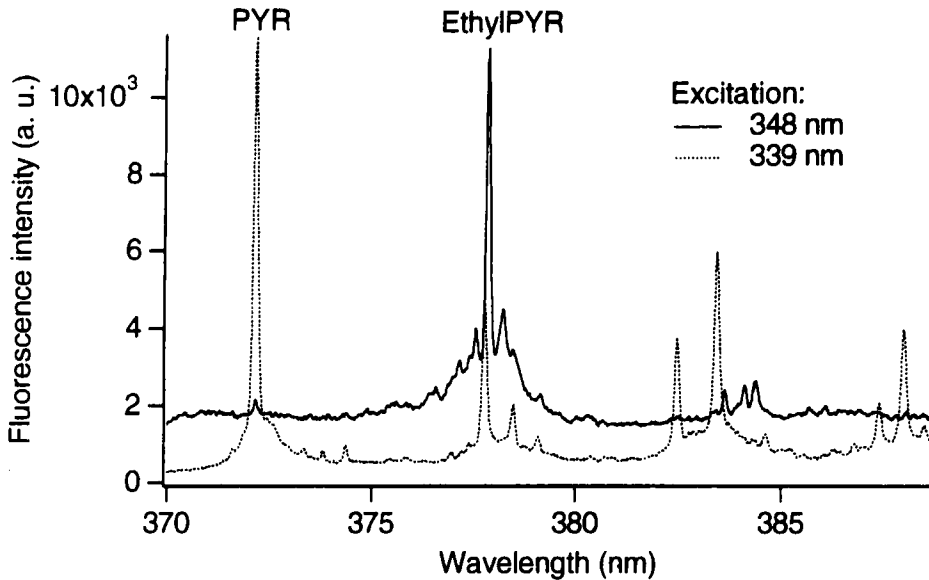


Figure 5 Shpol'skii fluorescence spectra of pyrene and ethyl-pyrene (tentatively identified on the basis of a low temperature excitation spectrum; see text) in a crude extract of a sediment sample (SC6). Lamp excitation, n-octane, T = 26K.

cases Shpol'skii spectra exhibit a site structure caused by a partial analyte-matrix incompatibility, which decreases the inherent selectivity of the technique, laser excitation is also advantageous for eliminating this site structure. In the ideal situation, when selective site (laser) excitation is utilized, the fluorescence spectrum attributed to one separate sites is observed (Figure 7).

Table 2 PAH contents (mg/kg D.W.) in soil and sediments from selected locations in the Netherlands determined by lamp and laser excited Shpol'skii spectroscopy.

Compounds	Samples ^a				
	SC1	SC3	SC4	SC5	SC6
1-MPyrene	0.29	0.035	0.36	1.7	11.8
2-MPyrene	0.42	0.082	0.24	0.7	6.5
4-MPyrene	0.61	N.A. ^b	0.26	N.A.	N.A.
E-Pyrene	1.0	N.A.	2.1	4.1	11.0
B[e]Pyrene	8.2	0.46	3.9	9.5	12.6
Perylene	0.89	0.25	0.96	1.5	4.4
Anthanthrene	1.9	0.21	1.9	1.4	4.5
DB[a,h]Pyrene	0.12	0.010	0.22	0.3	0.58
DB[a,i]Pyrene ^c	0.36	0.012	0.54	0.64	0.87
DB[a,l]Pyrene ^d	0.011	N.A.	0.017	N.A.	0.33

^a - For the description of the sample see Table 1.

^b - Identification was not attempted

^c - Identified using both lamp and laser excitation

^d - Identified only by LESS.

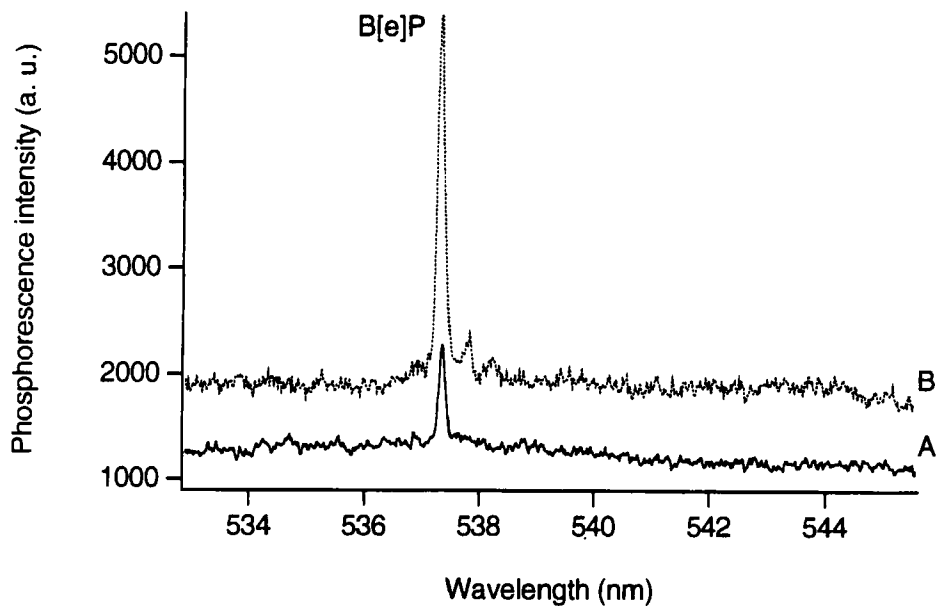


Figure 6 Shpol'skii phosphorescence spectra of benzo[e]pyrene: A – crude extract of a sediment sample (SC5), B – standard n-octane solution ($1.8 \cdot 10^{-7}$ M). Lamp excitation at 333 nm, n-octane, $T = 26\text{K}$.

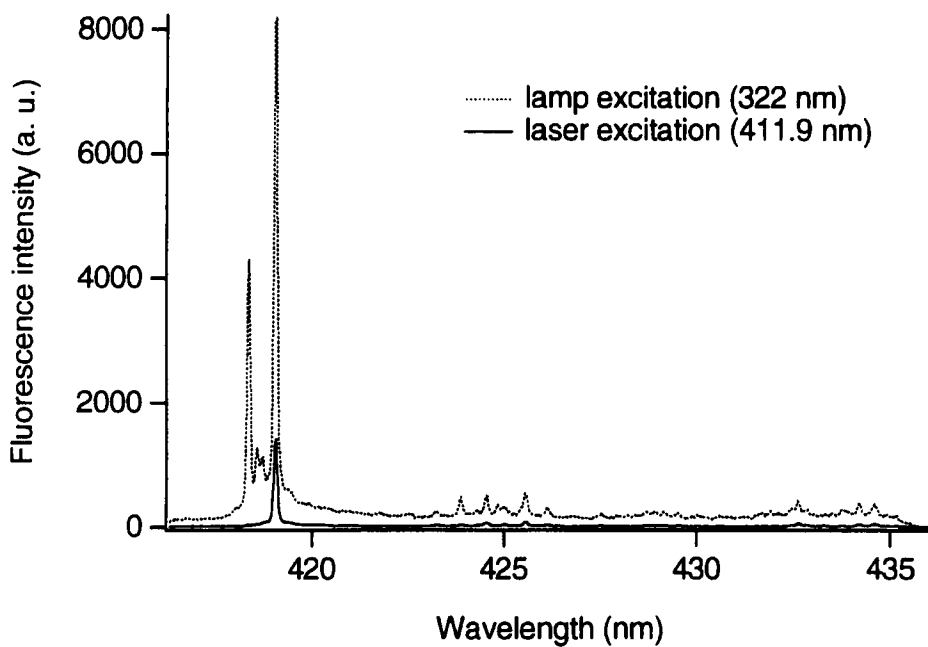


Figure 7 LESS spectra of dibenzo[a,l]pyrene ($2.4 \cdot 10^{-7}$ M) upon lamp and laser excitation. n-octane, $T = 26\text{K}$.

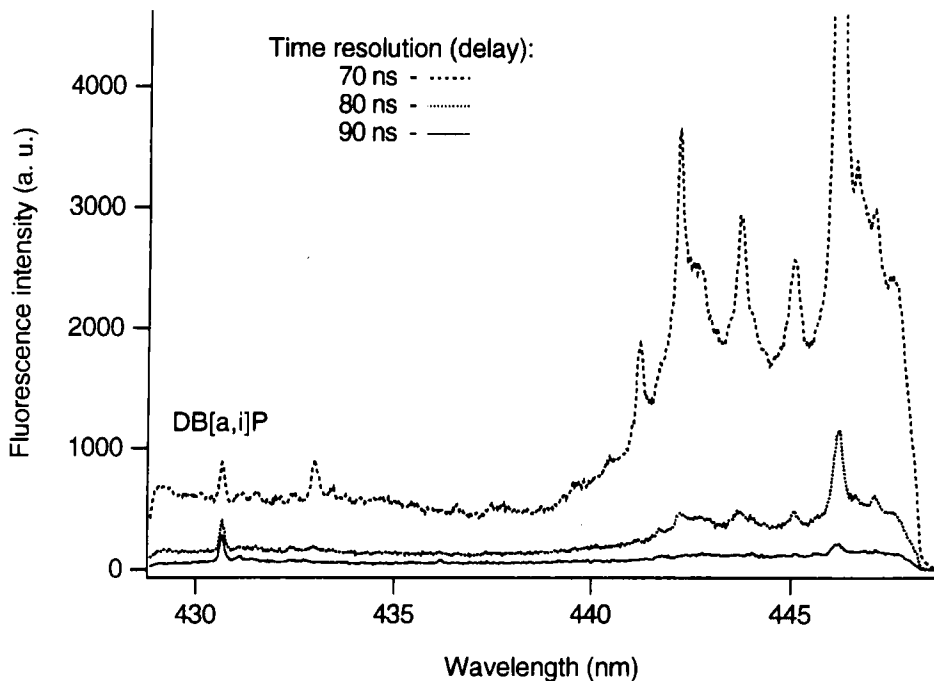


Figure 8 LESS spectra of dibenzo[a,i]pyrene in a crude extract of a soil sample (SC1). Laser excitation at 416.7 nm. Time-resolved detection. n-hexane, T = 26K.

Additional possibilities are offered when time-resolved fluorescence detection is applied to the analysis of compounds with long-living fluorescence properties. For example, Figure 8 demonstrates the influence of a delay time after each laser pulse before actual detection takes place. Depending on the delay time, a significant reduction of background noise and a strongly reduced interference caused by emission from short-living fluorophores is achieved.

The described principles are appropriate for the direct, selective determination of the most potent carcinogenic PAH known today – dibenzo[a,i]pyrene in crude extracts of soil and sediment samples with different level of pollution. Time-resolved LESS spectra of DB[a,i]P in the unfractionated n-octane extract of a soil sample is depicted in Figure 9. The absolute limit of detection achieved was as low as 2 femtomole (10 μ l sample volume). The amount of DB[a,i]P found in the examined soil and sediment samples (see Table 2) was roughly two orders of magnitude lower than those of the major priority PAHs (e.g.: B[a]P).

CONCLUSIONS

Shpol'skii spectroscopy as an independent analytical technique has been demonstrated to be capable of direct unambiguous identification of various structural isomers of PAHs in complex extracts of environmental samples. Without preliminary fractionation or clean-up procedures a wide range of individual parent PAHs and alkyl-substituted

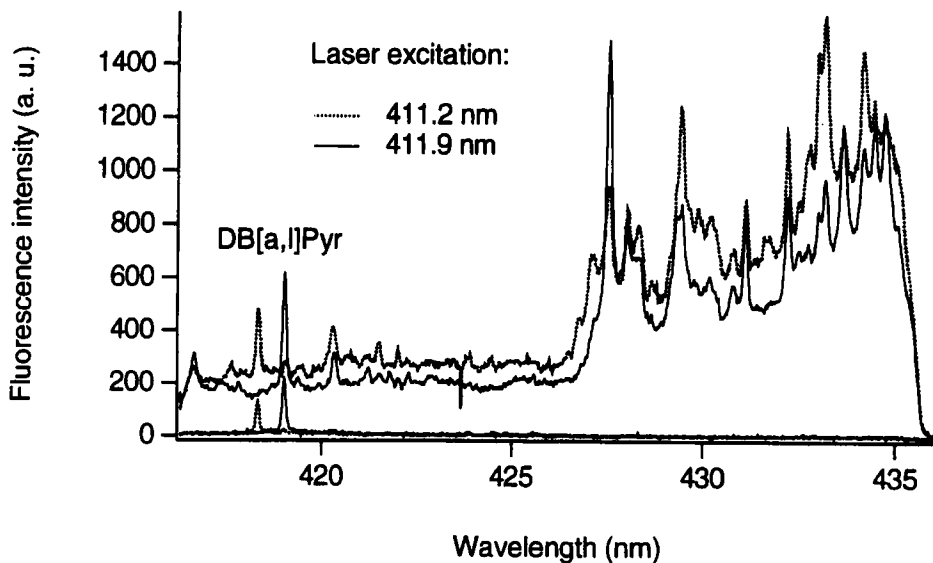


Figure 9 LESS spectra of a crude extract of a soil sample (SC1) (upper curves), and of a standard solution of dibenzo[a,l]pyrene (2.4 nM, lower curves). Time-resolved detection (100 ns delay), n-octane, T = 26K.

derivatives can be selectively determined by employing easy-to-use conventional (lamp excited) Shpol'skii fluorescence measurements. Although the analytical potential of this technique is limited by the availability of the reference spectra, additional possibilities of tentative identification are offered if low temperature excitation spectra are recorded. With lamp excitation, the range of identified compounds can be extended by utilizing phosphorescence detection.

Direct ultra-selective and sensitive determination of PAH isomers in crude extracts of soil and sediment samples can be achieved by means of laser excited Shpol'skii spectroscopy. In this technique, time-resolved fluorescence detection is especially advantageous for the analysis of compounds present at very low concentrations in complex mixtures.

In general, a combination of different experimental modes of Shpol'skii spectroscopy seems to be a highly-efficient analytical tool for environmental and toxicological studies.

Acknowledgements

This research was conducted within the long-term fellowship program financed by European Environmental Research Organization (EERO).

References

1. R. P. Singh, I. D. Brindle, T. R. B. Jones, J. M. Miller and M. Chiba, *J. Am. Soc. Mass Spectrom.*, **4**, 898 (1993).
2. G. Castello and T. C. Gerbino, *J. Chromatogr.*, **642**, 351 (1993).
3. J. Slobodnik, S. J. F. Hoekstra-Oussoren and U. A. Th. Brinkman, (1995) [In preparation].

4. E. L. Cavalieri, S. Higginbotham and E. G. Rogan, *Polycyclic Aromatic Compounds*, **6**, 177–183 (1994).
5. R. I. Personov, in: *Laser Analytical Spectrochemistry* (V. S. Letokhov, ed.; Adam Hilger, Bristol and Boston, 1985), chap. 6.
6. L. A. Nakhimovskii, M. Lamotte and J. Joussot-Dubien, *Handbook of low temperature electronic spectra of polycyclic aromatic hydrocarbons* (Elsevier, Amsterdam, 1989).
7. C. Gooijer, F. Ariese, J. W. Hofstraat and N. H. Velthorst, *Trends in analytical chemistry*, **13**(2), 53–61 (1994).
8. J. W. Hofstraat, H. J. M. Jansen, G. Ph. Hoonweg, C. Gooijer, N. H. Velthorst and W. P. Cofino, *Intern. J. Environ. Anal. Chem.*, **21**, 299–332 (1985).
9. F. Ariese, C. Gooijer, N. H. Velthorst and J. W. Hofstraat, *Fresenius J. Anal. Chem.*, **339**, 722–724 (1991).
10. P. Garrigues and M. Ewald, *Intern. J. Environ. Anal. Chem.*, **21**, 185 (1985).
11. A. E. Elsaïid, R. Walker, S. Weeks, A. P. D'Silva and V. A. Fassel, *Applied Spectroscopy*, **42**(5), 731–740 (1988).
12. F. Ariese, S. J. Kok, C. Gooijer, N. H. Velthorst and J. W. Hofstraat, in: *Polycyclic Aromatic Hydrocarbons: synthesis, properties, analysis, occurrence and biological effects* (P. Garrigues, M. Lamotte, eds.; Gordon and Breach, London, 1993), pp. 761–768.