J. Braz. Chem. Soc., Vol. 16, No. 2, 220-226, 2005.
Printed in Brazil - ©2005 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

dGMP-BPDE DNA Adduct Investigation in Environmentally Exposed Rural Workers by Capillary Electrophoresis with Laser-Induced Fluorescence Detection

Emanuel Carrilho*[*,a], Ana Paula Formenton-Catai [a], Fernando M. Lanças[a] and Delma M. Pereira[b]

[a] *Instituto de Química de São Carlos, Universidade de São Paulo, CP 780, 13560-970 São Carlos - SP, Brazil*

[b] *Instituto de Química, Universidade Federal de Goiás, CP 131, 74001-970 Goiânia - GO, Brazil*

Um método alternativo à pos-marcação com ^{32}P radioativo foi proposta para a determinação do aduto de desoxiguanosina monofosfato com epóxido de benzo[a]pirenodiol (dGMP-BPDE), um biomarcador para exposição humana à hidrocarbonetos policíclicos aromáticos (PAH) carcinogênicos, usando eletroforese capilar com fluorescência induzida a laser (CE-LIF). O instrumento CE-LIF modificado foi ajustado para operar com um laser UV de He/Cd (325 nm) para detecção da fluorescência nativa dos adutos de BPDE. O método foi linear por mais de três ordens de grandeza e apresentou limites de detecção de $2,5 \times 10^{-9}$ mol L^{-1} com relação sinal/ruído igual a 3 após diluições sucessivas do padrão de dGMP-BPDE. Neste nível de concentração, a recuperação foi de 1 aduto para cada 10^7 bases não modificadas. Os valores de CV% para ensaios inter- e intra-dias foi melhor que 7% e os estudos de recuperação em três níveis diferentes renderam valores em torno de 50%. Este método foi validado e aplicado pela primeira vez a determinação de dGMP-BPDE em amostras de sangue provenientes de trabalhadores rurais Brasileiros, os quais foram expostos a PAH na colheita de cana-de-açucar e fornos de produção de carvão vegetal.

An alternative method to ^{32}P-postlabeling has been proposed for sensitive detection and quantitation of deoxyguanosine monophosphate - benzo[a]pyrenediol epoxide (dGMP-BPDE), a biomarker for human exposure to carcinogenic polycyclic aromatic hydrocarbons (PAH), using capillary electrophoresis with laser-induced fluorescence detection (CE-LIF). A modified CE-LIF instrument was adjusted to operate with a He/Cd UV laser (325 nm) for native fluorescence detection from BPDE adducts. The method was linear over three decades in concentration, with the detection limit of 2.5×10^{-9} mol L^{-1} at the signal-to-noise ratio of three after consecutive dilution of the dGMP-BPDE standard. At this level, recovery of 1 adduct per 10^7 normal nucleotides was possible. The RSD values for inter- and intra-day determination were better than 7% and recovery studies at three different levels yielded values around 50%. This method has been validated and for the first time applied to determination of dGMP-BPDE in blood samples from Brazilian rural workers, which were exposed to PAH in sugar-cane plantation harvesting and charcoal-production ovens.

Keywords: BPDE, carcinogenic risk, capillary electrophoresis, environmental exposure

Introduction

Human exposure to carcinogenic compounds such as polycyclic aromatic hydrocarbons (PAH) has been directly related to the incidence of cancer.[1] Such studies have related the development of cancer and other behavioral effects in population from highly polluted areas such as in iron foundry due the presence of the PAH. In Brazil, two typical cases in which workers are exposed to PAH are during the harvesting of the sugar-cane plantations and in charcoal-production ovens. In order to make the process of harvesting easier, the whole sugar-cane crop is set on fire. The burning produces a large quantity of fly soot that remains for long time in suspension in the air, thus affecting all neighboring populations, or adsorbed to the burnt sugar-cane straw. The analysis of sugar-cane soot has shown the presence of high quantities of PAH, including benzo[a]pyrene, a potent carcinogenic and mutagenic compound.[2] Therefore, the workers employed for the harvesting process are potentially exposed to polynuclear aromatics substances. In the second case, in many instances in Brazil, charcoal is still used as an energy source. The production of charcoal is laborious and handcraft based. Hundreds of wood sticks and small logs are placed in

* e-mail: emanuel@iqsc.usp.br

house-size brick ovens and are let burn not-to-completion. About half ton of charcoal is removed from the ovens by barely-dressed workers because of the excessive heat (hot weather plus the remaining heat from the oven). The whole environment is smoke and soot full, indicating that there is a high potential for PAH exposure.[3]

Once in the organism, the PAH are initially activated through an oxidative metabolic pathway to electrophilic intermediates capable of covalent binding to DNA, thus forming DNA adducts. If such adducts are not cleaved and repaired by the organism, they may initiate gene mutations and lead to adverse health effects in humans.[4] Benzo[a]pyrene (BaP) is the most studied PAH due its high carcinogenicity and is metabolized to *anti*-7,8,9,10-tetrahydrobenzo[a]pyrene-7,8-diol-9,10-epoxide, commonly known as BPDE, which can react with DNA either *in vivo* or *in vitro*.[5] The adduct formation involves the reaction of the exocyclic amino group of guanidine to the benzylic carbon of the epoxide. Others PAH-DNA adducts are also formed but in lower proportions.[6] DNA adducts have been classified as biomarkers of the exposure of organisms to PAH, therefore the measurement of such adducts can determine the risk for cancer development. Assays for detection of DNA adducts have been carried out by ^{32}P-postlabeling[7] and immunoassays.[8] The most sensitive assay for DNA adducts analysis is the ^{32}P-postlabeling method, which is capable of detecting adducts present at levels as low as 1 adduct in 10^9 – 10^{10} normal nucleotides. However, analysis by ^{32}P-postlabeling technique presents some disadvantages such as working with radioactive phosphorus and the time-consuming chromatographic separation procedures. Capillary electrophoresis (CE) is a technique that provides fast analysis time with superior separation efficiencies, ease of use, and low analysis cost. Several DNA adducts, especially PAH derivates such as BPDE, have been separated and analyzed by CE using UV,[9] mass spectrometry (MS),[10] and laser-induced fluorescence (LIF) detection.[11] Fluorescence detection methods are known to be very sensitive and when this detection mode is used together with CE low adduct levels can be detected.[12] The use of laser as an excitation source yields an increase in the fluorescence intensity; therefore LIF detection can increase the sensitivity of fluorescence-detection methods by several orders of magnitude, thus enabling CE-LIF as a viable tool to measure up to 1 adduct to 10^8 normal bases in theory.

Even though several modes of CE have been used to separate DNA adducts formed *in vitro*,[13] CE has never been fully exploited as a validated analytical method for adduct determination *in vivo*. In this study, such analytical method was developed using CE with LIF detection to analyze dGMP-BPDE adduct in blood samples. Several figures of merit for the proposed analytical method were determined and the method was applied to the analysis of blood samples from rural workers environmentally exposed to PAH.

Experimental

Reagents

(±)-*Anti*-benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide (BPDE) was obtained from the National Cancer Institute (NCI) Chemical Carcinogen Reference Standard Repository at Midwest Research Institute (Kansas City, MO, USA) at 97.5% purity, determined by HPLC. 2'-deoxyguanosine-5'-(mono)phosphate (dGMP), DNase I, snake venom phosphodiesterase I, RNase and proteinase K were obtained from Amersham Pharmacia Biotech (Piscataway, NJ, USA). Acetonitrile, acetone, methanol, ethyl acetate, diethyl ether, sodium chloride and magnesium chloride were obtained from Mallinckrodt (Phillipsburg, NJ, USA) and the Tris/TAPS buffers, SDS and EDTA were obtained from Sigma (Saint Louis, MO, USA).

Caution. It is well established that BPDE is a potent mutagenic and/or carcinogenic agent and should be handled with care.

Apparatus

Capillary electrophoresis Beckman P/ACE 5000 system (Fullerton, CA, USA) with laser-induced fluorescence (LIF) detection was used for DNA adducts analysis during method development. An Omnichrome He/Cd laser (Melles Griot, Carlsbad, CA, USA) was used for LIF excitation at 325 nm and a glass-made optical rejection filter at 375 nm (70% of transmittance) was used for selection of the fluorescent light. All separations were carried out using a polyvinylalcohol (PVA) coated fused-silica capillary column with suppressed electroosmotic flow (EOF). The capillary dimensions were 75 μm i.d. and 47 cm of total length (40 cm from injection to detection point). Hydrodynamic injections conditions were 5 s at 0.5 psi (35 mbar) and the separations were conducted applying reverse polarity (–20 kV) at the injection end. Separation was carried out in a medium with 50 mmol L^{-1} Tris-TAPS pH 8.3 buffer. The electrophoresis conditions were optimized and details were given elsewhere.[13]

Synthesis of adduct standard with 2'-deoxyguanosine 5'-(mono)phosphate (dGMP)

The deoxynucleotide dGMP (40 mg) was mixed with

1 mL of 10 mmol L^{-1} Tris-HCl buffer (pH 7.0) and this solution was added to a solution containing 500 μg of (±)-*anti*-BPDE in 0.45 mL of acetone. This reaction mixture was then incubated overnight at 37 °C. The excess of BPDE that did not react with dGMP was removed from solution with three extractions with ethyl acetate and one extraction with diethyl ether.[14] The separation of unmodified nucleotides from modified nucleotides (adducts) was carried out with SPE cartridges containing 100 mg of C18 phase (Amersham Life Sciences, Buckinghamshire, UK). This purification was conducted adding 500 μL of the adduct solution in the cartridge and then 2 mL of water for elution of the unmodified nucleotides and 2 mL of water/methanol (6:4 v/v) for elution of the modified nucleotides. The methanol from this fraction was totally evaporated and 100 μL of 5 mmol L^{-1} Tris-TAPS pH 8.3 buffer was added. Details for the synthesis and the purification development are presented elsewhere.[15]

Method validation

Calibration curve: linearity, limits of detection (LOD) and quantitation (LOQ). For quantitative analysis a calibration curve was obtained by successive dilution of the adduct stock solution which was prepared by taking an aliquot of 500 μL of the adduct standard solution after the purification and diluted three-fold to a total volume of 1.5 mL. In order to determine the concentration of the adduct in the stock solution, a UV-Vis spectrum was obtained. The adduct concentration was calculated measuring the maximum absorption at 345 nm. Using the molar absorption coefficient (ε) of the 2.9×10^4 L mol^{-1} cm^{-1} obtained from the literature,[16] the concentration value for the adduct stock solution was 2.9×10^{-5} mol L^{-1}. The following concentrations were obtained by dilution with water until a given concentration corresponded to the detection limit (3 times the signal/noise ratio). The linearity of the calibration curve was evaluated over a concentration range from 2.5×10^{-9} mol L^{-1} to 6.3×10^{-6} mol L^{-1}. All analyses were carried out in triplicates.

Precision and accuracy. The intra- and inter-day precision analyses were carried out by adding three adduct concentration levels in the DNA extracted from calf blood (control sample). Intra-day analysis was carried out in triplicate in the same day and the inter-day analysis was carried out in triplicate in three consecutives days. The concentration levels of added dGMP-BPDE used for precision evaluation were: 6.3×10^{-9}, 1.9×10^{-8}, and 5.0×10^{-8} mol L^{-1}.

Recovery of dGMP-BPDE by solid-phase extraction. The assessment of the recovery for the analytical method was made in two steps. The first experiment was carried out with the addition of three adduct concentration levels in a solution containing DNA retired from calf blood and before of the digestion of DNA into nucleotides. In the second experiment the addition of the adduct solution was carried out after the digestion of DNA, but before of the purification step. In this way, it was possible to sort out the contribution of each step in the overall recovery. The analyses were carried out in triplicate for three adduct concentrations: 6.3×10^{-9}, 1.9×10^{-8}, and 5.0×10^{-8} mol L^{-1}.

Sample preparation

Blood sampling and isolation of leukocytes. Rural workers from both the sugar-cane plantation and charcoal production volunteered for blood donation of samples. Three samples were taken from 15 different workers. In a Vacutainer™tube, approximately 5 mL of blood was drawn and 20 mL of solution A (20 mmol L^{-1} Tris-TAPS buffer (pH 7.6), 20 mmol L^{-1} NaCl, and 10 mmol L^{-1} $MgCl_2$) was added. The resulting solution was mixed and centrifuged for 10 min at 2500 rpm. The supernatant was discarded and another 10 mL aliquot of the solution A was added and the precipitate was mixed and centrifuged. This procedure was repeated until it was obtained only leukocytes. The final precipitate was suspended with 500 μL of a solution A and centrifuged by 10 min at 10000 rpm and the supernatant was discarded before the next step.

Extraction of DNA. After discarding the supernatant, the precipitate was suspended with 500 μL of a solution B, (10 mmol L^{-1} Tris-TAPS (pH 8.0), 100 mmol L^{-1} NaCl, 10 mmol L^{-1} EDTA pH 8.0, 20% SDS and 20 units Proteinase K). The solution was heated to 55 ºC for 6 h and after that it was added 500 μL of solution B and 316 μL of 5.0 mol L^{-1} NaCl. The resulting solution was centrifuged for 20 min at 10000 rpm and the supernatant was transferred to two tubes of 1.5 mL. To this solution it was added 200 μL of 70% cold ethanol and centrifuged for 10 min at 10000 rpm. The supernatant was discarded and it was added 250 μL of 10 mmol L^{-1} Tris-TAPS pH 7.6 and 1 mL RNase. This solution was heated to 37 ºC for 1 h. The total DNA was quantitated by measuring the maximum absorption at 260 nm and calculated according to the ratio of 1 absorbance unit for 50 μg ml^{-1} of DNA.

DNA digestion. For each mg of DNA it was added 260 Kunits of DNase and the solution was heated to 37 ºC for 6 h. After digestion, 1 mL of 0.1 mol L^{-1} Tris-TAPS pH 9.0 and 0.1 units of snake venom phosphodiesterase was added. This solution was heated to 37 ºC for 48 h.

Purification of the adduct. The DNA extracted from

blood samples from workers was digested and purified in order to obtain only dGMP-BPDE adduct biomarker. The unmodified nucleotides were separated from modified nucleotides as previously described and the resulting fraction was evaporated to a final volume of 100 μL.

Results and Discussion

In this study a method for DNA adducts analysis by CE-LIF was developed. The blood samples utilized were obtained from rural workers known to be occupationally exposed to PAH either during sugar-cane harvesting or in charcoal-production ovens. Initially, a standard of dGMP-BPDE adduct was synthesized, purified, and spectroscopically characterized in-house.[15] The purified standard was used to prepare the stock solutions as well as was used in the method development and validation. The separation conditions were previously established and several CE modes of separation were evaluated in terms of sensitivity, efficiency and analysis time.[13]

Method validation

Figure 1 shows the calibration curve obtained from dGMP-BPDE adduct analysis by CE-LIF for both peak height and peak area as a function of adduct molar concentration. The concentration range of adduct was varied from 2.5×10^{-9} mol L^{-1} to 6.3×10^{-6} mol L^{-1} and the linearity was better than r = 0.998 over three decades in molar concentration. The calibration curves were constructed based on peak height *vs*. concentration and peak area *vs*. concentration in order to compare the method that would yield best figures of merit. Although the linearity was fairly the same in both cases, it is possible to see that the lower adduct concentration (the effective limit of detection) presented an value of area which deviates from linearity while the correspondent value in peak height is well fit by the linear regression curve. This is due to difficulties in defining the start-stop integration peak parameters at the detection limit, therefore, peak height was the analytical parameter used throughout the validation and analysis. As described, the adduct stock solution was successively diluted in order to reach a measurable detection limit and not only and estimation based on extrapolation of a concentrated adduct level. The LOD was 2.5×10^{-9} mol L^{-1} considering a signal/noise ratio of 3 based on peak-to-peak noise. Using the same approach, the quantitation limit found was 7.5×10^{-9} mol L^{-1}. Such detection limit represents an approximate measurement of 1 adduct to 10^7 nucleotides. The intra- and inter-day precision data are presented in the Table 1. For this study, three concentrations were used to evaluate

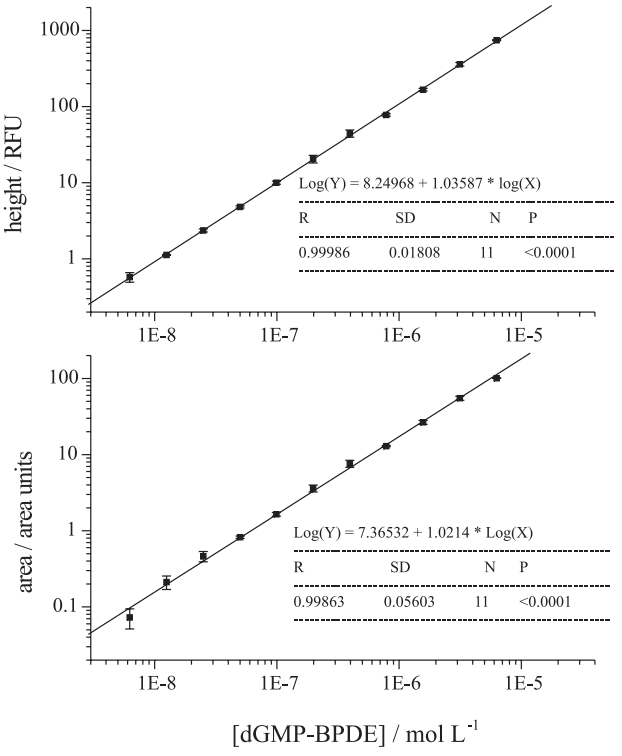

Figure 1. Calibration curves for determination of dGMP-BPDE by CE-LIF. The plot was constructed based on a) peak height *vs.* concentration and b) peak area *vs.* concentration. All separations were carried out using a PVA coated column. The capillary dimensions were 75 μm i.d. and 47 cm of total length (40 cm from injection to detection point). Samples were hydrodynamically injected for 5 s at 0.5 psi and the separations were conducted at –20 kV at the injection end (reverse polarity). Separation was carried out in 50 mmol L^{-1} Tris-TAPS pH 8.3 buffer.

Table 1. Precision data for migration time and peak height in intra- and inter-day experiments

	Intra-day[a]				Inter-day[b]			
[dGMP-BPDE] (mol L^{-1})	Migration time (min)	RSD (%)	Peak height (RFU)	RSD (%)	Migration time (min)	RSD (%)	Peak height (RFU)	RSD (%)
5.0×10^{-8}	14.78 ± 0.06	0.41	4.70 ± 0.10	2.12	14.73 ± 0.07	0.47	5.09 ± 0.31	6.09
1.9×10^{-8}	14.75 ± 0.05	0.39	1.73 ± 0.02	1.16	14.72 ± 0.07	0.48	1.72 ± 0.09	5.23
6.3×10^{-9}	14.70 ± 0.04	0.27	0.71 ± 0.03	4.22	14.74 ± 0.09	0.61	0.65 ± 0.04	6.15

[a] n = 3; [b] n = 3 in 3 consecutive days.

the precision of the method. The RSD values for the intra-day assay for both migration time and peak height were calculated and presented values ≤ 0.50% and 5.00%, respectively. For the inter-day experiment, the RSD values were ≤ 0.75% and 7.00%, respectively for migration time and peak height. Such results are satisfactory and comply with current regulations.[17]

The studies for recovery of the adduct were carried out in two experiments. In a first experiment three concentrations of dGMP-BPDE adduct were added in a solution of calf DNA before the DNA digestion. In the second experiment the same concentrations of dGMP-BPDE adduct were added in a solution of DNA calf after DNA digestion, therefore, before the SPE extraction and clean-up. The results are presented in the Table 2 and show a maximum recovery of 53% for the highest level with spiking of the adduct before the SPE procedure. Although it may appear that such recoveries are low similar values were found by Norwood in his detailed study.[9] This relatively small recovery values could be due to some degradation of the adduct with its manipulation. Nevertheless, it is clear that the SPE procedure alone contribute less to the RSD than the two steps combined, *i.e.,* digestion of DNA and the SPE clean-up. What was interesting to note was that this SPE procedure was absolutely the same one used to purify the dGMP-BPDE adduct and no other peak was observed when the adduct alone, or spiked to a blank sample, was analyzed.[15]

Table 2. Results comparing the recovery studies in three different levels for addition of adduct standard in two different steps of the analytical procedure

Adduct level (mol L^{-1})	Recovery[a] (%)			
	Before DNA digestion	RSD (%)	After DNA digestion	RSD (%)
5.0×10^{-8}	45 ± 15	33	53 ± 9	17
1.9×10^{-8}	38 ± 9.0	24	48 ± 4	8.3
6.3×10^{-9}	45 ± 10	22	43 ± 4	9.3

[a] n = 3.

In Figure 2 is presented the electropherograms obtained from the calf blood DNA (Figure 2a), used as a control blank sample, and the same blank spiked with dGMP-BPDE standard to a final concentration of 5.0×10^{-8} mol L^{-1} (Figure 2b) to evaluate selectivity and sensitivity. The addition of the standard dGMP-BPDE adduct in the blank solution produced only an intense peak in 14.7 min, as expected. The signal/noise ratio obtained in Figure 2b was about 30, roughly, 10 fold the LOD. The recovery studies were carried out at three different adduct levels, 6.3×10^{-9}, 1.9×10^{-8}, and 5.0×10^{-8} mol L^{-1}, and the results are shown in Figures 3a to 3c, respectively. In Figure 3d is shown the same sample recovered from the 1.9×10^{-8} mol L^{-1} level (Figure 3b) plus spiking of 3.8×10^{-13} moles of adduct standard. If the recovery would be 100%, the final concentration would be 2.5×10^{-8} mol L^{-1}, which is an intermediary value between the middle and the upper adduct levels.

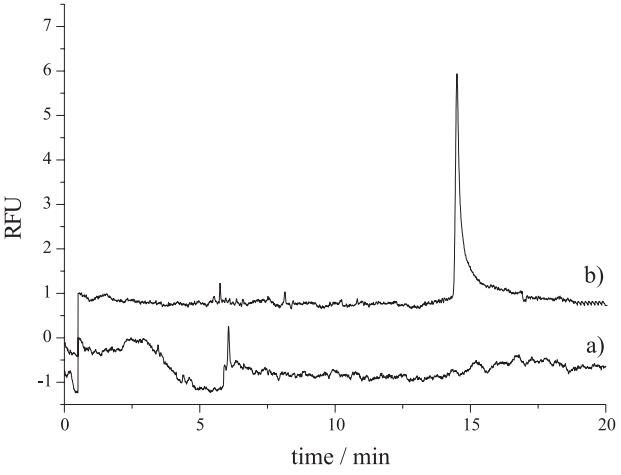

Figure 2. a) Capillary electrophoresis separation of sample obtained from a blank control from calf blood and b) the same blank sample spiked with adduct standard to a final concentration of 5.0×10^{-8} mol L^{-1}. Analysis conditions were the same as in Figure 1.

In all separations in Figure 3, regarding the recovery of spiked adduct in the DNA extracted from blood, it was possible to detect various peaks, which were not detected in the blank sample (Figure 2a) neither in the adduct standard.[13] Such peaks could be due to the degradation of the added adduct during the process of digestion and the

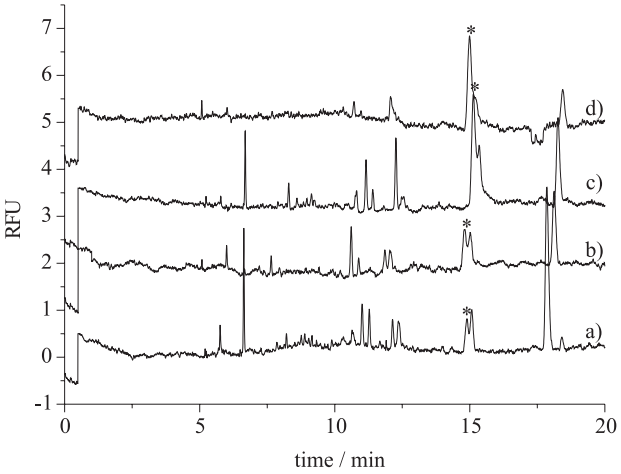

Figure 3. Capillary electrophoresis separation of dGMP-BPDE adducts obtained from the recovery studies at 3 concentration levels in calf-blood DNA extract: a) recovery from 6.3×10^{-9} mol L^{-1} level; b) recovery from 1.9×10^{-8} mol L^{-1} level; c) recovery from 5.0×10^{-8} mol L^{-1} level; and d) the 1.9×10^{-8} mol L^{-1} recovery level plus spiking of 3.8×10^{-13} moles of adduct standard. dGMP-BPDE peak is labeled with an asterisk (*). Analysis conditions were the same as in Figure 1.

SPE purification and are the reason for relatively low recovery levels (*ca.* 50%). The total fluorescence was not integrated since there is no evidence about the quantum yield of each peak being detected. Evidently, all peaks must be BPDE and BPDE-adduct degradation products to exhibit fluorescence at this excitation wavelength (325 nm). Since two peaks were present at nearly the same migration time of the adduct in the recovered samples, in order to proceed with the quantitation, we have spiked a similar amount of adduct to the recovered samples. In this way, we could be sure to pick the right peak for quantitation. All peaks labeled with an asterisk (*) represented the dGMP-BPDE adduct.

Analysis of DNA from rural workers environmentally exposed to PAH

Blood from workers environmentally exposed to PAH were collected by certified nurses. All workers were volunteers for blood donation and completed a donation query in an anonymous manner. For every donor, three Vacutainer™ flasks of 5 ml each were drawn; one flask was submitted to clinical analysis for diagnostics of any contagious disease, one flask was submitted to DNA extraction, and the last flask was kept in ultra-low temperature freezer after separation of blood. From the 15 samples, 6 samples were obtained from the sugar-cane harvesting workers while 9 samples were obtained from workers from the charcoal-production ovens. Randomly, 10 out 15 samples were taken and submitted to the proposed method for analysis of dGMP-BPDE by CE-LIF.

In analysis of environmental and occupational human exposure, the evaluation of the exposure risk is determined by number of modified nucleotide bases in relation to the normal, non-modified bases.[18] The exposure level varies from 1 adduct in 10^6 to 10^9 normal bases. Assuming that the concentration of adduct formed in the organism of 1 adduct formed in 10^7 normal bases being of considerably high risk for cancer development,[1] the DNA extraction from 10 mL of blood yields about 2 mg of DNA. Therefore, the quantity of adduct should be 400 pg or 600 femtomoles.[19] Considering that the sample volume after purification was 100 μL, the adduct concentration would be of 6.0×10^{-9} mol L^{-1}, which is within the LOD, however, below the LOQ. Figure 3 presents a representative electropherogram from a sample obtained from a worker exposed to PAH. All samples from workers gave similar results and no dGMP-BPDE adduct was detected. It can be seen, however, a peak in ~6 min, which could also be seen in the blank sample (Figure 2a). The peak referent to the dGMP-BPDE was not detected, indicating that the workers environmentally exposed to PAH may present dGMP-BPDE adduct marker at concentrations below the LOD for the present methodology. It is important to point out that in this work, the sample was dissolved in diluted buffer, and no on-column preconcentration mechanisms (such as sample stacking) were used, thus leaving some opportunity for improvement to lower even further the detection limits. Nevertheless, at the present sensitivity level, the assessed risk for cancer development can be considered low[1] and the studied method would be suitable as is.

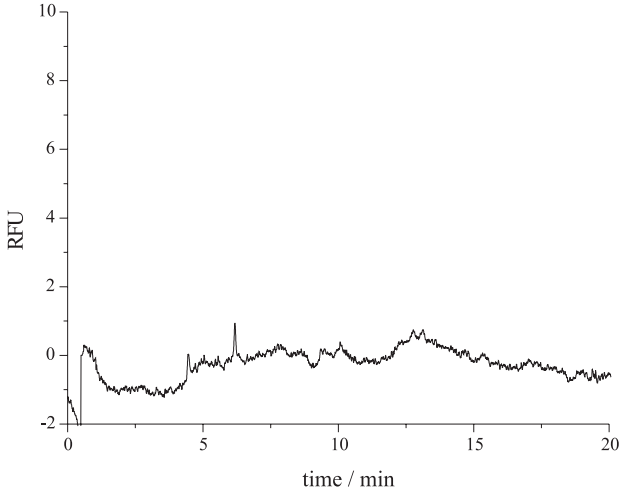

Figure 4. Typical electropherogram obtained from a DNA sample extracted from a 5 mL aliquot of blood from a worker exposed to PAH in charcoal ovens. Analysis conditions were the same as in Figure 1.

Conclusions

Capillary electrophoresis with laser-induced fluorescence detection has shown to be a powerful and sensitive technique for the detection of dGMP-BPDE adducts, as well as other DNA adducts and biomarkers of human exposure to carcinogenic and mutagenic substances. The Brazilian rural workers, although working in very unpleasant conditions and exposed to PAH, apparently are not developing a high risk of cancer development since no sample presented dGMP-BPDE above the detection limit. However, this is an important subject matter and a more comprehensive study, with a larger population should be carried out.

Acknowledgments

The authors are thankful to the contributions of Dr. Jyh-Ming Lin from the American Health Foundation for valuable suggestions and discussions, Dr. Harold Seifried from the National Cancer Institute – NCI, for providing the BPDE from the Midwest Research Institute Repository,

and Dr. Barry L. Karger from the Barnett Institute and Northeastern University for his kind donation of the PVA coated capillaries. This work has been supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP, under grant number 98/12385-2 and a scholarship 99/05481-8 (A.P.F.-C.). One of the authors (E.C.) is thankful to Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNPq, for a fellowship.

References

1. Grimmer, G.; Brune, H.; Deutschwenzel, R.; Dettbarn, G.; Misfeld, J.; Abel, U.; Timm, J.; *Cancer Lett.* **1985,** *28*, 203; Binková, B.; Lewtas, J.; Mísková, I.; Rossner, P.; Cerná, M.; Mrácková, G.; Peterková, K.; Mumford, J.; Meyer, S.; Srám, R.; *Environ. Health Perspect.* **1996**, *104*, 591; Lioy, P. J.; Greenberg, A.; *Toxicol. Ind. Health* **1990**, *6*, 209.
2. Lanças, F. M.; Matta, M. H. R.; Hayasida, L. J.; Carrilho, E.; *J. High Resolut. Chromatogr.* **1991**, *14*, 633; Zamperlini, G. C. M.; Silva, M. R. S.; Vilegas, W.; *Chromatographia* **1997**, *46*, 655; Godoi, A. F.L.; Ravindra, K.; Godoi, R. H. M.; Andrade, S. J.; Santiago-Silva, M.; Van Vaeck, L.; Van Grieken, R.; *J. Chromatogr. A* **2004**, *1027*, 49.
3. Poppi, N. R.; Santiago-Silva, M.; *Chromatographia* **2002**, *55*, 475.
4. Lawley, P. D.; *Mut. Res*. **1989**, *213*, 3; Hemminki, K.; *Carcinogenesis* **1993**, *14*, 2007.
5. Sims, P.; Grover, P. L.; Swaislan, A.; Pal, K.; Hewer, A; *Nature* **1974**, *252*, 326.
6. Jeffrey, A. M.; Jannette, K. W.; Blobstein, S. H.; Weinstein, I. B.; Beland, F. A.; Harvey, R. G.; Kasai, H.; Miura, I.; Nakanishi, K.; *J. Am. Chem. Soc.* **1976**, *98*, 5714; Jeffrey, A. M.; Weinstein, I. B.; Jannette, K. W.; Grzeskowiak, K.; Nakanishi, K.; Harvey, R. G.; Autrup. H.; Harris, C.; *Nature* **1977,** *269*, 348; Koreeda, M.; Moore, P. D.; Wislocki, P. G.; Levin, W.; Conney, A. H.; Yagi, H.; Jerina, D. M.; *Science* **1978,** *199*, 778.
7. Gupta, R. C.; Reddy, M. V.; Randerath, K.; *Carcinogenesis* **1982**, *3*, 1081; Gupta, R. C.; *Cancer Res*. **1985**, *45*, 5656; Reddy, M. V.; Randerath, K.; *Carcinogenesis* **1986**, *7*, 1543.
8. Reddy, M. V.; Gupta, R. C.; Randerath, K.; *Carcinogenesis* **1984**, *5*, 231; Poirier, M. C.; Santella, R.; Weinstein, I. B.; Grunberger, D.; Yuspa, S.H.; *Cancer Res.* **1980**, *40*, 412.
9. Norwood, C. B.; *Anal. Biochem.* **1999**, *272*, 100.
10. Barry, J. P.; Norwood, C.; Vouros, P.; *Anal. Chem*. **1996**, *68*, 1432; Willems, A.V. ; Deforce, D. L.; Van den Eeckhout, E. G.; Lambert, W. E.; Van Peteghem, C. H.; De Leenheer, A. P.; Van Bocxlaer, J. F.; *Electrophoresis* **2002**, *23*, 4092.
11. Penmetsa, K. V.; Shea, D.; Leidy, R. B.; Bond, J. A. J.; *HRC - J. High Res. Chrom.* **1995**, *18*, 719.
12. Schmtiz, O. J.; Wörth, C. C. T.; Stach, D. Wie²ler, M.; *Angew. Chem. Int. Ed.* **2002**, *41*, 445.
13. Fomenton-Catai, A.P.; Carrilho, E.; *Anal. Bioanal. Chem.* **2003**, *376*, 138.
14. Lin, M.; Desai, D.; Chung, L.; Hecht, S. S.; Amin, S.; *Chem. Res. Toxicol.* **1999**, *12*, 341.
15. Formenton-Catai, A. P.; Machado, R. P.; Lanças, F.M.; Carrilho, E.; *J. Braz. Chem. Soc.* **2004**, in press.
16. Weinstein, I. B.; Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Harvey, R. G.; Harris, C.; Autrup, H.; Kasai, H.; Nakanishi, K.; *Science* **1976**, *193*, 592.
17. Guidance for Industry, *Bioanalytical Method Validation* (U.S. Department of Health and Human Services, U.S. Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine, Rockville, Maryland, May 2001).
18. Hemminki, K.; Dickey, C.; Karlsson, S.; Bell, D.; Hsu, Y. Z.; Savela, K.; *Carcinogenesis* **1997**, *18*, 345.
19. Harvey, S. D.; Bean R. M.; Udseth, H.R.; *J. Microcolumn Sep.* **1992**, *4*, 191.

Received: February 29, 2004
Published on the web: February 21, 2005

FAPESP helped in meeting the publication costs of this article.