Use of response surface design methodology to optimize the separation of 8 PAHs in capillary zone electrophoresis with laser-induced fluorescence detection

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INTRODUCTION
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, many of which show carcinogenic effects. There is thus a need for reliable analytical methods to quantify these contaminants at trace levels in complex environmental and food samples. This project aims at developing an electrophoretic microchip dedicated to PAH analysis. Due to their miniaturized format, microsystems allow fast analysis and low reagent consumption. The electrophoretic separation method used was first developed with classical capillary format. A new cyclodextrin-modified (CD) capillary zone electrophoresis (CZE) method, inspired by the work of Brown et al. [1] was therefore developed. Using a univariate method, a dual CD system, involving one neutral CD (Methyl-β-CD, Me-β-CD) and one anionic CD (Sodiumethyl-β-CD, SBE-β-CD) allowed the separation of 19 PAHs in less than 15 min [2]. As a high number of experimental factors is generally involved in CE, the one-variable-at-a-time approach is often time-consuming. More and more multivariate analysis techniques, which allow to achieve the global optimum with a small number of experiments, are therefore used, today, in the search for optimum separation conditions in CE. In this study, an experimental design was carried out to gain a deeper insight into separation conditions and to optimize the final analysis of the 8 most studied PAHs (listed as priority pollutants by the United States Environmental Protection Agency and the European Food Safety Authority).

MULTIVARIATE APPROACH

1) Design of experiments
Choice of the factors
Factors most influencing PAH electrophoretic behavior:
- [SBE-β-CD] • [Me-β-CD] • %MeOH
Fixed parameters: T = 25 °C, [borate] = 10 mM, [urea] = 600 mM, λ = 450 nm and AV = 14 kV (no jode effect)
Choice of DOE
Central Composite Design with 3 factors

2) Coefficients for modeling of PAH electrophoretic migration

| Coefficients for modeling of normalized migration times: |
| - always significant: [SBE-β-CD], [Me-β-CD], [Me-β-CD] |
| - sometimes significant: [SBE-β-CD] x [Me-β-CD], [SBE-β-CD] x %MeOH, [Me-β-CD] x %MeOH |

CONCLUSION
Using a central composite design, a simple, selective and fast CD-CZE was optimized for the analysis of 8 PAHs. Under optimal conditions, baseline resolutions (Rs > 4) of all PAHs were obtained in less than 8 min with efficiencies greater than 1.5 x 10^6. Typical RSD variations, determined from 6 repetitions, gave values between 0.2 and 1.2 % for normalized migration times and between 2.5 and 6.2 % for normalized corrected peak areas. In the near future, this analytical method will be validated quantitatively and real food extracts will be analyzed.

EXPERIMENTAL SECTION

Material and methods
Agilent Technologies HP-3D CE, bare fused-silica capillaries 50 μm ID, x 49 cm (detection at 33.5 cm).
LIF detection: Evolution, Picocontainers with He-Cd Laser (λex = 325 nm, PFM = 750 V, RT: 0.2 s).
Electrolytes prepared from 3 stock solutions: 10 mM sodium tetraborate decadahydrate with 2.5 M urea, 100 mM SBE-β-CD (DS 6.2) and 100 mM Me-β-CD (DS 12.6).
Standard mixtures of PAHs in 1:9 (v/v) ACN/background electrolyte (BGE): 100 ppb dibenzo[a]anthracene (DBahA), logP 6.7, Mw 278 g/mol, 200 ppb chrysene (CHR), logP 5.6, Mw 228 g/mol, 100 ppb benzo[a]amphiphase (BaA), logP 5.7, Mw 228 g/mol, 25 ppb benzo[k]fluoranthene (BkFA), logP 6.3, Mw 252 g/mol, 200 ppb benzo(a)pyrene (BaP), logP 6.0, Mw 252 g/mol, 50 ppb benzo[b]fluoranthene (BbFA), logP 6.6, Mw 252 g/mol, 350 ppb indeno[1,2,3-cd]pyrene (IP), logP 6.6, Mw 276 g/mol, 50 ppb benzo(g)chrysene (BghiP), logP 6.7, Mw 266 g/mol, 162 ppb umbelliferone (US).
Statistical data analyses performed with JMP 10.0 and Excel 2007.

REFERENCES