

Application of capillary electrophoresis–fluorescence line-narrowing spectroscopy for on-line spectral characterization of closely related analytes

D. Zamzow, C.-H. Lin, G.J. Small, R. Jankowiak*

Ames Laboratory – USDOE and Department of Chemistry, Iowa State University, Ames, IA 50011, USA

Abstract

Capillary electrophoresis (CE) interfaced with low-temperature (4.2 K) fluorescence line-narrowing spectroscopy (FLNS) is used for the separation and spectral characterization of closely related analytes. In this paper, the CE–FLNS system is applied to the analysis of a mixture of deuterated and protonated benzo[*a*]pyrene, a mixture of structurally similar benzo[*a*]pyrene and benzo[*e*]pyrene, and mixtures of dibenzo[*a,l*]pyrene-derived adenine DNA adducts. The CE–FLNS system provides on-line separation and high-resolution spectroscopic identification of CE-separated analytes, via fingerprint structure of vibrationally resolved FLN spectra at 4.2 K. The combination of the separation power of CE and the spectral selectivity of FLNS provide a methodology that has potential to become a powerful tool for molecular analyte characterization. The main applications of the CE–FLNS system, due to its selectivity, should be in the chemical analysis of structurally similar analytes and applications where analyte purity and detailed structural characterization are required. © 1997 Elsevier Science B.V.

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1. Introduction

It is well recognized that in the analysis of chemically complex samples, two-step analytical approaches (separation followed by analyte characterization) are often required. This is why, in addition to the development of separation methods and protocols using HPLC and capillary electrophoresis (CE) techniques, the development of selective detection methods that are capable of providing structural information on separated molecular analytes is an area of active research. For example, HPLC has been coupled with NMR spectroscopy for direct analysis of complex mixtures from both synthetic and biological origins [1]. Interfacing CE with mass

spectroscopy (MS) has been demonstrated [2,3]; capillary zone electrophoresis coupled with electro-spray MS has been used for separation and subsequent detection of DNA adducts [4,5]. Capillary electrochromatography has been coupled to MS for analysis of pharmaceutical drugs [6]. Very recently, CE has also been interfaced with fluorescence line-narrowing spectroscopy (FLNS) for on-line separation and characterization of fluorescent molecular analytes [7]. The motivation for developing these techniques is the need for analytical approaches that can provide selective and unambiguous determination of separated analytes. Recent advances in on-line sample concentration techniques [8–10] used in CE (e.g. focusing, stacking, isotachopheresis) should allow effective application of these methods for the analysis of real-world samples.

*Corresponding author.

CE in particular, has found widespread applications in analytical and bioanalytical separation research. A diverse spectrum of samples has been analyzed; small ions and large biomolecules can be separated [2,11]. This powerful separation technique has been successfully applied to the analysis of DNA adducts [4,5,12] and nucleic acid bases and DNA oligonucleotides [13], and for gene mapping [14], DNA sequencing [15], and cell analysis [16,17]; new applications are rapidly advancing. CE has also begun to have an impact in clinical analytical laboratories [18].

FLNS is a high-resolution fluorescence-based detection method that provides spectral resolution sufficient for structural characterization of molecular analytes and distinction between structurally similar species [19–21]. In regard to selectivity, it has been shown that various stereoisomers and different conformations of polycyclic aromatic hydrocarbon (PAH)–DNA adducts can be resolved by FLNS [22,23]. Concerning versatility, FLNS has been successfully applied to PAHs [7,12] and nucleoside/nucleotide DNA adducts from *in vitro* [24,25] and *in vivo* [25–27] experiments, where one is limited to picomole or smaller quantities of bound metabolite. A variety of other fluorescent analytes including thiazole orange dyes [28], squaraine dyes [29], chlorophylls [30], heme proteins [31], iron-free cytochrome [32], and bacteriochlorophylls [33] have also been characterized by FLNS. This paper further explores the potential and versatility of the recently assembled CE–FLNS system, and shows new examples of its application to the separation and on-line spectral characterization of PAHs and PAH–DNA adducts.

2. Experimental

The components of the CE–FLNS apparatus have been described in detail recently [7]. However, due to the novelty of the system, a brief description is included here; a schematic diagram is shown in Fig. 1. The system consists of a modular CE apparatus (ATI Unicam Model 310) coupled to a capillary cryostat (CC) and a high-resolution spectrometer system. The CC (Janis Research) consists of a double-walled quartz cell with inlet and return lines

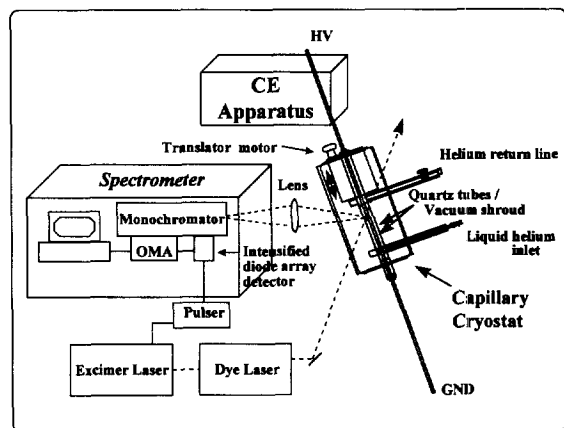


Fig. 1. Schematic diagram of the CE–FLNS system used for structural characterization of CE-separated analytes.

for introducing liquid nitrogen or liquid helium; the outer portion of the CC is evacuated. The capillary, positioned in the central region of the CC, can be cooled to 77 or 4.2 K after the CE separation is complete, by a continuous flow of liquid nitrogen or liquid helium through the cryostat. Liquid helium temperature is required for laser-excited FLNS experiments. Non-line-narrowed (NLN) fluorescence spectra acquired at 77 K provide information including the position (wavelength) of the fluorescence (0,0) band and the vibronic band structure of CE-separated analytes. Once the fluorescence origin band position is known (from the NLN spectra), appropriate laser excitation wavelengths can be selected for FLNS characterization of the analyte.

For the separation of PAHs and PAH–DNA adducts discussed in this paper, an acetonitrile–water solution (30:70, v/v) containing 40 mM sodium bis(2-ethylhexyl) sulfosuccinate and 8 mM sodium borate, adjusted to pH 9 using phosphoric acid, is used as the CE buffer. Separation of analytes is achieved on the basis of micellar electrokinetic chromatography (MECC), with sodium bis(2-ethylhexyl) sulfosuccinate as the surfactant [34]. UV-transparent fused-silica capillary tubing (Polymicro Technologies), 95 cm×75 μm I.D., is used for electrophoretic separations. Samples are injected hydrodynamically, and separations are typically performed at an applied voltage of 25 kV. An ab-

sorbance detector, positioned 40 cm from the inlet of the capillary (not shown in Fig. 1), provides absorption electropherograms. Fluorescence electropherograms are obtained using either an excimer laser or argon-ion laser for excitation of the CE-separated analytes within the CC, approximately 85 cm from the capillary inlet. When the separation is complete, the voltage is turned off and the capillary is cooled to low temperature.

CE-separated analytes are characterized by low-temperature fluorescence spectroscopy. For FLNS measurements, a 1-m focal-length monochromator (McPherson Model 2061) is equipped with a 2400-G/mm grating, providing a resolution of 0.08 nm and a spectral window of approximately 10 nm. An excimer-pumped dye laser system (Lambda Physik LEXtra excimer and FL-2002 dye laser) is used for excitation. Fluorescence line-narrowed (FLN) spectra are generated using a series of laser wavelengths that selectively excite regions of the $S_1 \leftarrow S_0$ transition of the analyte, each of which reveals a portion of the excited-state vibrational frequencies of the molecule [19,20]. Fluorescence is collected at a right angle to the laser excitation beam, dispersed by the monochromator, and detected by a photodiode array (Princeton Instruments IRY-1024/GRB intensified array); the diode array is operated in a gated detection mode, using the output of a photodiode to trigger a high-voltage pulse generator (Princeton Instruments FG-100). To discriminate against scattered and reflected laser light, the CC is tilted 20° with respect to the laser beam. The CC is attached to a translation stage (New England Affiliated Technologies Model TM-200-SM); translation of the CC and capillary allows the CE-separated analytes to be sequentially characterized by NLN spectroscopy and FLNS.

3. Results and discussion

To further evaluate the feasibility of the CE-FLNS approach and apparatus, we discuss below the cooling rate of separated analytes within the capillary cryostat and show several examples of separation and on-line spectral characterization of closely related compounds using the CE-FLNS system.

3.1. Cooling rate in the capillary cryostat

The small dimensions of the capillary cryostat (22 cm × 4 mm I.D.) and the low thermal capacity of the capillary section to be cooled should ensure rapid cooling of analytes after the CE separation is complete. In Ref. [7] we estimated that cooling of the capillary to 4.2 K takes several minutes. To address this issue we have studied the cooling rate in more detail. Fluorescence spectra of pyrene, acquired as a function of time after opening the valve of the helium transfer line to the CC, are shown in Fig. 2. Spectrum (a) was obtained at room temperature, and spectra (b)–(e) were obtained 30, 35, 40, and 50 s after opening the valve. The five spectra, plotted using the same y-axis scale expansion (offset for clarity), illustrate a very fast cooling rate and a significant increase in fluorescence quantum yield with decreasing temperature. Spectrum (e), obtained at 50 s, is identical to the FLN spectrum of pyrene obtained in a regular helium immersion cryostat. This clearly indicates that the capillary and CC can be cooled to 4.2 K in less than 1 min. As a result, after the separation is complete, the CE-separated

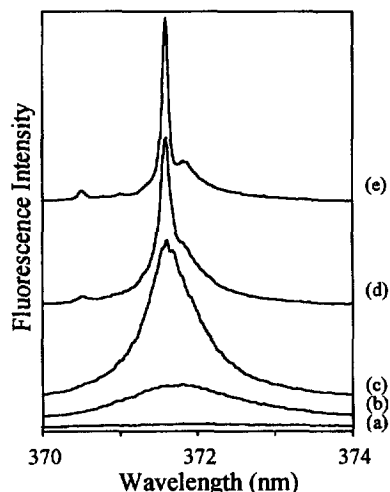


Fig. 2. Fluorescence spectra of pyrene as a function of time (temperature) after opening the valve of the helium transfer line to the capillary cryostat; $\lambda_{\text{ex}} = 369.6$ nm. Spectrum (a) was obtained at room temperature and spectra (b)–(e) were obtained 30, 35, 40, and 50 s, respectively, after opening the valve of the helium transfer line.

analytes can be rapidly frozen in place in the CC, leading to very minimal band dispersion. Once frozen, arbitrary detection times can be used to completely characterize the separated analytes. When the fluorescence analysis is complete, efficient warming of the CC and capillary can be achieved by closing the valve of the helium transfer line and introducing (warm) helium gas into the CC.

3.2. CE–FLNS analysis of perprotio- and perdeuterio-benzo[*a*]pyrenes

Separation and spectral characterization of a mixture of deuterated and protonated benzo[*a*]pyrene (B[*a*]P) by CE–FLNS is shown in Fig. 3. Frame A is a portion of the room-temperature fluorescence electropherogram for the B[*a*]P-d₁₂/B[*a*]P mixture ($c \sim 10^{-5} M$), generated from a three-dimensional plot of fluorescence emission (intensity and wavelength) versus time. The excimer laser was used for excitation (308 nm), and the total fluorescence emission signal in the 350–500-nm region was integrated to generate the fluorescence electropherogram shown in Fig. 3A. The wavelengths of the fluorescence origin bands obtained for peaks (a) and (b) were 403 and 404 nm, respectively (data not shown).

FLN spectra for CE-separated peaks (a) and (b) using selective laser excitation at 395.7 nm at 4.2 K

are shown in Fig. 3, Frames B (spectrum a) and C (spectrum b), respectively. While some of the excited-state vibrational modes are similar in the two spectra, there are some obvious differences. For B[*a*]P-d₁₂, there are strong modes at 353, 493, and 558 cm⁻¹; for B[*a*]P, the strong modes are at 510 and 580 cm⁻¹. Thus, spectra (a) and (b) are clearly distinguishable with a different pattern of vibrational frequencies and intensities, revealing differences in analyte composition. These data (and other FLN spectra obtained using different laser excitation wavelengths; not shown here) can be used as ‘fingerprints’ for spectral identification of a compound. The identity of CE-separated analytes is obtained by comparison of the FLN spectra acquired with the available library of FLN spectra for PAHs. Comparison of the CE–FLNS spectra in Fig. 3 shows that spectrum (a) is virtually indistinguishable from spectrum (c), the B[*a*]P-d₁₂ standard, and spectrum (b) is identical to spectrum (d), the B[*a*]P standard. Therefore, peaks (a) and (b) of the electropherogram can be unambiguously assigned as deuterated B[*a*]P and protonated B[*a*]P, respectively. Thus, deuteration leads to a blue-shift in the fluorescence spectra of PAHs, as has been shown for perylene and B[*a*]P in *n*-octane Shpol’skii matrices [35]. The fact that the migration time for B[*a*]P-d₁₂ is shorter than that for B[*a*]P in Fig. 3A is consistent with previous MECC

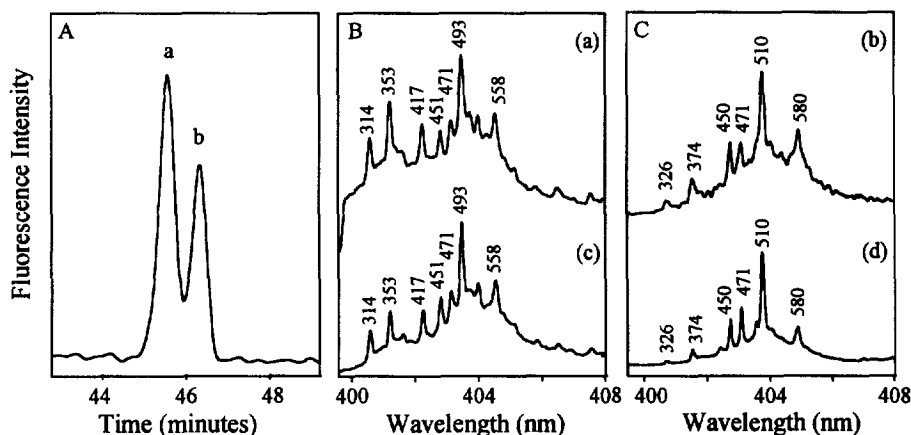


Fig. 3. (A) Room-temperature fluorescence electropherogram for a mixture of (a) B[*a*]P-d₁₂ and (b) B[*a*]P using a CE buffer consisting of 40 mM sodium bis(2-ethylhexyl) sulfosuccinate and 8 mM sodium borate in acetonitrile–water (30:70, v/v), pH 9; capillary, 85 cm×75 μm I.D.; applied voltage, 25 kV; current, 50 μA. (B and C) FLN spectra in the CE-buffer matrix at $T=4.2 K$, $\lambda_{exc}=395.7 nm$, were obtained for the CE-separated analytes (a) and (b). Spectra (c) and (d) are from the library of FLN spectra of PAHs for B[*a*]P-d₁₂ and B[*a*]P, respectively. The FLN peaks are labeled with their S_1 vibrational frequencies, in cm⁻¹.

separations of dansylated methylamine and dansylated methyl- d_3 -amine [36], and indicates that the deuterated analog is more hydrophilic than the protonated compound.

3.3. Analysis of incompletely separated analytes

An example of the CE–FLNS analysis of incompletely resolved analytes is shown in Fig. 4 for a mixture of benzo[*e*]pyrene (B[*e*]P) and B[*a*]P. The fluorescence electropherogram in Frame A shows that these two isomers are not baseline-resolved in the CE separation. Low-temperature fluorescence detection reveals the presence of two analytes via two distinct fluorescence origin bands at 376 and 403 nm, respectively (data not shown). This would also be the case if the analytes were not separated (i.e. if B[*e*]P and B[*a*]P comigrated). CE–FLN spectra for B[*e*]P and B[*a*]P are shown in Fig. 4, Frames B and C, respectively. Since the (0,0) band for B[*e*]P is located at 376 nm, selective laser excitation in the range of approximately 371–357 nm (~ 350 – 1400 cm^{-1} above the origin band) can be used to generate FLN spectra for B[*e*]P; two of these are shown in Fig. 4B. For B[*a*]P, which has its (0,0) band at 403 nm, selective laser excitation in the 397–381 nm range can be used to generate FLN spectra; two of these are shown in Fig. 4C. By changing the laser excitation wavelength, a series of FLN spectra can

be obtained that map out all of the excited-state vibrational frequencies of the molecule. It is this series of fingerprint FLN spectra that provide selective, unambiguous structural characterization of fluorescent analytes. Thus we conclude that in some cases the selectivity of FLNS alone is sufficient to characterize mixtures of analytes, even if they are not completely resolved in the CE separation.

3.4. Confirmation of analyte purity

An application of the CE–FLNS system in the area of confirmation of analyte purity is shown in Fig. 5. Fluorescence electropherograms for two different samples of a dibenzo[*a,l*]pyrene–adenine adduct (3-(dibenzo[*a,l*]pyren-10-yl)–adenine; DBP-N3Ade) are shown in Frames A and B. Both of these samples had been subjected to purification by two-dimensional HPLC [24,37] and were then analyzed by CE–FLNS. For sample 1 (Frame A), there are two major peaks (I and II) plus a number of smaller, minor contaminants. For sample 2 (Frame B), the electropherogram shows the presence of only one prominent peak (II). FLN spectra obtained at 4.2 K using 416.0 nm excitation for the major peaks are shown in Frames C and D. The FLN spectra for analyte II in both samples match the spectra of a DBP-N3Ade standard adduct, whose structure had been confirmed by MS [37]. Thus, analyte II can be

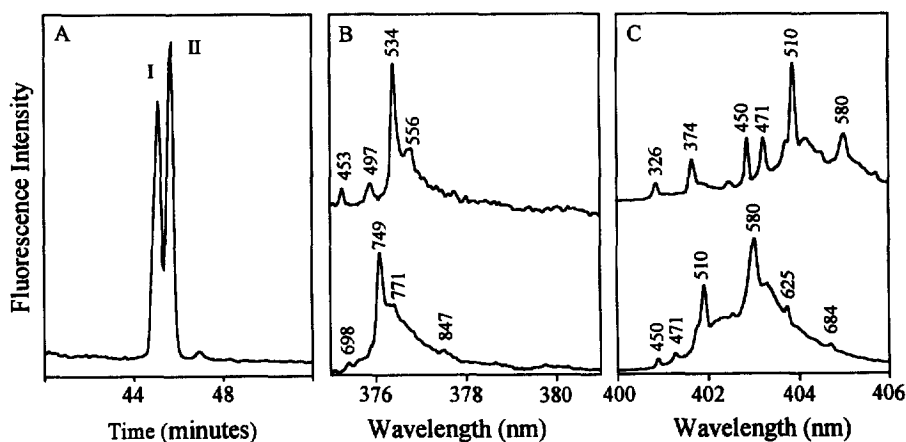


Fig. 4. (A) Room-temperature fluorescence electropherogram for a mixture of (I) B[*e*]P and (II) B[*a*]P. (B) FLN spectra of CE-separated B[*e*]P (I) obtained in the CE-buffer matrix at $T=4.2\text{ K}$ using $\lambda_{ex}=369.0\text{ nm}$ (upper) and $\lambda_{ex}=365.8\text{ nm}$ (lower spectrum). (C) FLN spectra of CE-separated B[*a*]P (II) obtained in the CE-buffer matrix at $T=4.2\text{ K}$ using $\lambda_{ex}=395.7\text{ nm}$ (upper) and $\lambda_{ex}=393.8\text{ nm}$ (lower spectrum). The FLNS peaks are labeled with their S_1 vibrational frequencies, in cm^{-1} .

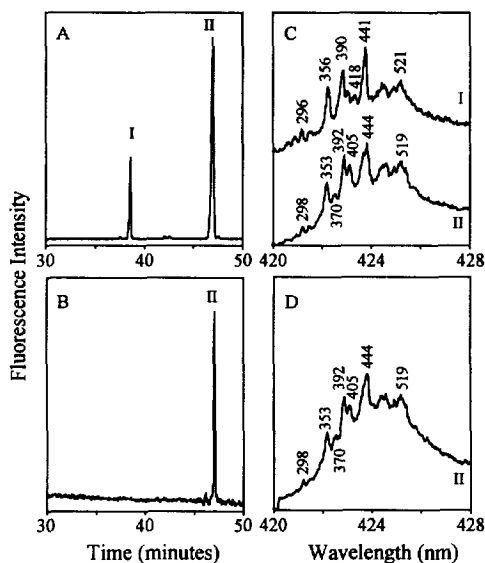


Fig. 5. Room-temperature fluorescence electropherograms (Frames A and B) obtained for two different DBP-N3Ade adduct samples. FLN spectra for the CE-separated analytes (I and II) are shown in Frames C and D using $\lambda_{ex}=416.0$ nm, $T=4.2$ K. The FLN peaks are labeled with their S_1 vibrational frequencies, in cm^{-1} . See text for discussion.

assigned as the DBP-N3Ade adduct. The FLN spectra for analyte I show a different pattern of vibrational frequencies and intensities, so analyte I cannot be DBP-N3Ade. Although the identity of analyte I has not been determined, it is more hydrophilic than DBP-N3Ade (based on the CE retention times) yet possesses a substituted DBP fluorescent chromophore (based on the FLN spectra). This example shows that on-line characterization by CE-FLNS affords a number of advantages compared to HPLC separation, fraction collection, and subsequent (off-line) analysis. Imprecise timing of fraction collection, leading to impure HPLC fractions, is likely responsible for the differences in these two DBP-N3Ade samples. As shown in Fig. 5, on-line analysis by CE-FLNS, combining the high resolving power of CE and the spectral selectivity of FLNS, provides an excellent method to determine the purity of analytes and analyte fractions.

3.5. Analysis of structurally similar DNA adducts

Another example of the selective detection pro-

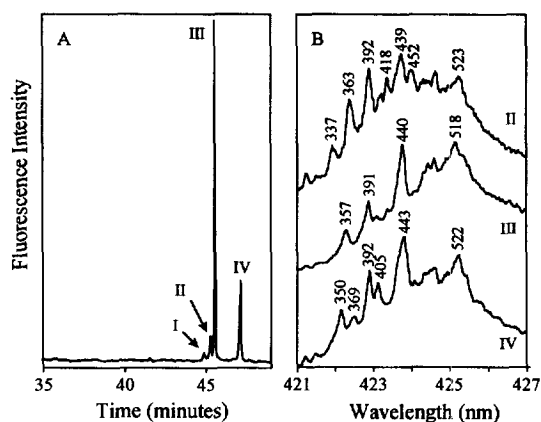


Fig. 6. (A) Room-temperature fluorescence electropherogram obtained during separation of a mixture of (II) DBP-N7Ade, (III) DPB-N1Ade, and (IV) DBP-N3Ade. An unidentified impurity is labeled as peak (I). (B) FLN spectra for the three CE-separated adducts, obtained at 4.2 K using selective laser excitation at 416.0 nm. The FLN peaks are labeled with their S_1 vibrational frequencies, in cm^{-1} .

vided by the combination of the separation power of CE and the spectral selectivity of FLNS is shown in Fig. 6 for the analysis of three dibenzo[*a,l*]pyrene-adenine adducts, DBP-N1Ade (1-(dibenzo[*a,l*]pyren-10-yl)-adenine), DBP-N3Ade (3-(dibenzo[*a,l*]pyren-10-yl)-adenine), and DBP-N7Ade (7-(dibenzo[*a,l*]pyren-10-yl)-adenine). Since these three adducts differ only in the position at which binding of DBP to adenine occurs, they are structurally very similar. Previous FLNS analysis of these three DBP-adenine adducts resulted in selective identification of the individual adducts; that is, the individual adducts could be distinguished (unpublished results). However, a mixture of these adducts could not be resolved by FLNS alone. By combining CE (for separation) and FLNS (for spectral characterization), a mixture of these three adducts can be resolved, as shown in Fig. 6. The room-temperature fluorescence electropherogram obtained during CE separation of the DBP-adenine adduct mixture is shown in Frame A. Four peaks (labeled I, II, III, and IV) are observed, indicating that one is an impurity present in the mixture. CE-FLNS spectra obtained for the three major separated analytes (II, III, and IV) are shown in Fig. 6B. These spectra were obtained in the CE buffer matrix at 4.2 K, using selective laser excitation at 416.0 nm. Comparison of the FLN

spectra in Fig. 6 with the library of FLN spectra generated for DNA adducts obtained in the CE buffer matrix in a regular helium immersion dewar showed that peaks II, III, and IV correspond to DBP-N7Ade, DBP-N1Ade, and DBP-N3Ade, respectively. Since the adducts of interest were identified, no attempt was made to characterize the impurity peak (I).

4. Concluding remarks

We have demonstrated that mixtures of structurally similar PAHs and closely related DNA adducts can be separated and analyzed directly on-line by CE–FLNS. The combination of the separation power of CE and the spectral selectivity of FLNS provides a valuable technique for characterizing analytes in complex mixtures. Structural characterization is obtained via comparison of the high-resolution FLN fingerprint spectra with the available library of FLN spectra of standard samples. We have shown that the CE–FLNS approach provides identification of analytes based not simply on a comparison of retention times of analytes and standards but rather on characteristic, high-resolution FLN spectra. Since the CE–FLNS methodology provides excellent selectivity and subfemtomole detection levels, this approach should be particularly valuable in the analysis of PAH–DNA adduct reaction mixtures from *in vitro* and *in vivo* experiments. With improvements in the current CE–FLNS system, attomole detection levels are projected and many applications are anticipated where analyte purity and detailed structural characterization are required.

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References

- [1] J.P. Shockcor, S.E. Unger, I.D. Wilson, P.J.D. Foxall, J.K. Nicholson, J.C. Lindon, *Anal. Chem.* 68 (1996) 4431.
- [2] C.E. Kientz, A.G. Hulst, A.L. De Jong, E.R.J. Wils, *Anal. Chem.* 68 (1995) 675.
- [3] L. Licklider, W.G. Kuhr, M.P. Lacey, T. Keough, M.P. Purdon, R. Takigiku, *Anal. Chem.* 67 (1995) 4170.
- [4] J.P. Barry, C. Norwood, P. Vouros, *Anal. Chem.* 68 (1996) 1432.
- [5] D.L.D. Deforce, F.P.K. Ryniers, E.G. Van den Eeckhout, F. Lemiere, E.L. Esmans, *Anal. Chem.* 68 (1996) 3575.
- [6] K. Schmeer, B. Behnke, E. Bayer, *Anal. Chem.* 67 (1995) 3656.
- [7] R. Jankowiak, D. Zamzow, W. Ding, G.J. Small, *Anal. Chem.* 68 (1996) 2549.
- [8] T.J. Thompson, F. Foret, P. Vouros, B.L. Karger, *Anal. Chem.* 65 (1995) 900.
- [9] R.L. Chien, D.S. Burgi, *Anal. Chem.* 64 (1992) 1046.
- [10] R.L. Chien, D.S. Burgi, *Anal. Chem.* 64 (1992) 489A.
- [11] J. Tehrani, R. Macomber, L. Day, *J. High Resolut. Chromatogr.* 14 (1991) 10.
- [12] D. Zamzow, G.J. Small, R. Jankowiak, *Mol. Cryst. Liq. Cryst.* 291 (1996) 155.
- [13] R.E. Milofsky, E.S. Yeung, *Anal. Chem.* 65 (1993) 153.
- [14] X.C. Huang, M.A. Quesada, R.A. Mathies, *Anal. Chem.* 64 (1992) 967.
- [15] H. Swerdlow, J.Z. Zhang, D.Y. Chen, H.R. Harke, R. Grey, S. Wu, N.J. Dovichi, C. Fuller, *Anal. Chem.* 63 (1991) 2835.
- [16] B.L. Hogan, E.S. Yeung, *Anal. Chem.* 64 (1992) 2841.
- [17] B.L. Hogan, E.S. Yeung, *Trends Anal. Chem.* 12 (1993) 4.
- [18] J.P. Landers, *Clin. Chem.* 41 (1995) 495.
- [19] R. Jankowiak, G.J. Small, *Anal. Chem.* 61 (1989) 1023A.
- [20] R. Jankowiak, G.J. Small, *Chem. Res. Toxicol.* 4 (1991) 256.
- [21] M.J. Sanders, R.S. Cooper, G.J. Small, V. Heisig, A.M. Jeffrey, *Anal. Chem.* 57 (1985) 1148.
- [22] F. Ariese, G.J. Small, R. Jankowiak, *Carcinogenesis* 17 (1996) 829.
- [23] M. Suh, F. Ariese, G.J. Small, R. Jankowiak, T.-M. Liu, N.E. Geacintov, *Biophys. Chem.* 56 (1995) 2561.
- [24] K.M. Li, R. Todorovic, E.G. Rogan, E.L. Cavalieri, F. Ariese, M. Suh, R. Jankowiak, G.J. Small, *Biochemistry* 34 (1995) 8043.
- [25] E.G. Rogan, P.D. Devanesan, N. RamaKrishna, S. Higginbotham, N.S. Padmavathi, K. Chapman, E.L. Cavalieri, H. Jeong, R. Jankowiak, G.J. Small, *Chem. Res. Toxicol.* 6 (1993) 356.
- [26] P.D. Devanesan, N. RamaKrishna, N.S. Padmavathi, S. Higginbotham, E.G. Rogan, E.L. Cavalieri, G.A. Marsh, R. Jankowiak, G.J. Small, *Chem. Res. Toxicol.* 6 (1993) 364.

- [27] M. Suh, F. Ariese, G.J. Small, R. Jankowiak, A. Hower, D.H. Phillips, *Carcinogenesis* 16 (1995) 2561.
- [28] N. Milanovich, M. Shu, J.M. Hayes, G.J. Small, *Biospectroscopy* 2 (1996) 125.
- [29] J. Wolf, K.-Y. Law, A.B. Myers, *J. Phys. Chem.* 100 (1996) 11870.
- [30] R.A. Avarmaa, K.K. Rebane, *Spectrochim. Acta* 41A (1985) 1365.
- [31] H. Koloczek, J. Fidy, J.M. Vanderkooi, *J. Chem. Phys.* 87 (1987) 4388.
- [32] P.J. Angiolillo, J.S. Leigh, J.M. Vanderkooi, *Photochem. Photobiol.* 63 (1982) 133.
- [33] I. Renge, K. Mairing, R. Avarmaa, *J. Lum.* 37 (1987) 207.
- [34] Y. Shi, J.S. Fritz, *Anal. Chem.* 67 (1995) 3023.
- [35] Y. Yang, A.P. D'Silva, V. Fassel, *Anal. Chem.* 53 (1981) 2107.
- [36] M.M. Bushey, J.W. Jorgenson, *Anal. Chem.* 61 (1989) 491.
- [37] N. RamaKrishna, N.S. Padmavathi, E.L. Cavalieri, E.G. Rogan, R. L. Cerny, M.L. Gross, *Chem. Res. Toxicol.* 6 (1993) 554.