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Near-Field Thermal Lens Detection at 257 nm as an Alternative to Absorption Spectrometric Detection in Combination with Electromigrative Separation Techniques

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A device is presented that permits detection of analytes absorbing electromagnetic radiation at $\lambda = 257$ nm (in fused-silica capillaries with 75-μm i.d.) via the near-field thermal lens effect. The detector was realized by using a frequency-doubled argon ion laser as pump laser and a laser diode (emission wavelength, 633 nm) coupled into a monomode optical fiber as probe laser. Comparing the performance of this detector to the performance of a commercial absorption spectrometric detector working at $\lambda = 257$ nm equipped with a unit for on-column detection in fused-silica capillaries showed a substantial improvement in detection limits (up to 30-fold improvement) for the near-field thermal lens detector (NF-TLD). The feasibility of the NF-TLD for sensitive detection of nonfluorescent analytes in real samples after separation by micellar electrokinetic chromatography was shown taking the determination of nitroaromatic compounds in contaminated water from a former ammunition plant as an example. Dependence of the thermal lens signal on pump laser power, velocity of the mobile phase, and chopper frequency was investigated. A linear calibration range over 2 orders of magnitude was obtained.

Electromigrative separation techniques (i.e., capillary electrophoresis (CE), capillary gel electrophoresis (CGE), (micellar) electrokinetic chromatography ((M)EKC), capillary isotachophoresis (CITP), and capillary isoelectric focusing (CIEF)) are highly efficient separation techniques that are performed in capillaries with inner diameters usually in the range of 50−75 μm. Capillary electrochromatography (CEC) is usually performed with 100-μm capillaries. To avoid extra-column band broadening, sample injection volumes have to be restricted to a few nanoliters. Only if on-line focusing techniques can be applied can larger injection volumes be tolerated. Similar restrictions are valid for detection. To avoid instrumental band broadening, optical detection is performed mainly in a section of the capillary (on-column detection). In case of absorption spectrometric detection, the maximum optical path length corresponds to the inner diameter of the separation capillary. These restrictions of the sample injection volume and detection volume result in detection limits (absorption spectrometric detection) that are significantly lower for an analysis method employing high-performance liquid chromatography (HPLC) than for an alternative method employing an electromigrative separation technique.

High detection limits and low signal-to-noise ratios are drawbacks of electromigrative separation techniques that often restrict the application range of these highly efficient techniques to the determination of constituents with concentrations higher than 0.1 mg L^{-1} in the sample solution. Lower signal-to-noise ratios also result in lower precision of quantification. Low precision of quantification might be incompatible with legislation requirements.

Methods to improve precision and detection limits include the use of capillaries with widened inner diameter at position of detection,[1] z-shaped detection cells,[2] and multireflection detection cells.[3] However, to avoid excessive instrumental band broadening and, hence, intolerable loss in efficiency, a 10-fold improvement in detection limits with these methods appears to be the upper limit.[4] Other strategies comprise on-line focusing of the injected sample zone by stacking, sweeping, chromatographic procedures, or on-line coupling to isotachophoresis.[5] Although in some cases, very impressive improvements of the limits of detection have been obtained with on-line preconcentration methods, they are not applicable in all cases, depending on the composition of the sample matrix.

To exploit the full potential of capillary electromigrative separation methods, detection techniques more sensitive than those based on the measurement of absorbance are highly desirable. One detection technique that can be very powerful in combination with capillary electromigration separation methods is amperometric detection;[6,7] however, this technique is restricted

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to analytes that are either electrochemically active or can be converted into electrochemically active compounds. Alternatively, detection limits several orders of magnitude lower than those obtained with absorption spectrometric detection can be achieved with (laser-induced) fluorescence detection.[8] Laser-induced fluorescence detection can be regarded as the most sensitive small-volume detection method developed so far. However, if the analyte to be determined has no significant fluorescence quantum yield, it has to be converted into a fluorescent derivative. Derivatization reactions are not always available for the analyte of interest. Generally, because of matrix effects, derivatization can result in less precise quantification. It should be mentioned that extremely low detection limits have often been obtained with samples that were derivatized at a high concentration of analyte before being diluted. Of course, limits of detection obtained in such manner are not representative for samples containing trace concentrations of the analyte of interest. Consequently, even for analytes that can be derivatized, a detection technique applicable to the nonderivatized analyte is still highly desirable.

Photothermal detection methods have been proven to offer higher sensitivity and lower detection limits than absorption spectrometric detection for the detection of analytes absorbing in the UV or visible range in low volume rectangular detection cells[9] or micrometer-diameter capillary tubes.[10] These detection methods are based on the fact that the absorption of radiation corresponds to the absorption of energy. The fraction of energy that is not released as radiation (fluorescence, phosphorescence) is converted into heat released into the surrounding medium, increasing locally the temperature in the detection volume. This increase in temperature is measured indirectly by optical means.[11,12]

The successful use of photothermal detection in combination with capillary electromigration separation has been demonstrated by several workers. In 1992, Waldron and Dovichi[13] reported excellent detection limits for phenylthiohydantoin- (PTH-) amino acids separated by micellar electrokinetic chromatography (MEKC) and detected with a laboratory-built photothermal detector. Detection was at 248 nm employing a KrF excimer laser as pump laser. With a similar experimental arrangement, Waldron and Li[14] reported the successful determination of food preservatives after separation by CE. In addition, the use of photothermal detection in combination with CEC[15] and in combination with nonaqueous CE (NACE)[16] has been reported. It should be emphasized that the signal height with photothermal detection is largely dependent on solvent properties, such as specific heat capacity, heat conductivity and dn/dT (refractive index change with temperature).

Briefly, the principal idea of thermal lensing is as follows: an excitation light beam passes through a sample of interest, the wavelength of the excitation light beam is tuned to an absorption line of the analyte, and a part of the optical energy is absorbed by the sample.[9,12,17]

By absorption of radiation, molecules are excited into vibrational, rotational, or electronic states. By means of nonradiative relaxation processes, the excited molecules release the excitation energy in the form of heat. This causes heating of the medium in which the analyte is dissolved that is associated with a change in the refractive index. This effect can be monitored with a second light beam (probe beam with wavelength not absorbed by the sample) as a local change in the beam intensity (e.g., passing through a pinhole). This indirectly monitored thermal lens signal depends on the refractive index profile induced in the sample by the excitation (pump) beam. At steady-state conditions, the change in refractive index of the medium is related to the temperature change through the differential equation

$$\frac{dn}{dT} = \left(\frac{dn}{dT}\right)_\rho + \left(\frac{dn}{d\rho}\right)_T \left(\frac{d\rho}{dT}\right)_p \qquad (1)$$

where n is the refractive index; T, the temperature; ρ, the density of the sample; and p, the pressure. The temperature coefficient of the index of refraction, dn/dT is determined primarily by changes in the sample density (second term). Most liquids expand when heated, resulting in a negative value of dn/dT.

Krattiger et al.[18] have demonstrated for the determination of nucleosides and nucleotides by MEKC in 20-μm-i.d. capillaries that the replacement of a commercial absorbance spectrometric detector with a hologram-based photothermal detector (frequency-doubled argon ion laser as pump laser, emission line 257 nm, and HeNe laser as probe laser) results in an improvement of the concentration limits of detection of ~2.4−30. These large differences in improvement ratios are ascribed to optical bleaching effects.

On the basis of the theoretical work of Wu and Dovichi,[19] of Power,[20] and of Li et al.,[21] Seidel et al.[22] presented a photothermal detector (near-field thermal lens detection, NF-TLD) for detection in capillaries,. The detector presented had a crossed-beam configuration of the pump (argon ion laser) and probe laser (HeNe laser) beams. In this experiment, the thermal lens effect was observed in the near-field mode via a photodiode placed behind a pinhole that is placed only 4 mm apart from the separation capillary. With this detector working with an emission line of the pump laser of $\lambda = 364$ nm, a ~50-fold improvement in concentration detection limits for nitrophenol pesticides that have been separated by MEKC has been obtained (i.d. separation capillary, 75 μm), compared to detection limits measured with the same separation method and a commercial absorption spectrometric detector working at $\lambda = 364$ nm. The detector presented was also applied to the determination of metal cations via complexation

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and separation by capillary electrophoresis.[23] A modified version of the presented detection unit (fiber optics) was applied to determine amino acids derivatized with 4-dimethylaminoazobenzene-4-sulfonyl (dabsyl) chloride separated by capillary electrophoresis.[24]

In the present paper, we investigate whether near-field thermal lens detection at a detection wavelength of 257 nm offers advantages over conventional absorption spectrometric detection in combination with capillary electromigrative separation techniques. A very compact capillary detector based on the near-field thermal lens effect working with a frequency-doubled argon ion laser (emission wavelength, 257 nm) as pump laser and a laser diode ($\lambda = 633$ nm) as probe laser is presented. The dependence of the signal height on several instrumental parameters of the detector is investigated.

The determination of nitroaromatic compounds that are of importance as possible pollutants at the sites of former ammunition plants has been taken as an example. Nitroaromatic compounds cannot be quantified by fluorescence detection because of the strong fluorescence quenching caused by nitro groups. In a previous paper, Kleiböhmer et al.[25] demonstrated that MEKC offers advantages over HPLC (concerning resolution and separation speed) in the determination of explosives residues in soil. However, with conventional absorption, spectrometric limits of detection are at least 1 order of magnitude higher employing the MEKC method than with the HPLC method, restricting the application of the MEKC method to samples of heavily polluted soil.

In the present paper, we investigate whether the detection limits for the solutes of interest can be improved (compared to absorption spectrometric detection with a commercial detector) by photothermal detection with the detection unit developed in our laboratories. The MEKC-NF-TLD method developed is applied to a real sample, and results are compared to those obtained with an established reference method.

EXPERIMENTAL SECTION

Chemicals. All test solutes were used as received without further purification. With the exception of 1-fluoro-2,4-dinitrobenzene (Merck, Darmstadt, Germany), all solutes were obtained either as pure standards or as solutions ($c = 1000 \pm 30$ mg L^{-1}) in acetonitrile from Promochem, Wesel, Germany.

Separation buffers were prepared from disodium tetraborate (p.A., Fluka, Seelze, Germany), boric acid (suprapure, Merck, Darmstadt, Germany), and sodium dodecyl sulfate (SDS) (p.A., Fluka, Seelze, Germany). Standard solutions were prepared in acetonitrile and diluted by separation buffer to the final concentration.

Liquid Chromatographic Measurements. The HPLC system used consisted of a Gynkotek 580 pump, a Gynkotek 340S UV detector, and a Dionex ASI 100 autoinjector. The column (Nucleosil 120-3 C18, 250 × 3 mm) was from CS-Chromatographie-Service, Langerwehe, Germany. As mobile phase, a methanol/water gradient was run (starting with 100% water, with linear increase to water/methanol 45:55 (v/v) in 30 min) at a constant flow rate of 0.4 mL min^{-1}. Peak identification was achieved by comparing retention times and absorption spectra. The quantification was performed by calculating peak areas at a detection wavelength of 230 nm and comparison with data obtained by an external calibration. Injection volume was 250 μL (untreated aqueous sample).

MEKC. The SpectraPhORESIS 100 capillary electrophoretic (CE) system was from ThermoQuest and was equipped with either the home-built photothermal detector (see section "Thermal Lens Detection") or (for comparison) a conventional UV absorbance detector (Linear UVIS 200, Spectra Physics). Fused-silica capillaries (Supelco and CS-Chromatographie-Service) of 75-μm-i.d., 104 cm total length (68 cm to the detector), and 360-μm o.d. were used. The polyimide coating was removed by heating at the detection window and was washed with acetone. Samples were injected in the hydrodynamic mode, applying vacuum for 0.3 s. Thiourea was used as a marker of the hold-up time.

Separations were performed using an aqueous buffer with c(SDS) = 25 mmol L^{-1}, $c(Na_2B_4O_7)$ = 2.5 mmol L^{-1}, and $c(H_3BO_3)$ = 12.5 mmol L^{-1} (pH = 8.9) as separation electrolyte and a separation voltage of 30 kV.

Thermal Lens Detection. The latest version of our laboratory-developed photothermal detection device for capillary electrophoresis contains a frequency-doubled Ar^+ laser (Lexel 95 SHG) that is used as pump beam source. This laser system delivers up to 120 mW cw at $\lambda = 257$ nm. Attenuation is achieved by a thin, reflective aluminum film.

A sketch of the optical pathways of the pump and the probe beams through the capillary is shown in Figure 1: the pump laser beam impinges horizontally at the bottom of the photothermal detection device on an intensity-modulating ultraminiature chopping head (model 360 H, Scitec Instruments), which is integrated in the photothermal detector block. The chopper simultaneously gives the reference signal of the lock-in amplifier to permit phase sensitive detection. After passing the chopper, the pump beam is vertically deflected upward by a 45° mirror and focused by a lens ($f = 25$ mm) into the capillary, whereby (as a result of a local increase in temperature as a consequence of light absorption) the refractive index of the flowing medium is gradually altered and a so-called thermal lens is formed. A second laser diode, intensity-stabilized and guided by a monomode optical fiber (Schaeffer and Kirchhoff) is used for the detection of the change in the refractive index. This probe beam enters via an optical fiber in a cross-beam experimental setup (90°) focused by a lens ($f = 15$ mm) into the fixed capillary and overlaps with the pump laser beam in a very small volume determined by the inner diameter (75 μm) of the capillary. The optimum sizes of the waists of the pump and probe laser beams (ratio 1:10) were determined according to ref 26. After passing the capillary (dotted lines), the probe beam diverges, and the thermal lens signal is detected with a photodiode behind a pinhole ($d = 200$ μm), which is located ~1 cm behind the capillary. The thermal lens signal is preamplified and fed into a lock-in microcontroller, which is integrated in the rear section of the photothermal detector head. The data signal output is fed via an R 232 interface into a personal computer for measurement control and data treatment. With a second photodiode, the intensity of the pump beam is monitored. Both power and pointing stability

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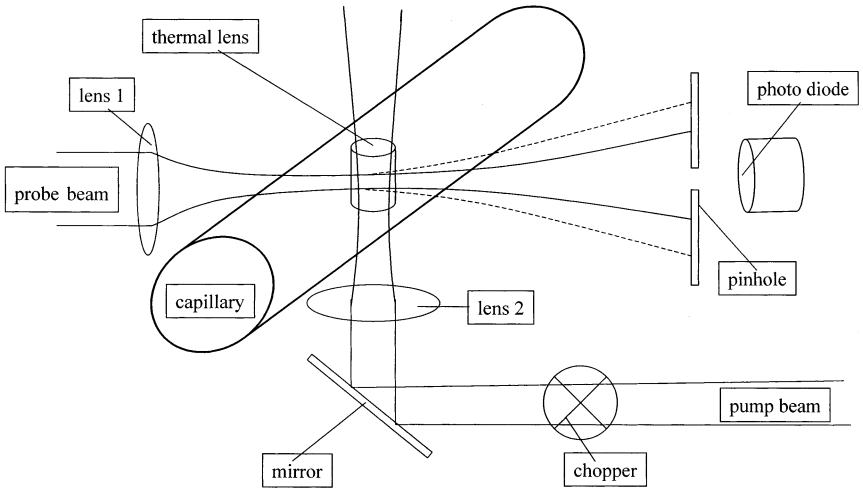

Figure 1. Schematic view of the optical pathways (of pump and probe beams through the capillary) used in the thermal lens detection unit

of the pump laser, however, proved to be sufficient so that no further means of signal normalization were required.

Because of the small detection volume (a few nanoliters), a transverse arrangement of the two laser beams has advantages over a collinear setup. As a result of the short interfering path, there is no gain in sensitivity by a collinear setup. However, with a transverse arrangement fewer optical components are needed. The capillary holder permits an accurate (in the micrometer range) three-dimensional alignment of the pump and the probe beam.

RESULTS AND DISCUSSION

Near-Field Thermal Lens Detector. Detector parameters were optimized in terms of the signal-to-noise ratio obtained for the signal generated by a solution of 1,3-dinitrobenzene ($c = 0.04$ g L^{-1}) (taken as example), as compared to the noise generated by the nonabsorbing separation buffer *with the separation capillary being completely filled with the solution of interest*.

For 1,3-dinitrobenzene, the thermal lens signal (at constant velocity of the solution in the capillary) is proportional to the pump laser power (Figure 2) up to a laser power of 120 mW. However, for a different analyte, 1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane (HMX), there is strong deviation from linearity under identical experimental conditions. Dynamic optical bleaching and photolysis might be responsible for this nonlinearity.[12] An intermediate pump laser power of 105 mW was chosen for further experiments,[13,18] because it is well-known that at extreme energy density, the sample is decomposed, the solution can be boiled, and even the cell might be degraded.

The chopper frequency has a large impact on the signal generated by the lock-in amplifier. In Figure 3 the dependence of the thermal lens signal on the chopper frequency for several voltages (between the ends of the capillary) is depicted. There is an increase in the amplified signal with decreasing chopper frequency.

The time-resolved signal (output of the photodiode behind a pinhole recorded with an oscilloscope) generated by the chopped beam can be characterized by (i) an exponential decrease of the measured beam intensity (approaching a saturation limit) due to the formation of the diffracting element (thermal lens) in the

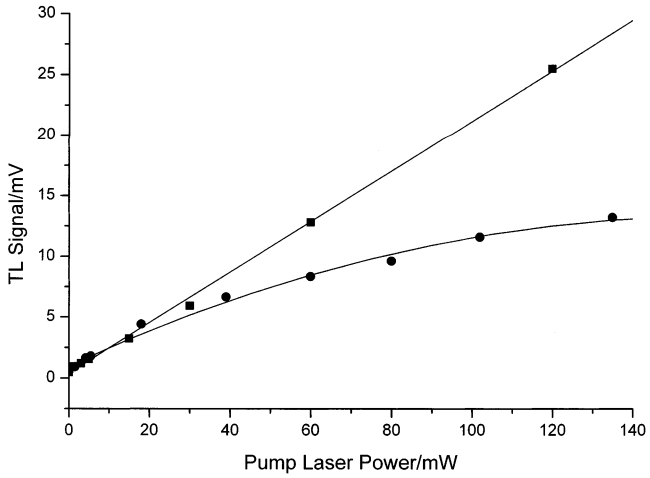

Figure 2. Dependence of the thermal lens signal on pump laser power. Capillary completely filled with a solution of analyte ($c = 0.04$ g L^{-1}) in separation buffer (disodium tetraborate 2.5 mmol L^{-1}, boric acid 12.5 mmol L^{-1}, SDS 25 mmol L^{-1}). ■, 1,3-dinitrobenzene; ●, HMX; chopper frequency, 100 Hz; voltage, 30 kV. Capillary: 104 (68) cm, 75-μm i.d.

detection volume and (ii) an inverse exponential increase of the measured beam intensity (approaching a saturation limit) due to the dissipation of heat from the solution into the capillary wall and the surrounding medium (air). It has to be emphasized that this type of function has been obtained for all analytes investigated. The periodic time-resolved signal has a frequency determined by the periodicity of the chopper. The amplitude (i.e., the height of the periodic signal change) is proportional to the signal generated by the lock-in amplifier. It is evident that the amplitude of the periodic time-resolved signal becomes smaller with increased frequency. Hence, (if the noise is independent of the chopper frequency) the chopper frequency should be selected as low as possible taking into consideration that with very sharp peaks (as they have to be detected in electromigrative separation techniques), the sampling rate must be sufficiently high (~5−10 Hz in general). An intermediate chopper frequency of 85 Hz has been selected for further studies, because this frequency allowed the highest signal-to-noise ratio under the conditions described in the legend of Figure 3.

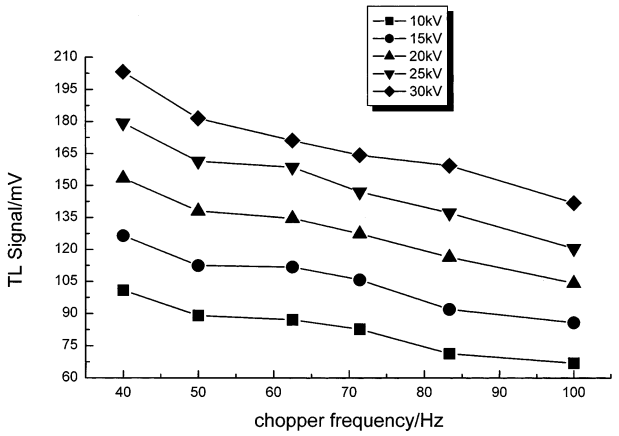

Figure 3. Dependence of the thermal lens signal on chopper frequency for different voltages applied. Capillary completely filled with a solution of 1,3-dinitrobenzene (c = 0.04 g L^{-1}) in separation buffer (disodium tetraborate 2.5 mmol L^{-1}, boric acid 12.5 mmol L^{-1}, SDS 25 mmol L^{-1}). Pump laser power = 105 mW. Capillary: 104 (68) cm, 75-μm i.d.

If the response time of the recording device is sufficiently short, the time-resolved signal is dominated by the heat flow rather than by the signal recording process. In fused-silica capillaries filled with a buffer of pH = 8.9, there is a flow of liquid (the electroosmotic flow, EOF) toward the cathode. The velocity of the EOF is directly proportional to the electric field strength in the capillary. At a constant length of the capillary, the electric field strength is directly proportional to the voltage applied; hence, the velocity of the liquid phase in the capillary (transporting heat by convection versus the cathode) is directly proportional to the voltage applied.

In Figure 3, the signal generated by the lock-in amplifier for various chopper frequencies is also plotted for different voltages applied. It has to be emphasized that in all of these measurements, the capillary is completely filled with the solution of interest so that these effects described cannot be attributed to different velocities of the analyte zone passing the detector or to peak broadening dependent on varying resident times in the capillary. As can be seen in Figure 3, the recorded thermal lens signal increases proportionally to the voltage applied. It has to be emphasized that for each measuring point shown in Figure 3, the alignment of the two laser beams has been manually optimized. Measurements in streaming liquids have shown that for flowing samples, the optimum signal is found when the probe beam is located downstream from the pump beam axis.[27] One possible explanation for the observation that the photothermal signal is enhanced with an increase in separation voltage is that with higher liquid-phase velocity, the negative impact of optical bleaching and of photolysis on the signal height is reduced.

It is evident that the accelerated heat flow also reduces the increase in the time-resolved signal during the formation of the diffracting optical element. Liquid flow acts to remove heat from the probe beam region, which decreases the magnitude and curvature of the temperature gradient formed locally.[27] Consequently, we would expect the thermal lens signal to be decreased as the applied voltage is increased.[28] Taking both effects optical bleaching or photolysis and accelerated heat flow into consideration, the thermal lens signal can be expected to go through a maximum if the velocity of the flowing medium is increased. Because of the limited output of the HV generator used, no measurements with higher voltage have been possible. However, in a similar experiment employing a NF-TL detector as the detector in high performance liquid chromatography or flow injection analysis, Steinle[29] found a maximum of the function describing the dependence of the registered thermal lens signal (amplified by a lock-in amplifier) on the flow rate of the mobile phase containing an absorbing analyte at constant concentration.

It should be noted that with electroosmotically driven liquid flow, the actual velocity of the liquid is not a function of the distance to the capillary wall, because electroosmosis produces a so-called pluglike flow (i.e., the velocity of a liquid segment is virtually independent of the position in the capillary). This independence of the actual velocity of the distance to the capillary wall is in contrast to the laminar flow produced in pressure-driven liquid chromatographic systems.[28]

Limits of Detection, Linearity of Calibration Function. Nitroaromatic compounds have been identified as contaminants in soil and surface water samples taken at the sites of former ammunition plants and firing ranges. Determination of these constituents is required for risk assessment and drinking water quality control. Because of the fluorescence quenching of the nitro group, this class of compounds can be neither detected by fluorescence nor converted into fluorescing derivatives.

Kleiböhmer et al.[25] have developed a method for the separation of 11 nitroaromatic compounds by MEKC. A modified method has been used by us to separate by MEKC 14 nitroaromatic compounds of environmental interest. In Figure 4, the separation of standards is shown. A list of compounds and abbreviations used in this text is given in Table 1. To improve precision of determination, 1-fluoro-2,4-dinitrobenzene has been selected as an internal standard, absorbing at λ = 257 nm and coeluting with none of the compounds of interest. In Figure 5, the structural formulas of the analytes of interest together with their abbreviations are given.

The usefulness of MEKC with absorption spectrometric detection (UVD) for the separation and identification of organic constituents in gunshot and explosive materials has been also demonstrated by Northrop et al.[30] They conclude that "the excellent mass detection limits, low cost, rapid analysis time, superior resolution, and extremely small sample requirements [of MEKC] make this method attractive for the forensic analysis of organic constituents of gunshot and explosive materials." MEKC with UVD has also been evaluated as a useful method for biodegradation studies of 2,4,6-trinitrotoluene (TNT).[31] In these studies, the determination of TNT and its transformation products in various matrixes is mandatory. In addition, capillary electrochromatography (CEC) has been successfully employed for the separation of 14 nitroaromatic and nitramine explosive compounds in under 7 min, featuring efficiencies exceeding 500 000 plates/m.[32]

Although the separation obtained for nitroaromatic compounds by MEKC or CEC is excellent, the limits of detection (LOD) for

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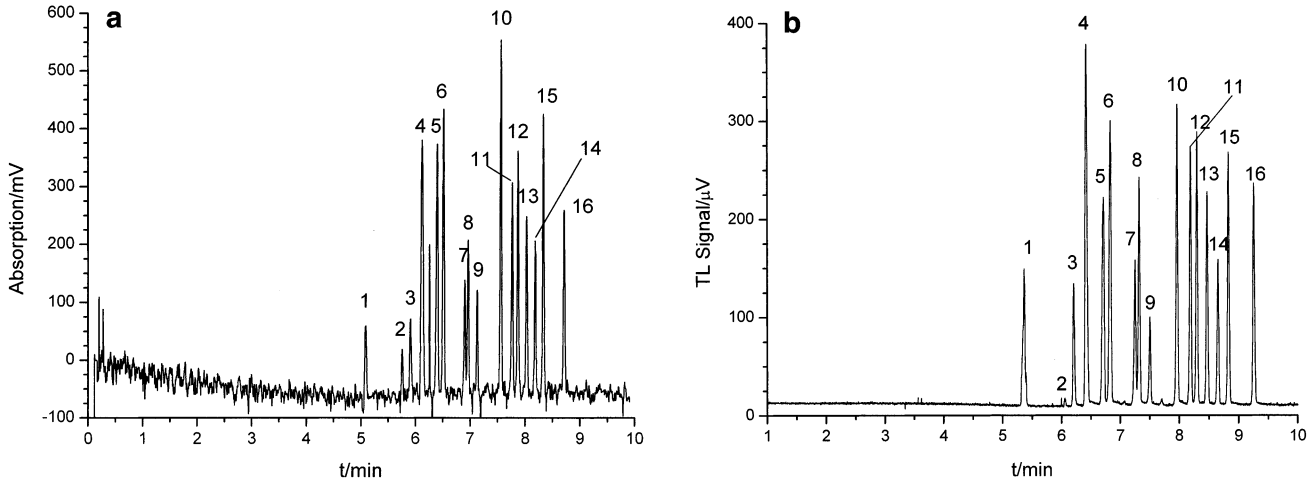

Figure 4. Separation of standards: (a) UV-detection at $\lambda = 257$ nm, (b) thermal lens detection (pump laser power, 105 mW; chopper frequency, 85 Hz) by MEKC (separation buffer: disodium tetraborate 2.5 mmol L^{-1}, boric acid 12.5 mmol L^{-1}, SDS 25 mmol L^{-1}, pH = 8.9. For peak assignment, see Table 1. Capillary: 104 (68) cm, 75-μm i.d. Voltage, 30 kV. Injection, vacuum 0.3 s).

Table 1. Limits of Detection Obtained for Compounds Shown in Figure 5 for UV-Detection ($\lambda = 257$ nm) and TL-Detection under Identical Separation and Injection Conditions[a]

no.	abbrev	compd	LOD TL detector mg L^{-1}	LOD UV detector mg L^{-1}	improvement factor
1	EOF	system peak	n.d.[b]	n.d.	n.d.
2	HMX	1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane	1.4	6.0	4.3
3	1,3,5-TNB	1,3,5-trinitrobenzene	0.11	3.6	33
4	1-F-2,4-DNB	1-fluoro-2,4-dinitrobenzene	0.075	2.0	27
5	1,3-DNB	1,3-dinitrobenzene	0.13	2.1	16
6	NB	nitrobenzene	0.14	2.8	20
7	2,4,6-TNT	2,4,6-trinitrotoluene	0.092	2.3	25
8	Tetryl	2-4-6-*N*-tetranitro-*N*-methylaniline	0.059	1.7	29
9	2-M-3-NA	2-methyl-3-nitroaniline	0.32	5.1	16
10	2,4-DNT	2,4-dinitrotoluene	0.091	1.5	16
11	2,6-DNT	2,6-dinitrotoluene	0.10	2.5	25
12	2-NT	2-nitrotoluene	0.15	3.4	23
13	4-NT	4-nitrotoluene	0.19	4.6	24
14	2,3-DNT	2,3-dinitrotoluene	0.19	3.4	18
15	2-A-4,6-DNT	2-amino-4,6-dinitrotoluene	0.11	1.9	17
16	4-A-2,6-DNT	4-amino-2,6-dinitrotoluene	0.13	2.9	22

[a] For experimental parameters, refer to Figure 4a,b. [b] Not determined.

an MEKC-UVD method are ~1 order of magnitude higher than those measured for an alternative HPLC-UVD method (at the same detection wavelength.)[33,34] Consequently, for environmental studies requiring low LOD, an MEKC or a CEC-UVD method has a clear disadvantage, as compared to an alternative HPLC-UVD method.

We, therefore, evaluated whether NF-TLD can assist in improving limits of detection obtainable with methods employing MEKC. In Figure 4, electropherograms obtained with the same sample ((compounds 2, 3, 7, 8) $c = 10$ mg L^{-1}; (compounds 4, 5, 9−11, 14−16) $c = 20$ mg L^{-1}; (compounds 6, 12, 13) $c = 30$ mg L^{-1}) under identical sample injection and separation conditions recorded by UVD ($\lambda = 257$ nm) and by NF-TLD (emission wavelength of pump laser = 257 nm) are compared to each other. The large improvement in signal-to-noise ratios due to the replacement of the UV detector by the laboratory-built NF-TL detector is obvious.

It is also obvious that the peak height ratios are different in the two electropherograms shown. Such a change in peak height ratios was also observed by Krattiger et al.,[18] who compared electropherograms of the same sample generated under identical sample injection and separation conditions recorded either with a laboratory-built hologram-based thermooptical absorbance detector (pump laser emission wavelength, 257 nm) or a commercial UVD ($\lambda = 257$ nm). The observed change in peak height ratios was ascribed to optical bleaching. This assumption is strongly supported by recording the thermooptical signal dependent on the pump laser power. If the capillary is completely filled with a solution containing the analyte at constant concentration and the dependence of the signal on the pump laser power (solution pumped at constant velocity, constant chopper frequency) is recorded, then a straight line is obtained for the solute not affected by optical bleaching, whereas for a solute the signal of which is strongly discriminated in thermooptical measurements, a nonlinear behavior is found.

Consistent with the experiments conducted by Krattiger et al.,[18] also in the present case, discrimination effects due to optical

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(34) Mussenbrock, E. Ph.D. Thesis, University of Münster (Germany), 1994.

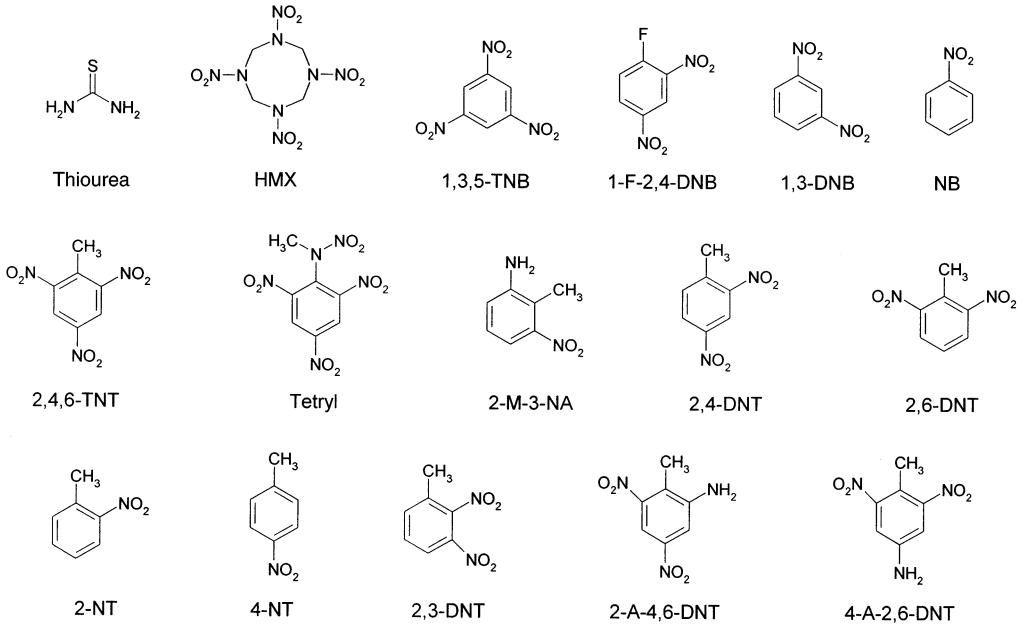

Figure 5. Structural formulas of analytes and the abbreviations used.

Table 2. Theoretical Plate Numbers Calculated for Recorded Peaks from Peak Width at Half Height and Migration Time[a]

			plate numbers	
no.	abbrev	compd	TL detector	UV detector
1	EOF	system peak	n.d.[b]	n.d.
2	HMX	1,3,5,7-tetranitro-1,3,5,7-tetraazacyclooctane	200 000	200 000
3	1,3,5-TNB	1,3,5-trinitrobenzene	210 000	210 000
4	1-F-2,4-DNB	1-fluoro-2,4-dinitrobenzene	160 000	150 000
5	1,3-DNB	1,3-dinitrobenzene	120 000	280 000
6	NB	nitrobenzene	160 000	340 000
7	2,4,6-TNT	2,4,6-trinitrotoluene	270 000	420 000
8	Tetryl	2,4,6-*N*-tetranitro-*N*-methylaniline	260 000	300 000
9	2-M-3-NA	2-methyl-3-nitroaniline	230 000	450 000
10	2,4-DNT	2,4-dinitrotoluene	350 000	540 000
11	2,6-DNT	2,6-dinitrotoluene	410 000	370 000
12	2-NT	2-nitrotoluene	420 000	380 000
13	4-NT	4-nitrotoluene	360 000	400 000
14	2,3-DNT	2,3-dinitrotoluene	370 000	410 000
15	2-A-4,6-DNT	2-amino-4,6-dinitrotoluene	430 000	430 000
16	4-A-2,6-DNT	4-amino-2,6-dinitrotoluene	390 000	390 000

[a] Mean value for five consecutive runs. For experimental parameters, refer to Figure 4. [b] Not determined.

bleaching or photolysis can be assumed. However, they should neither reduce the linearity of the calibration function nor affect the accuracy of quantification based on peak areas, corrected peak areas, or peak heights.

A comparison of the electropherograms given in Figure 4 also suggests that there is no extra-column band broadening induced by replacing the UV detector by the laboratory-built NF-TL detector. Additional band broadening would be induced if the effective detection volume or the signal rise time would be increased. The plate numbers for the peaks shown in Figure 4 are independent of the detection technique employed (see Table 2). Resolution of peaks is not affected by the type of detector used. Consequently, the NF-TL detector studied in this paper provides a spatial resolution, rise time, and sampling rate that does not induce additional band broadening, as compared to results obtained with commercial CE instrumentation.

In Table 1, LODs are given (signal = $3\times \sigma$ of the background noise, mean of five consecutive measurements) that were calculated from 1200 data points of the original data file (noise) and from values for the peak heights for $c = 10-30$ mg L^{-1}. With the exception of HMX, the limits of detection have been significantly improved, replacing the commercial UV detector with the laboratory-built NF-TL detection unit. In most cases, a 20−33-fold improvement has been obtained. It can be assumed that this improvement factor is associated with either the stability of the compound toward photodegradation (photolysis) or with the kinetics of relaxation to the ground state (dynamic optical bleaching).

Calibration functions (peak area (mean of three consecutive runs) versus concentration in sample) were measured for the compounds 1,3-DNB and 1-F-2,4-DNB in the range of 0.5−50 mg L^{-1}. For the two compounds, linear calibration functions have been

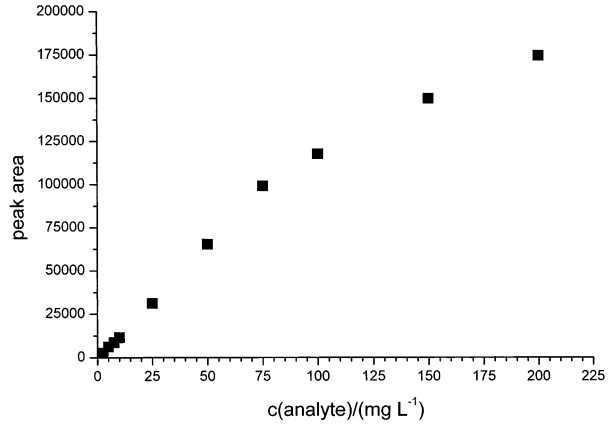

Figure 6. Calibration function (thermal lens detection) for 1,3-dinitrobenzene in the range of 0.5−225 mg L^{-1}. For experimental conditions, see Figure 4b.

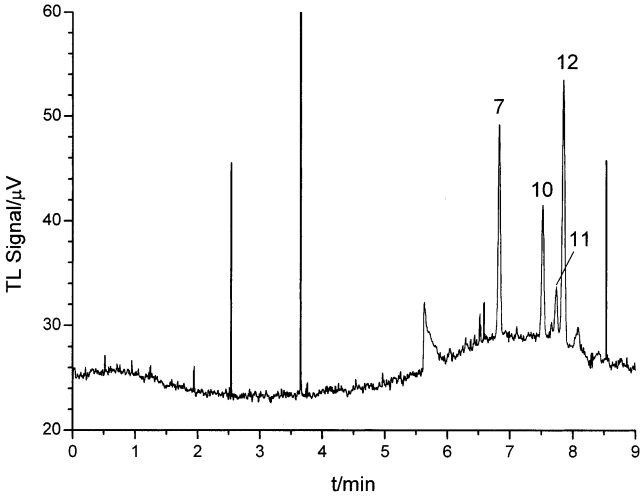

Figure 7. Analysis of a contaminated water sample using MEKC and thermal lens detection Direct injection, vacuum 0.5 s. For remaining experimental parameters, refer to Figure 4b.

obtained. The regression coefficients for the two lines (data points = mean of three consecutive runs) are 0.9999 in both cases. For 1,3-DNB, the maximum linear calibration range is 2 orders of magnitude. For a wider calibration range (Figure 6), there is deviation from linearity (data points = mean of three consecutive runs).

Analysis of a Surface Water Sample. A surface water sample taken from the site of a former ammunition plant has been analyzed employing the MEKC-NF-TLD method developed in our laboratory and for comparison employing an HPLC-UVD method routinely applied to surface water samples contaminated with nitroaromatic compounds. Experimental details of the HPLC-method are given in the Experimental Section.

With both methods, the aqueous sample was directly injected without any sample preparation step. In Figure 7 the recorded trace for the MEKC-NF-TLD separation is given. Four compounds could be clearly identified via spiking. It has to be emphasized that detection and identification of these compounds is not possible if the NF-TL detector is replaced by a commercial UV detector. The quantitative results obtained (1-F-2,4-DNB (c = 1.98 mg L^{-1}) as internal standard, addition of 10 μL of solution of 1-F-2,4-DNB to 1 mL of aqueous sample, calculation of peak area ratios) for five consecutive measurements are given in Table 3, together with the standard deviations.

Table 3. Comparison of Quantitative Results for the Surface Water Sample Obtained with the Proposed MEKC-TLD Method[a] and an Alternative HPLC-UVD Method[b]

compd	MEKC-TLD, ng mL^{-1}	HPLC-UVD, ng mL^{-1}
2,4,6-TNT	850 ± 37	790
2,4-DNT	670 ± 75	580
2,6-DNT	260 ± 18	190
2-NT	2300 ± 230	2100

[a] Arithmetic mean of five consecutive measurements and standard deviation. For experimental parameters, see Figure 7. [b] For experimental conditions, refer to Experimental Section.

These results do not deviate significantly from those obtained by the alternative HPLC method (uncertainty range, ± 5%). Slightly higher values have been obtained by the MEKC-NF-TLD method for 2,6-DNT, possibly as a result of integration uncertainties (low signal-to-noise ratio) or coelution of an interfering compound. With an optimized detection wavelength of 230 nm, the LOD for the HPLC method is ∼1 ng of compound/volume injected. In the present case, 250 μL of the aqueous sample was injected. Such a large injection volume does not deteriorate the chromatographic efficiency, because the injection zone is very effectively focused on the head of the packed column as a result of the extremely low elution strength of water. Assuming an injection volume of 10 μL (necessary, if no focusing possible) in the calculation, the LOD for the compounds determined is ∼0.1 mg L^{-1}, on the same order of magnitude as the LODs calculated for the MEKC-NF-TLD method.

CONCLUSIONS

Near-field thermal lens detection can be regarded as a powerful alternative to absorption spectrometric detection if methods employing an electromigrative separation technique must have lower limits of detection than those obtainable with currently available one-wavelength absorption spectrometric detectors. With a frequency-doubled argon ion laser as pump laser, sensitive NF-TL detection in the UV region below 300 nm is possible. The emission lines (e.g., 257 nm) of this laser allow sensitive detection of many organic compounds and also compounds of bioanalytical interest (e.g., nucleosides, nucleotides, and oligonucleotides[18]). The concept of this detector is not restricted to measurement of absorption at 257 nm. Any wavelength is principally possible if a source of radiation is available that provides radiation that results in a sufficiently sensitive thermal lens effect.

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