Photothermal and light emitting diodes as detectors for trace detection in capillary electrophoresis

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Capillary electrophoresis is a microvolume separation technique increasingly achieving recognition for use in the separation of inorganic and organic compounds due to its short analysis time, and sample volumes in the nanoliter to picoliter range. Photothermal techniques and light emitting diodes have important advantages to offer in detection devices. This overview discusses the applications of these detectors to trace detection and determination of pharmaceuticals, pesticides, metal ions, environmental pollutants, amino acids, *etc*. The basic principles and advances in these detector systems and their applications using capillary electrophoresis in terms of increasing detection limits are also discussed.

1 Introduction

In recent years, there has been a rapid development in capillary electrophoresis (CE) as an analytical technique.[1] The growing interest in analyzing minute complex samples in which more than one species must be determined is one of the driving forces for the rapid development of capillary electrophoresis. The popularity of the use of capillary electrophoresis in the various analytical fields has been accelerated by its simplicity, high efficiency, selectivity, large separation capacity, and relatively low cost. The advantages of capillary electrophoresis are well known: heat dissipation in capillary tubes is good and temperature gradients can be very small, this makes it possible to increase the voltage applied thus considerably decreasing the analysis time and allowing a greater resolution. The separation efficiency is mostly influenced by the inner diameter of the capillary which is of the order of 25 to 250 micrometers. Excellent separation efficiencies are easily obtainable in qualitative form although quantitative results may not be obtainable owing to a shortage of sensitive detection schemes. The primary reason for this is that an extreme degree of miniaturization is involved.

Detection in capillary electrophoresis is a significant challenge due to the small dimensions of a capillary. A number of detection methods (UV–VIS absorption, light emitting diodes (LEDs), photothermal, laser induced fluorescence, electrochemistry, mass spectrometry, inductively coupled plasma-mass spectrometry, nuclear magnetic resonance) are employed in capillary electrophoresis to meet this challenge, many of which are similar to those employed in liquid column chromatography. Direct or indirect optical absorption in the UV–VIS region of the spectrum is the most usual method for detection in capillary electrophoresis. Most of the commercial instruments incorporate a deuterium or tungsten lamp, a monochromator and a photomultiplier tube which is the most complicated part of a very simple system. When LEDs, which are nearly monochromatic light sources, are coupled to photodiodes they provide a very simple alternative detection device in analytical chemistry. Laser induced fluorescence spectroscopy is one of the most sensitive optical methods, but is not always suitable, because not all molecules show fluores-

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cence. In an additional step non-fluorescent molecules can be fluorescently labeled, but it requires sample pretreatment, which is undesirable. Therefore, photothermal techniques have some important advantages and can be useful detection systems. Photothermal techniques have great potential as a routine method for process control or environmental surveillance. The advantage lies in miniaturization of systems for the following reasons: excellent optical quality; fixed, adjustment free components; high reproducibility due to the use of masks; high level of integration due to planar technology; very low costs and the sample volumes being in the nanoliter to microliter range. The main disadvantage of using photothermal methods is that these require many time consuming steps to align the laser beams, which can be done only by highly skilled staff.

Although capillary electrophoresis is considered to be a major advance in separation technology and many reviews have appeared, the applications of photothermal and light emitting diodes as detectors have not been covered yet. This overview gives the basic principles of photothermal and light emitting diodes as detector systems used in capillary electrophoresis. There are many applications of capillary electrophoresis using these detectors, but only a few which appear to be significant are mentioned here to demonstrate their uses.

2 Photothermal detectors

2.1 Thermal lensing

Thermal lensing (TL) was reported first by Gordon *et al.*[2] He accidentally observed the TL effect during the study of laser Raman scattering. He put a sample cell of 1 cm pathlength into the cavity of a HeNe laser and, noticing the change in thermo-optical properties, one year later proposed TL as a method for measuring small absorption coefficients.[3] The first dual-beam experiment was conducted by Grabiner *et al.*[4] in 1972 with a CO_2-laser as pump beam and a continuous HeNe laser as probe beam. He observed the change in the refractive index caused by heating from vibrational relaxation processes of gases at a reduced pressure. This marked the first activity in trace analysis in gases. The thermal lens technique is based on absorption spectroscopy in which a rise in temperature occurs in an illuminated liquid induced by the absorption of small amounts of energy from a laser beam passing through the liquid. The localized temperature change brings about a transverse gradient of the refractive index which is called the thermal lens.

Hu and Whinnery[5] optimized the TL-signal by positioning the probe beam waist one confocal length in front of the minimum beam waist of the pump beam. Most of the thermal lens instruments described are based on far-field detection. This is a major drawback because the set-up needs long deflection pathlengths of the order of a few meters. Miniaturization of thermal lens instruments began with the development of near-field detection by Long and Bialkowski,[6] and Power.[7] It now became possible to build miniaturized sensor systems which were easy to align.[8–10]

2.2 Basic principles

The idea underlying photothermal methods[10] (Fig. 1) is this: an excitation light beam passes through the sample of interest, the light is tuned to an absorption line of the analyte, and the optical energy is absorbed by the medium. If the collisional quenching rate in the analyte is significantly higher than the radiative rate, most of the energy appears in the electronic states or vibrational and rotational-translation modes of the molecules. Heating the medium changes the refractive index. The change in refractive index of the medium can be detected either directly by means of an interferometer (photothermal phase shift spectroscopy or photothermal interferometry), or by a probe laser beam which changes its shape (converging or diverging) (thermal lensing) or is deflected (photothermal deflection spectroscopy) when passing the region excited by the pump beam. In photoacoustics a microphone for gases and solids or a piezoelectric transducer for liquids, which are in acoustic contact with the sample, are used as detectors to measure the amplitudes of resultant acoustic waves.

The photothermal effect can be monitored as a change in intensity of the probe beam by a photodiode after passing through a pinhole (TL) or as a deflection of the probe laser beam (PDS), caused by the change in refractive index, behind the sample cell by a position-sensitive detector. The distance between the sample and the pinhole determines the size of the detector. For practical application we have succeeded in shortening the distance between pinhole and sample cell from the typical 400 mm to 0.4 mm. The latest experiments with the so-called near field thermal lens were performed with a newly constructed rugged and compact system which is easy to use with a capillary electrophoresis system. Photoacoustic and photothermal techniques, such as photoacoustic spectroscopy (PAS), thermal lensing (TL), photothermal deflection spectroscopy (PDS), and photothermal phase shift spectroscopy (PTPS), have proved in the past few years to be valuable analytical tools for measuring very small concentrations of analytes in liquids. PAS especially is preferred in analytical measurements to photothermal techniques, because very fast and easy alignment procedures allow it to be used sporadically. Our own experimental studies of the performance of the four related techniques mentioned above have shown photothermal techniques to be superior to PAS and conventional spectrophotometry in assays for environmental pollutants.[11]

2.3 Theory

The theory of the thermal lens effect has been described frequently. The models include various different excitation sources, various geometries of the pump and probe beams (collinear, transverse or shifted by a small angle) and flow of the observed media. Fig. 2 shows a typical thermal lens detection device. A modulated pump beam produces the time-dependent thermal lens. A transverse probe beam detects the lens by changing its intensity, which is measured with a calibrated photodiode behind a pinhole. In the experimental setup, the laser light is condensed and impinges into the capillary containing sample solution. By the absorption of the pump beam a temperature distribution is created and influenced by convection and thermal diffusion. It is described by the solution of the differential equation (1).[12,15]

$$\frac{\partial T(r,t)}{\partial t} = D\nabla^2 T(r,t) - v_x \frac{\partial T(r,t)}{\partial x} + \frac{1}{\rho \times c_p} Q(r,t) \qquad (1)$$

where $T(r,t)$ is the temperature distribution, D is the thermal diffusivity, v_x is the flow velocity of the medium in the x-

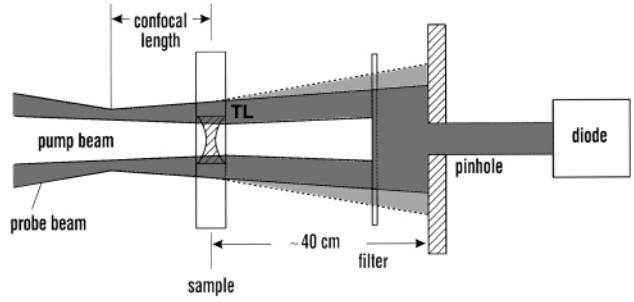

Fig. 1 Schematic diagram of the photothermal principle. (Reproduced with permission from ref. 10.)

direction, ρ is the density, c_p is the specific heat at constant pressure, and $Q(r,t)$ is the temperature source term, which is introduced by the absorption of the pump beam. The terms on the right-hand side respectively represent the effects of thermal diffusion, flow-through order and the heating effect by the pump beam. For cw laser systems (such as the Ar^+-laser) the term, $Q(r,t)$, is given by eqn. (2),

$$Q(r,t) = \alpha \times I(r,t) = \frac{2\alpha P_{av}}{\pi \times a^2}\left[\exp(-2r^2/a^2)\right](1+\cos\omega t) \qquad (2)$$

where $I(r,t)$ is the intensity of the pump beam, α is the absorption coefficient of an optically thin medium, P_{av} is the power of the pump beam, oscillating between 0 and $2P_0$, a is the radius of the pump beam and the expression of the trigonometric function is the modulation of the beam with a frequency given by $\omega/2\pi$ to permit lock-in detection.

If the source term is known, eqn. (3) can be solved, and we obtain the time-dependent temperature distribution inside the sample cell, which is given by:

$$T(x,y,t) = \frac{2\alpha P_{av}}{\pi \times \rho \times c_p} \times \int_0^t \frac{1+\cos\omega\tau}{8D\times(t-\tau)+a^2} \times \exp\left(\frac{-2\left([x-v_x\times(t-\tau)]^2+y^2\right)}{8D\times(t-\tau)+a^2}\right)\mathrm{d}\tau \qquad (3)$$

A time-dependent refractive index gradient is generated inside the cell by this radial temperature distribution, which is described by eqn. (4),

$$n(x,y,t) = n_0 + \left(\frac{\partial n}{\partial T}\right) \times T(x,y,t) \qquad (4)$$

where $n(x,y,t)$ is the refractive index inside the cell and n_0 is the refractive index at the ambient temperature.

The region of the sample which is irradiated by the pump beam reacts like a thermal lens, which causes the probe beam to be deflected or to diverge. The sinusoidally modulated pump beam [eqn. (4)] causes the intensity of the probe beam to change with the same frequency, thus making possible phase-sensitive lockin detection. Therefore, the relative change of probe beam intensity is a direct measurement of the thermal lens signal $S(t)$, which is given for a Gaussian beam profile by eqn. (5),

$$S(t) = \frac{w^2(0) - w^2(t)}{w^2(0)}, \qquad (5)$$

where $w(0)$ is the radius of the undisturbed probe beam with a Gaussian beam profile, and $w(t)$ is the radius during the period of heating by the pump beam.

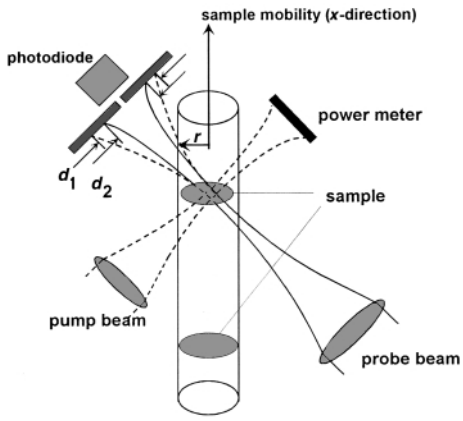

Fig. 2 Schematic view of the thermal lens effect, transverse dual beam setup; d_1 and d_2 indicate the HeNe laser probe beam diameters without and with thermal lens excitation, respectively. (Reproduced with permission from ref. 13.)

2.4 Photothermal sensors in combination with capillary electrophoresis

The applications of photothermal sensors in combination with capillary electrophoresis are very few. Earlier thermo-optical absorbance detectors were used for the detection of nucleotide monophosphate and protein.[1] Photothermal miniaturized detector systems have been developed by Faubel *et al.*[10,13] for capillary electrophoresis and applied to different systems. In Fig. 3 the schematic setup of the CE detector system is given. The capillary stage, CT, shown in Fig. 4(a) and (b), when combined with the Spindler & Hoyer microbank system, allows three-dimensional alignment of the beams to be achieved with a precision in the μm range.[9] The alignment procedure requires a walk-in procedure to be performed for the best TL signal.[14,16]

2.5 Applications of the capillary electrophoretic–thermal lens detector system

Some applications of capillary electrophoretic separations with thermal lensing as the detection system are described below:

2.5.1 Pesticides. There is great interest in detecting pesticides in the ppb range[17] because of their toxicity to humans. Different

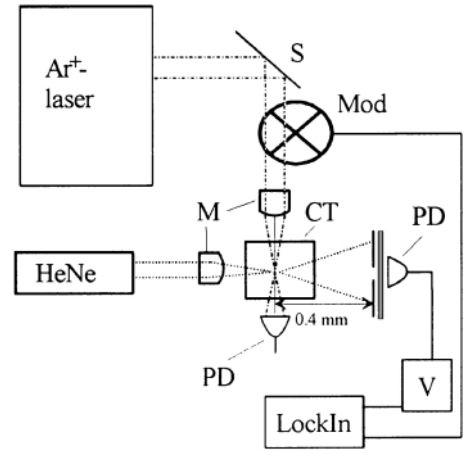

Fig. 3 Schematic setup of the CE–TL detector. S = mirror, Mod = chopper or acoustooptic modulator, M = microscope objective, CT = capillary tube, PD = photodiode, HeNe = helium–neon laser, V = preamplifier, LockIn = LockIn-amplifier. (Reproduced with permission from ref. 13.)

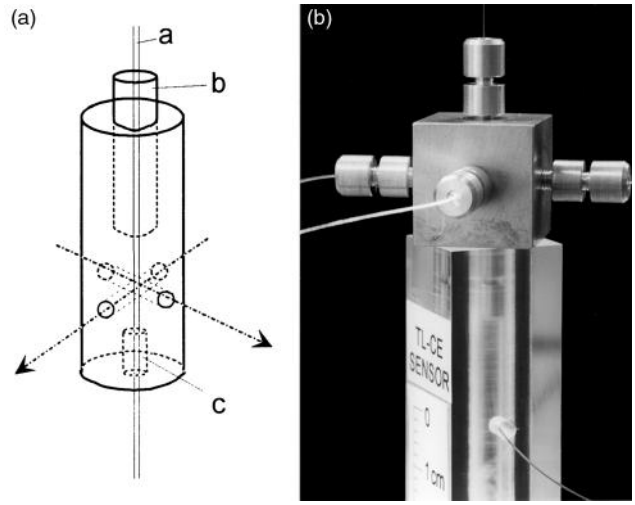

Fig. 4 (a) Sketch of a laboratory-made capillary stage; a = capillary, b = holder, c = center socket, the two arrows indicate the laser light beams. (b) Miniaturised detection head with capillary and fiber optic cables. (Reproduced with permission from refs. 13 and 20.)

separation principles, *e.g.*, micellar electrokinetic capillary chromatography, allow separation of relatively unpolar pesticides. Faubel *et al.*[13] have separated nitrophenol pesticides using micellar electrokinetic chromatography with thermal lens detection and compared their results with normal UV detection. The structures of the separated pesticides are shown in Fig. 5.

Micellar electrokinetic capillary chromatography (MECC) allows non-polar samples to be separated by a distribution of the substances between an aqueous electrophoretic buffer phase as carrier electrolyte and micelles (pseudostationary phase), which arise when the concentration of the surfactant is higher than critical micellar concentration. This method was first described by Terabe[18] in 1984.

The pesticides were separated by MECC and detected with a UV–VIS detector (linear UVIS 200, Spectra Physics) and the TL detector system. The anionic surfactant used was SDS (sodium dodecyl sulfate), an anionic detergent. As a carrier electrolyte, 50 mM SDS–10 mM Na phosphate was used with pH = 7.0. In Fig. 6, separation by the UV detector is shown; in Fig. 7, the TL detector was used. The signal-to-noise ratio was significantly improved by the TL detector, thus allowing detection in the ppb range. The UV detector was fixed at a wavelength of 364 ± 3 nm; the pump beam of the Tl detector delivered a power of 150 mW. The linearity of both methods for a 75 μm capillary was more than three orders of magnitude. The limit of detection with a signal-to-noise ratio of three was 1.1 ppm for DNOC for the UV–VIS detector and 23 ppb for the TL detector, which was a factor of 48 less than in usual absorbance spectroscopy (Table 1).

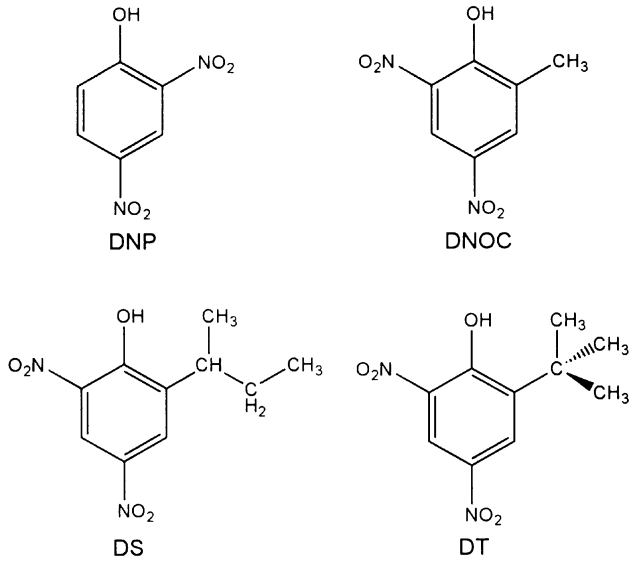

Fig. 5 Nitrophenol pesticides; DNP = 2,4-dinitrophenol, DNOC = 2-methyl-4,6-dinitrophenol, DS = 2-*sec*-butyl-4,6-dinitrophenol (dinoseb), DT = 2-*tert*-butyl-4,6-dinitrophenol (dinoterb). (Reproduced with permission from ref. 13.)

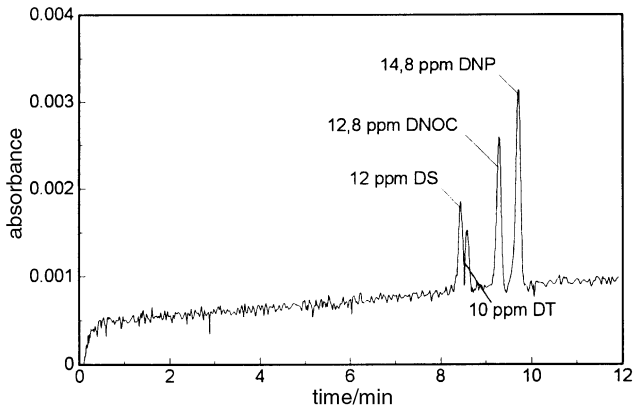

Fig. 6 Capillary electropherogram of a mixture of pesticides with UV-detection with a 75 μm capillary. (Reproduced with permission from ref. 13.)

2.5.2 Metal ions. Faubel *et al.*[19] have determined the natural iron concentrations in real water samples by using thermal lensing as a high performance capillary electrophoretic detector using 1,10-phenanthroline as a chromogenic reagent, which forms a stable complex with Fe(II) but not with Fe(III). The various ions do not interfere in the determination of iron. Fig. 8 illustrates the separation of iron(II) in the presence of Cu(II) using a thermal lens detector system. The method was applied to the determination of iron in rain water samples. The concentration of iron in rain water was found to be 0.9 μmol l^{-1}. The limit of detection for iron was 36 nmol l^{-1} with a signal-to-noise ratio of 7 : 1 using a 75 μm fused silica capillary.

2.5.3 Pharmaceuticals. Faubel *et al.*[20] separated a mixture of pharmaceuticals with photothermal detection using capillary

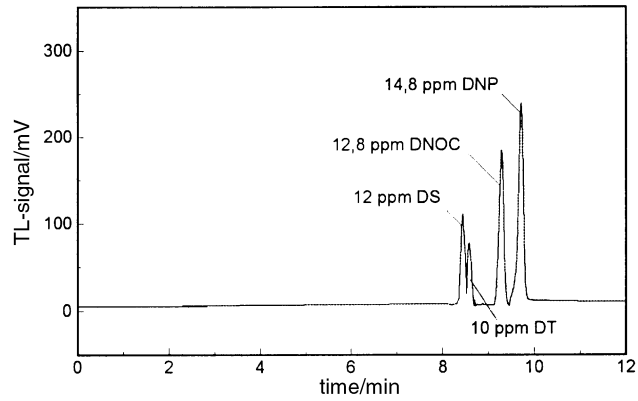

Fig. 7 Capillary electropherogram of a mixture of pesticides with TL-detection with a 75 μm capillary. (Reproduced with permission from ref. 13.)

Table 1 Comparison of the detection limits for different diameters of capillary using thermal lensing and UV–VIS spectrometry

Capillary internal diameter/μm	CE–capillary UV–VIS (ppb)	TL (ppb)
50	2550	45
75	1100	23
100	815	23
200	405	23

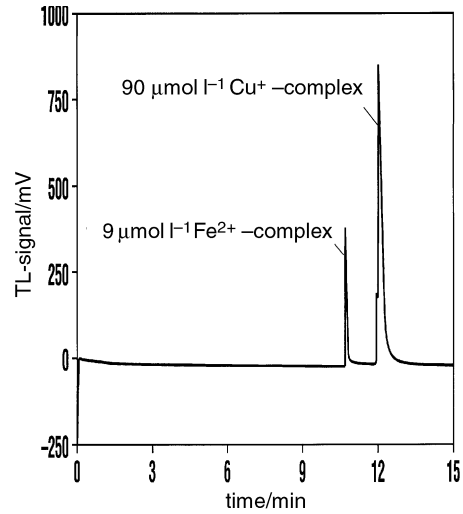

Fig. 8 Separation of iron–copper test solution. 75 μm capillary, 15 kV voltage, 0.3 s hydrodynamic injection time, thermal lens detection. (Reproduced with permission from ref. 19.)

electrophoresis. For the first time chloramphenicol, diclofenac, pentoxifyllin and oxprenolol were determined by thermal lens spectroscopy (Fig. 9). Direct and indirect separation techniques were used for different classes of substances with characteristic absorbance spectra. The combination of capillary electrophoresis and highly sensitive detection with thermal lens spectroscopy permits the analysis of nanoliter volume samples occurring in biomedical diagnostics. The limits of detection for the different drugs were 45 μmol l^{-1}, limited by pump laser stability.

2.5.4 Amino acids. At present, there is considerable interest in the sequence determination of minute quantities of proteins. The thermal lens detector system in combination with capillary electrophoresis handles the smallest amounts of the samples. Because the cross beam setup is independent of the optical pathlength inside the capillary, the smallest amounts of amino acids can be detected. Faubel *et al.*[21] have determined amino acids using a thermal lens as the detector system for capillary electrophoresis. The detector performance was demonstrated by monitoring mixtures of derivatised amino acids. Amino acids were labeled with 4-dimethylazobenzene-4-sulfonyl chloride (Reactive Red) as absorbance reagent. Thus, all the amino acids have a molar absorptivity in the visible region which because of its stability can be qualitatively and quantitatively detected with one excitation line. The separation of six amino acids (arginine, histidine, leucine, alanine, glycine, glutamic acid) is shown in Fig. 10 and the calibration linear range is two orders of magnitude with $r^2 = 0.998$. The limit of detection was 1.8×10^{-7} M and at a detection volume of 50 pl a detection limit of 10 amol was reported.

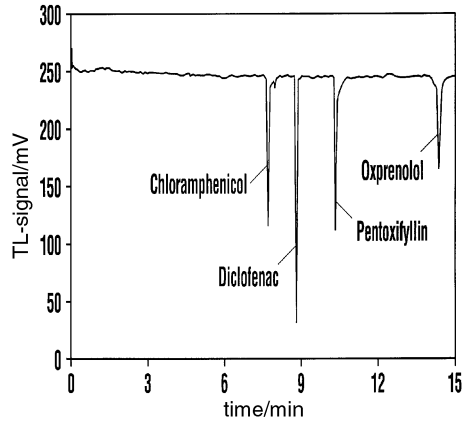

Fig. 9 Capillary electropherogram of pharmaceuticals using fused silica capillary 40/40 cm, i.d. 50 μm, voltage 15 kV. (Reproduced with permission from ref. 20.)

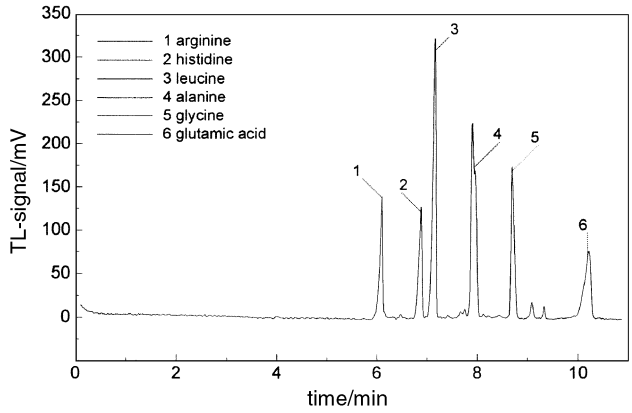

Fig. 10 CE-separation, buffer [100 mmol l^{-1} $NaBO_3$–NaOH, 50 mmol l^{-1} SDS with 15% methanol and 1% THF]; pH = 9.3; voltage: 25 kV, length of capillary = 55/35 cm, 50 μm i.d.; TL-detector, 458 nm; 150 mW; 80 Hz. (Reproduced with permission from ref. 21.)

Dovichi *et al.*[22] described the design of a simple, highly miniaturized instrument for manual microsequence analysis of proteins and peptides. The reaction chamber was made of fused silica capillary tubing with all reagents and solvents necessary for coupling and cleavage delivered *via* two valves and a syringe-based dispenser. Only two pressure regulators were required. A section of the flow-through reaction chamber was heated by thermoelectric modules to control the sequencing reaction temperature. Conversion of the extracted amino acid product to the more stable phenylthiohydantoin (PTH) form was performed so that it was dissolved in 1 μl buffer for the identification. Approximately 0.1% of this PTH product was analyzed by micellar electrokinetic capillary chromatography (MECC) with thermo-optical absorbance detection (TOAD), providing femtomole detection of the phenylthiohydantoin amino acids.

Deng *et al.*[23] described for the first time indirect thermo-optical detection for capillary electrophoresis. A 20 mW helium–neon laser (632.8 nm) was used to provide the pumping beam and a 2 mW helium–neon laser (632.8 nm) supplied the probe beam; Methylene Blue dye was used as a background absorber. The detection method was applied to the detection of amino acids separated by capillary electrophoresis. The detection limit for lysine was 5×10^{-6} mol l^{-1} (signal-to-noise ratio, 2).

2.5.5 Pollutants. A literature survey reveals that capillary electrophoresis has not been fully exploited for the analysis of environmental pollutants. Most of the applications of capillary electrophoresis in this field were to standards but not to real samples. The main disadvantage of capillary electrophoresis was the detector system. The applications of capillary electrophoresis for the analysis of pollutants were reviewed by Song and coworkers.[24] Photothermal methods have also been used for the detection of pollutants by Li *et al.*[25] They used a photothermal interferometric detection technique, in which an interference fringe pattern formed by two overlapping reflected probe beams from the front and rear surfaces of the sample was used to measure the photothermal signal. Its applications for microvolume trace analysis were also discussed.

3 Light emitting diodes as optical detectors

Light emitting diodes (LEDs) are small, inexpensive, power-efficient light sources that are widely used as indicator lamps for industrial instrumentation as well as in household appliances. These electroluminescent devices first became commercially available in the late 1960's. Today, it would be difficult to find an electronic instrument that does not sport an LED. Of special interest to the analytical chemist is that LEDs cover much of the visible and some near infrared (NIR) wavelengths of interest (470–1300 nm, 630–1550 nm) for laser diodes with acceptable monochromaticity for most applications. The expected lifetimes for LEDs are of orders of magnitude greater than for incandescent or discharge sources; mean time between failure for operation at 25 °C is typically 5×10^4 h or approximately 6 years of continuous service for typical LEDs; for some infrared LEDs expected lifetimes are more than 10^6 h. The short term spatial and intensity stabilities of a typical LED are respectively 30–200 and 50 times better than those of the He–Ne laser. With a battery operated power source, the intensity noise is stated to be about 2×10^{-3}% of the total amplitude. Assuming these as the noise sources in a complete detector, absolute noise levels of the order of 9×10^{-6} in absorbance and a detection limit (LOD) of 3×10^{-5} absorbance would be possible; this can be favourably compared with noise specifications of present day state-of-the-art commercial flow-through

absorption detectors (1×10^{-5} absorbance with rise time of 1.0 s).

3.1 Construction of the detector

Butler *et al.*[26] described the construction of a simple absorption detector for capillary electrophoresis using a light emitting diode and optical fibers. They used a bright blue–green light emitting diode as the source. Fiber optic coupling between light source, capillary and photodiode laser was employed. Due to the use of a high intensity light emitting diode, no optical focusing elements were required. The construction of the detector as proposed by Butler *et al.* is shown in Figs. 11 and 12.

3.2 Applications

The application of LEDs in optical instrumentation in analytical chemistry was first proposed in the 1970s. Flaschka *et al*. used the LED-phototransistor (PT) photometer with a 30 cm path length flow-through cell in 1973.[27] Since then, many reports and applications are reported for utilisation of LED-based absorbance detectors.[28] Reports on the use of LEDs in capillary electrophoresis have been limited. Liu *et al.*[29] have described the design of a detection cell for special capillaries of rectangular cross section incorporating an LED and photodiode, but no results for electrophoresis were reported.

Tong and Yeung[30] have presented a system in which a camera lens and a pair of ball lenses were used to focus the light from an LED through a capillary. A microscope lens was employed to concentrate the light into a reference beam and two photodiodes were used as signal and reference detectors. The two signals were processed numerically on a computer to form the ratio in order to remove the noise. The indirect detection of several inorganic anions using permanganate as absorbing dye in the running buffer was demonstrated.

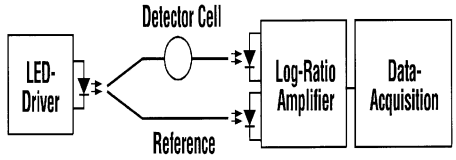

Fig. 11 Overall schematic diagram of the detector. (Reproduced with permission from ref. 26.)

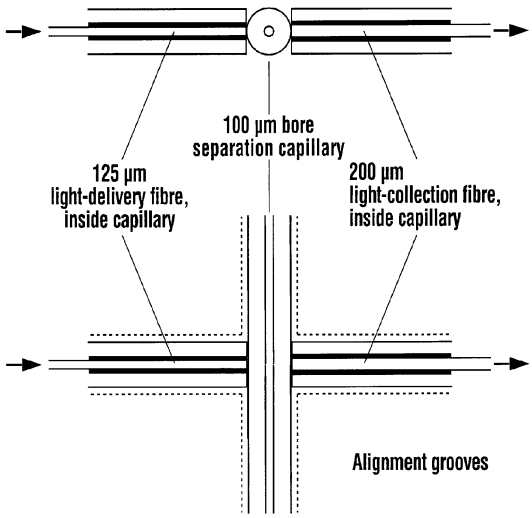

Fig. 12 Cross sectional schematic view from two angles of the detector cell. (Reproduced with permission from ref. 26.)

Bruno *et al.*[31] described a different approach to focus the light from an LED through a capillary. Gradient index (GRIN) lenses were butted directly to an LED and a photodiode and these were incorporated in an absorption cell incorporating a capillary fitted with apertures on either side. No applications of this system using capillary electrophoresis were demonstrated.

Macka *et al.*[32] have described the use of LEDs fitted into a commercial capillary electrophoresis detector in place of the standard light source. They demonstrated the determination of Mg, Ca, Sr, and Ba *via* the complexes with Arsenazo I using green and yellow LEDs. A comparison of noise, stray light and linearity with that of conventional light sources showed a favorable performance for LEDs.

Boring and Dasgupta[33] designed a purpose–made detector for CE. This cell is fitted with an aperture, can hold optical filters for wavelength selection and may be equipped with conventional light sources as well as LEDs. A comparative evaluation of performance in terms of base line noise, stray light and linearity also showed that LEDs can be useful light sources for capillary electrophoresis detectors.

The practical applicability of the detector system was also demonstrated by separating metal–PAR (4-(2-pyridylazo)-resorcinol)[26] chelates. A comparison of a detector using a light emitting diode with a commercial detector is shown in Fig. 13. It was observed that the response for a given concentration was 10–15% greater for the commercial detector, but equal detection limits, 5×10^{-7} M, were reported for both detectors (Figs. 14–16). The same detector system was applied for indirect detection of other inorganic cations and anions using organic dyes.

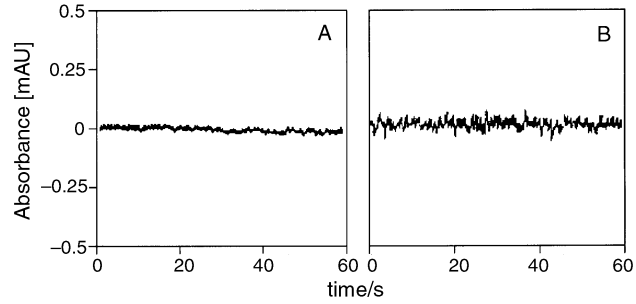

Fig. 13 Baseline noise recorded under static conditions. A, LED-based detector. B, commercial detector, 100 μm, i.d., plain fused silica capillary filled with water. (Reproduced with permission from ref. 26.)

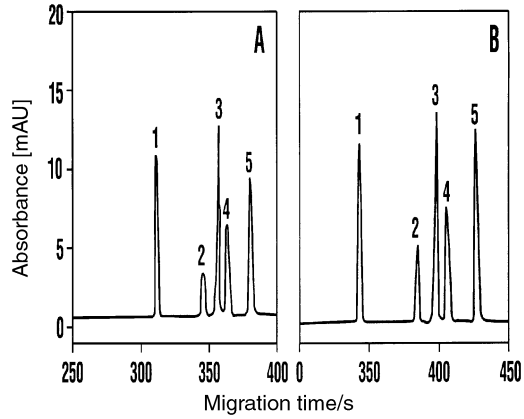

Fig. 14 Electropherogram of metal–PAR chelate separation using a plain fused-silica capillary of 100 μm i.d., 98 cm total length (70 cm to the detector): A, LED-based detector; B, commercial detector. Running buffer: 10 mmol dm^{-3} (pH 8.40) TAPS [*N*-[tris(hydroxymethyl)methyl]-3-amino-propanesulfonic acid] containing 0.1 mmol dm^{-3} PAR. Peaks: 1 = Co^{2+}; 2 = free PAR; 3 = Cu^{2+}; 4 = Fe^{2+}; 5 = Zn^{2+}, all metals 2.5×10^{-5} mol dm^{-3}. Separation voltage: 30 kV. Electrokinetic injection of ions in running buffer with elevated PAR concentration. 10 kV, 8 s. (Reproduced with permission from ref. 26.)

Lee *et al.*[34] determined five dithiocarbamates (DTC) (butyl-, diethyl-, octyl-, and pyrrolidine-1-dithiocarbamates) simultaneously by capillary electrophoresis (CE) with diode array detection. The dithiocarbamates gave linear calibration curves up to 50 μg ml^{-1} with 3σ detection limits in the 0.1–1 μg ml^{-1} range. Freitag and Brüggemann[35] applied photodiode array detection for polycyclic aromatic hydrocarbons and studied different parameters for the analysis of hydrocarbons in soil samples and machine oils.

Mank *et al.*[36] designed a diode laser-based absorption detector for conventional-size liquid chromatography (LC) and various detection setups and individual components were evaluated. A rationing system using a 10 mW 670 nm diode laser allowed detection of 6 × 10^{-10} M mitoxantrone [signal-to-noise (*S*/*N*) = 3; *N* = root-mean-square (rms) noise], an anti-tumor drug, in a biological matrix without any sample cleanup. Multipass detection and intensity modulation of the excitation light did not improve the detection limit. A fiber-optic detector cell, utilizing a gradient-index lens on the light-guiding fiber, was a good and robust alternative for the standard absorption detector cell. Detection limits with the use of the diode laser-based detector were 20-fold better than with an absorption detector for LC.

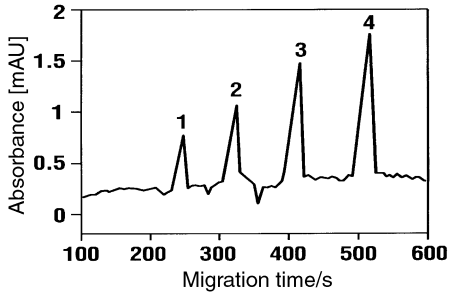

Fig. 15 Electropherogram of the indirect detection of anions using a neutral–coated fused silica capillary of 100 μm i.d., 96 cm total length (68 cm to the detector). Running buffer: 10 mmol dm^{-3} TRIS (pH 7.2). Containing 0.25 mmol dm^{-3} CPR. Peaks: 1 = Cl^-; 2 = SO_4^{2-}; 3 = ClO_4^- 4 = F^-. All 10^{-3} mmol dm^{-3}, except for SO_4^{2-}, 5 × 10^{-4} mmol dm^{-3}. Separation voltage: 15 kV. Electrokinetic injection of ions in running buffer: 10 kV, 4 s. (Reproduced with permission from ref. 26.)

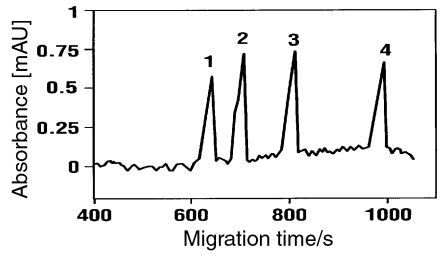

Fig. 16 Electropherogram of the indirect detection of cations using a plain fused-silica capillary of 100 μm i.d., 59 cm total length (40 cm to detector). Running buffer: 1 mmol dm^{-3} TRIS (pH 4.0) containing 0.15 mol dm^{-3} Pyronine G. Peaks: 1 = K^+; 2 = Ca^{2+}; 3 = Na^+; 4 = Li^+. All 10^{-3} mol dm^{-3}, except for K^+, 2 × 10^{-3} mol dm^{-3}. Separation voltage: 15 kV. Electrokinetic injection of ions in running buffer: 10 kV, 2 s. (Reproduced with permission from ref. 26.)

4 Conclusions

Capillary electrophoresis has established itself as a routine technique for the analysis of drugs, enantiomer separations, proteins and amino acids, pesticides, toxic metal ions (cations and anions), pollutants, *etc*. During the past few years many modifications in detector systems have been reported in the literature which increase the limit of detection. A comparison of detection limits for different detectors is given in Table 2. Combination of capillary electrophoresis with laser-induced fluorescence detection is highly promising but it is not applicable to all systems and therefore photothermal methods are more promising. Only a few applications of these photothermal methods to capillary electrophoresis are reported in the literature. Light emitting diode-based optical detectors provide inexpensive high performance life alternatives to continuum source detectors. For applications in which a reasonable match can be obtained between an LED wavelength and the analyte absorption, such detectors will generally outperform other available designs. Photothermal methods with a laser source are capable of very low noise levels. These methods do not involve a simple measurement of the change in transmission of a probe beam of desired wavelength. For the same absorbance change, the change in the signal is much greater at the transducer level in such measurements relative to that in conventional measurements transmission intensity. Unfortunately, practical affordable variable wavelength absorbance detectors based on photothermal principles are not on the horizon. But multi-wavelength detectors containing a multiplicity of emitter LEDs can be made easily and may constitute a high light throughput alternative to continuum sources coupled to a diode array detector. Several sequentially tuned LEDs can be serially placed along a tube opposite correspondingly placed pulsed detectors, constituting a series of radial path detectors with unusual flexibility in making kinetic measurements. In essence, the low

Table 2 Comparison of the detection limits for different spectroscopic detectors used for capillary electrophoresis

Detection mode	Approx. linear range/M (*S*/*N* = 2 or 3)	Approx. mass LOD/Mol	Applications	Advantages	Disadvantages
UV–VIS absorbance	10^{-6}–10^{-3}	10^{-15}	Nucleotides, peptides, cations and anions	Easy to use	Not so sensitive
Indirect UV–VIS absorbance	10^{-5}–10^{-3}	10^{-14}	Carbohydrates, ion analysis	Selective and sensitive	Comparatively less sensitive and imposes limits on choices of buffer
Optical absorbance (LED)	10^{-6}–10^{-3}	10^{-15}	Carbohydrates, ion analysis	Selective and sensitive	Comparatively less sensitive and selective in detection
Laser induced fluorescence	10^{-18}–10^{-12}	10^{-21}	Amino acids, peptides, proteins, nucleic acids	Highly sensitive	Selective and expensive
Mass spectrometry	10^{-12}–10^{-9}	10^{-15}	Amino acids, peptides	Highly sensitive	Selective and expensive
ICP-MS	10^{-12}–10^{-9}	10^{-21}	Amino acids, peptides	Highly sensitive	Selective and expensive
Nuclear magnetic resonance (NMR)	10^{-6}–10^{-3}	10^{-15}	Amino acids, peptides, clinical analysis	Highly sensitive	Low selectivity and expensive
Photothermal (thermal lensing)	10^{-18}–10^{-12}	10^{-21}	Ions, amino acids, peptides, proteins, nucleic acids	Highly sensitive, short analysis time	Expensive and difficult to handle

cost, small size and ease of fabrication of LED-based detectors hold unusual promise for those willing to experiment with them in an unconventional way.

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