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Comparison of monolithic capillary electrochromatography and micellar electrokinetic chromatography for the separation of polycyclic aromatic hydrocarbons

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ABSTRACT

Atmospheric pollution of anthropic origin is recognized as a major risk factor for health, in particular for respiratory and cardio-vascular systems. Among these pollutants, polycyclic aromatic hydrocarbons (PAHs) are placed on the list of US Environmental Protection Agency (EPA) as 'priority' pollutants and four of them are assigned as potential carcinogens by The International Agency for Research on Cancer (IARC). In the present work two capillary techniques—micellar electrokinetic chromatography (MEKC) and monolithic capillary electrochromatography (CEC)—were compared for the separation of eleven PAHs. Both techniques compared in the present work are fully compatible with every standard apparatus of capillary electrophoresis. For MEKC, enhancement of selectivity and decrease of the separation window of eleven PAHs were obtained with methanol:borate 25 mM (20/80, v/v) running buffer containing 10 mM of hydroxypropylated γ-cyclodextrins with low SDS content (25 mM). In case of CEC, two acrylate-based monolithic stationary phases (MSPs) were evaluated for their application in the separation of eleven PAHs. The best MSP based on butyl acrylate was compared with MEKC in terms of sample capacity, PAHs elution order, LOQ, efficiency and effect of pH. Influence of the hydrophobicity of mobile phase on the PAHs elution order was also studied.

1. Introduction

Most air toxics originate from human-made sources, including mobile sources (e.g., cars, trucks, buses) and stationary sources (e.g., factories, refineries, power plants), as well as indoor sources (e.g., some building materials and cleaning solvents). Some air toxics are also released from natural sources such as volcanic eruptions and forest fires. Combustion products of coal (soot and tars) were the first recognized chemical carcinogens. The earliest discovery that coal soot caused cancer in human chimney sweeps was reported by Percival Pott in 1775 followed by studies in animals in the 1920s and the discovery of carcinogenic polycyclic aromatic hydrocarbons (PAHs), e.g., benzo[*a*]pyrene (BaP), in the 1930s [1]. Since the reports of abnormally high death rates in the 1950s, atmospheric pollution of anthropic origin is recognized as a major risk factor for health, in particular for respiratory and cardio-vascular systems.

Ten PAHs out of 11 studied in the present work are placed on the list of US Environmental Protection Agency (EPA) as 'priority' pollutants, four of them are assigned as potential carcinogens by The International Agency for Research on Cancer (IARC) [2,3]. Since available sample volumes in environmental or epidemiologic studies are often limited and high efficiency is needed in order to increase sensitivity, capillary methods are of great interest.

PAHs are uncharged compounds. It is thus necessary to incorporate some additional agents interacting with analytes into the mobile phase in order to separate uncharged compounds by capillary electrophoresis. There are several methods to make CE suitable for the separation of uncharged compounds. The common approach in this case is the induction of electrophoretic mobility of neutral compounds by its *in situ* association with an appropriate component of running buffer by application of surfactants that, by formation of micelles, lead to separation of hydrophobic compounds on the basis of their specific micelle-solution partition coefficient [4–7]. Micelles using for example sodium dodecyl sulfate (SDS) form so called pseudostationary phase. Due to their

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surface charge, micelles migrate with different velocity than the electro-osmotic flow (EOF). Separation of neutral compounds is thus possible due to their distinctive values of the distribution coefficients between micelles and the running buffer. Several works dealing with PAH separation using SDS-based pseudostationary phases were published [4–8]. SDS may be used alone [5,7] or with various additives, e.g. β- or γ-CD [4,6] or fullerenes C60 and C70 [8]. The choice of surfactants suitable for PAHs separation is not limited to SDS. Kavran and Erim applied sodium dodecylbenzenesulfonate [9] while Norton and Shamsi polymeric surfactant poly(sodium undecenyl sulfate) [10]. Also cyclodextrins (ether-β and methyl-β type) were reported to be applied alone to separate PAHs [11].

Another possibility to separate uncharged compounds in capillary electrophoresis apparatus is to use monolithic stationary phases (MSPs) in capillary electrochromatography (CEC). MSPs applied in CEC combine EOF-related flat-flow profile with HPLC-like chromatographic properties of the stationary phase. Moreover, the EOF-generated velocity of the mobile phase is almost independent of the monolith's pore size. It renders separations with outstanding efficiencies (due to huge active surface) and maintains the time of analysis comparable to HPLC [12,13]. The first approach used to produce stationary CE-compatible capillary-format MSPs is to pack capillaries with silica-based particles. It requires problematic production of end-frits to avoid removal of the particles from the capillary [14,15]. To circumvent this problem, particle-sintered phases were designed [16]. A wide range of capillary-format stationary phases were successfully applied for PAHs separation, such as slurry pressure packed CEC-octadecylsilica [17], polymeric methacryloxypropyltrimethoxysilane-based hydrophobic stationary phase prepared in a sol–gel process [18] or isobutyl -modified polyhedral oligomeric silsesquioxanes-based monolith prepared in a process of suspension polymerization [19]. Recently, simple organic poly(meth)acrylate-based MSPs gained a huge interest due to relative simplicity of their preparation, wide range of commercially available monomers and unique chromatographic characteristics. Organic polymers are usually produced inside the capillary by copolymerization of various monomers based on esters of acrylic or methacrylic acid [20–26] or vinyl benzene derivatives [27] in the presence of a porogen solvent. Free radical polymerization is usually induced by incorporation into the mixture of UV and high temperature sensitive initiators like 2,2′-azobisisobutyronitrile (AIBN) [20–23,25] or ammonium persulfate (APS) with tetramethylethylenediamine (TEMED) enabling polymerization in room temperature [24,26]. Covalent attachment of the monolith to the capillary silica wall is required in order to retain MSPs into the capillary during analysis and thus to avoid retaining frits. This attachment is usually obtained by the use of 3-trimethoxy-silyl-propylacrylate (TMSPA, Fig. 2, 15), an organosilane compound providing anchoring sites for both silica wall and polymer [20,22–24,26]. Monoliths in CEC must fulfill two functions. The first one is to induce EOF by the presence of superficial groups bearing charges in the experimental conditions. This can be achieved by embedding into the monolith either negatively charged monomers, e.g. 2-acryloylamido-2-methylpropanesulfonic acid (AMPS) [22,25] (Fig. 2, 16), vinylsulfonic acid (VSA) [24,26] or positively charged monomers like [2-(acryloyloxy)ethyl]trimethylammonium methyl sulfate or chloride (AETMA) [20,21,26]. The second function of monoliths is to carry functionalities of the chromatographic stationary phase.

In this paper we compare for the first time CEC and MEKC in terms of selectivity, reproducibility, sensitivity, limit of detection and sample capacity for analysis of PAHs. Both techniques were tested on the same CE apparatus and were used in the optimal conditions for each approach. The objective here is to provide a comparison of MEKC and CEC to highlight advantages and disadvantages of both techniques.

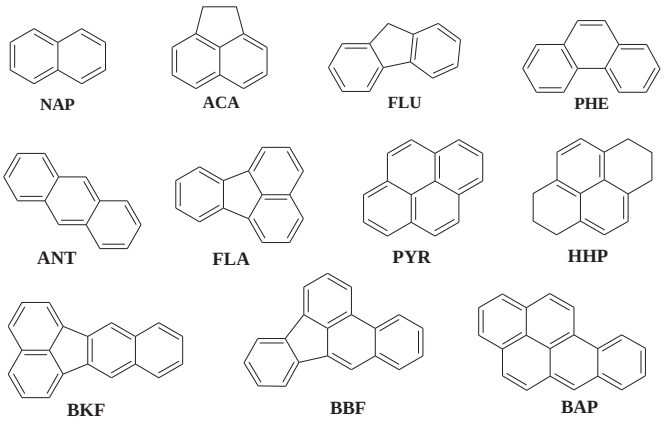

Fig. 1. Structures of polycyclic aromatic hydrocarbons (PAHs): naphthalene (NAP), acenaphthene (ACA), fluorene (FLU), phenanthrene (PHE), anthracene (ANT), fluoranthene (FLA), pyrene (PYR), 1,2,3,6,7,8-Hexahydropyrene (HHP), benzo [κ] fluoranthene (BKF), benzo [β]fluoranthene (BBF), benzo [α]pyrene (BAP).

2. Materials and methods

2.1. Chemicals, materials and standard solutions

PAHs assigned as FLA, PYR, BKF and BBF in Fig. 1 and all monomers and crosslinkers (butyl acrylate, BA; 1,4-butanediol diacrylate, BDDA; 2-acryloylamido-2-methylpropanesulfonic acid, AMPS, Fig. 2) were purchased from Sigma Aldrich (Isle-d'Abeau, France), PAHs assigned as NAP, FLU, ANT and BAP from Prolabo (Paris, France); ACA from BLB (Puteaux, France) and HHP from Dr. Theodor Schuchard (Munich, Germany). TMSPA, sodium hydroxide, acetic acid, hydroxylamine and orthophosphoric acid from Fluka (Isle-d'Abeau, France), organic solvents (HPLC-grade) from SDS (Val-de-Reuil, France), 2,2′-azobisisobutyronitrile, AIBN was obtained from Acros Organics (Noisy-Le-Grand, France), cyclodextrins from Wacker Cyclodextrins (Lyon, France). Water purified by PURELAB UHQ system (Antony, France).

For both MEKC and CEC, all working solutions of PAHs were prepared from the same stock solutions. Each stock solution was prepared by dissolving the given PAH in MeCN to obtain the final concentration on the level of 5.55 mM. Working solutions were prepared by 20-fold dilution of stock solutions by MeOH or MeCN (for MEKC and CEC experiments respectively). All solutions (both samples and running buffers/mobile phases) were filtered prior to use through 0.45 μm syringe filters. NAP, ACA, FLU, PHE, ANT, HHP and BAP (Fig. 1) were applied as standards for statistical considerations, in both CEC and MEKC; all of them for comparison of the CEC and MEKC for the sample capacity and elution order, while NAP and FLU for comparison of various monolith compositions.

Polyimide-coated capillaries (ID=75 μm and 50 μm, OD=365 μm) used during CE experiments and polytetrafluoroethylene-coated capillaries (ID=75 μm, OD=365 μm) used during CEC experiments were purchased from Polymicro Technologies (Phoenix, Arizona, USA).

2.2. Instruments

MEKC and CEC separations were performed on a 3D CE instrument (Agilent, Palo Alto, CA, USA) equipped with diode-array detector and ChemStation A.08.03 software. Detection was carried out at 220.8, 254.8 and 265.4 nm. Additionally, in most cases, DAD spectra were stored within the range 210–350 nm. Preliminary tests of various compositions of MSPs were conducted by means of Beckman P/ACE MDQ apparatus equipped with UV detector and 32 Karat 8.0 software. Detection was performed at 280 nm.

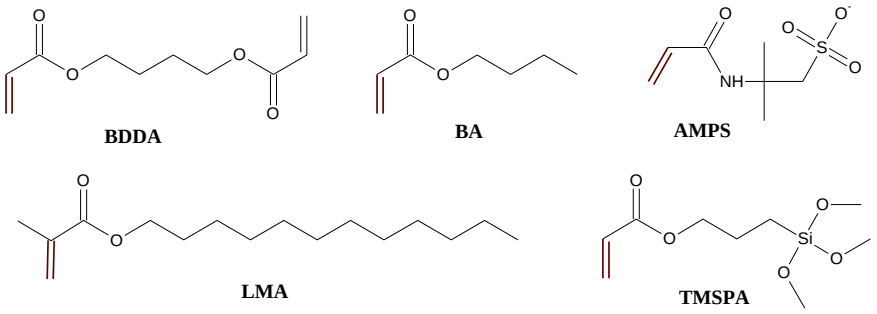

Fig. 2. Esters of acrylic/methacrylic used for monoliths preparation: linker: 1,4-butanediol diacrylate (BDDA); chromatographically useful monomers: butyl acrylate (BA), lauryl methacrylate (LMA); 3-(trimethoxysilyl)propyl acrylate (TMSPA) and EOF generator: 2-acryloylamido-2-methylpropanesulfonic acid (AMPS).

2.3. Micellar electrokinetic chromatography

Prior to use, capillaries were flushed in a pressure-assisted mode (50 mbar) with 0.1 M NaOH – 30 min, water – 15 min, running buffer: sodium borate (25 mM), SDS (25 mM), HP-γ-CD (10 mM) dissolved in MeOH/water=20/80 (v/v) – 15 min. Between subsequent separations, capillaries were flushed with the same sequence of solutions for 4 min for each solution. The length of capillary was 48 cm (40 cm between the inlet and the optical window).

2.4. Preparation of monolithic capillaries for CEC

All monolithic capillaries had following characteristics: overall length: 35 cm, including empty (monolith-free) part: 15 cm (7 cm from the end of the monolith to the detection window). All reagents were introduced in capillaries using a syringe pump from Harvard Apparatus PHD 2000 Infusion (Holliston, MA, USA). Teflon-coated fused silica capillary was first activated by flushing with 1 M NaOH for 30 min (200 μL/h) then pre-treated by the solution of TMSPA (Fig. 2) in 6 mM acetic acid (1/125, v/v), for 10 min (300 μL/h), and afterwards derivatised in static mode for 1 h at room temperature. After this process the capillary was flushed with water for 30 min (200 μL/h) to remove remaining compounds. Residual water was eliminated from the capillary by flushing it with air to prevent precipitation of the hydrophobic monomers. A mixture of monomers and crosslinker (TMSPA, 22.51 μmol; AMPS, 23.6 μmol; AIBN, 29.8 μmol; BDDA, 1.54 mmol; BA, 4.78 mmol, Fig. 2) diluted by 2 ml of porogen solvent (MeOH/MeCN/sodium phosphate buffer: 5 mM, pH 6.8: 20/60/20, v/v/v) was sonicated to remove dissolved air and then pumped through the capillary for 30 min (200 μL/h). Sonication of the mixture prevents from bubbling during the polymerization. Obtained capillary was capped at both ends by pieces of rubber and a part of the capillary to be empty (15 cm) was covered with aluminum foil to avoid the exposition on UV radiation. Polymerization process was conducted at room temperature into a UV light source (BLX E-365, 6 × 8 W, 365 nm, BIO-LINK Vilber Lourmat, Marne-la-Vallée, France) for 55 minutes (the total emitted energy equal to 6 J/cm^2). After the polymerization, the capillary was flushed with pure MeCN for 15 h by means of external HPLC pump (Jasco, model PU-2080 Plus, Tokyo, Japan) to remove unreacted monomers.

The capillary was flushed with desired mobile phase (in pressure and voltage assisted manner, 7.5 bar, 5–10 kV) after installation of into the CE cartridge until stabilization of both the baseline and current value (approx. 20–30 min). The completion of the replacement of MeCN by hydroxylamine/acetic acid buffer (10 mM, pH 6.8): MeCN (30/70, v/v) mobile phase was demonstrated by a rapid growth and stabilization of current value.

3. Results and discussion

3.1. Optimization of MEKC buffer and capillary properties

As the starting point of MEKC method development, the running buffer consisted in a mixture of equal volumes of acetonitrile and sodium borate buffer (8.5 mM, pH 9.9) containing 85 mM of SDS [7]. To our point of view, several parameters needed to be optimized. Long equilibration time was obtained because of low concentration of borate buffer. Simultaneously, high amount of SDS resulted in capillary obstruction after few separations. MeCN applied in the mobile phase caused degradation of capillary's polyimide coating. Narrow-bore capillaries (i.e. 50 μm I.D.) were tested with no success since the capillary clogged after few injections.

Thus, in order to promote interactions of PAHs with the micelles, the contribution of aqueous phase in the electrophoretic buffer was increased from 50% to 80%. Simultaneously, MeCN was replaced by methanol in order to protect the polyimide coating. We also increased the amount of sodium borate from 8.5 mM to 25 mM in order to reduce equilibration time. In parallel, to avoid clogging of the capillary, the concentration of SDS was decreased as much as possible, but was maintained above the level required to form micelles (25 mM) [28]. As a result, stable baseline and complete separation were obtained but the separation time grew by two times (more than 40 min) due to significant augmentation of the ionic strength of the buffer. Nevertheless, the decreased amount of SDS and lower organic solvent content in the running buffer led to partial precipitation of the most hydrophobic PAHs (HHP and BAP) visualized by severe peaks broadening and tailing.

First, 10 mM of γ-cyclodextrin (γ-CD) were added as auxiliary agent to the running buffer in order to increase PAHs solubility without increasing the contribution of MeOH. Because of its hydrophobic cavity, γ-CD actively competes with the micelles for hosting PAHs. This effect improved PAHs' solubility in buffer solution and simultaneously decreased the average time of interaction with micelles. Consequently, it reduces the overall migration time which resulted in a two times shorter run of analysis. Unfortunately peak tailing was still observed for HHP and BAP due to partial precipitation. So in a second time, hydroxypropylated γ-CD (HP-γ-CD) (10 mM) was used in order to increase PAHs solubility without affecting selectivity provided by micelles. Indeed, the substitution of γ-CD with hydroxypropyl groups led to a more hydrophobic entrance to the cavity and therefore to an easier interaction with PAHs. The overall time of analysis was again reduced by 15%. Attempts to increase HP-γ-CD concentration led to a deterioration of the separation quality.

In the final conditions injection was performed hydrodynamically during 0.5 s by applying 50 mbar pressure. The separation temperature was set to 30 °C and the voltage to 25 kV. The

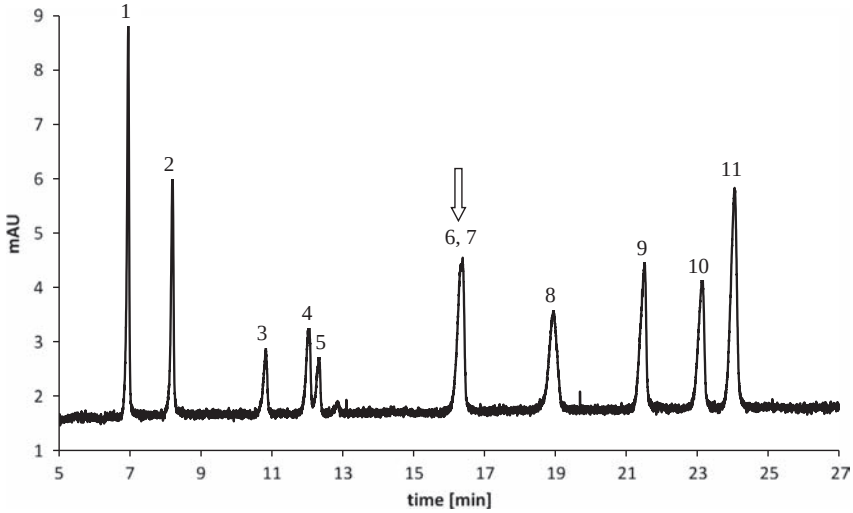

Fig. 3. Optimized separation of eleven PAHs by means of CD-MEKC method. Only one co-migration can be observed (shown by arrow). **1** – NAP, **2** – ACA, **3** – FLU, **4** – PHE, **5** – ANT, **6,7** – FLA+PYR, **8** – HHP, **9** – BKF, **10** – BAP, **11** – BBF. Injection: 0.5 s, 50 mbar; individual conc=approx. 50 ppm; separation: temperature=30 °C, voltage=25 kV. Capillary: total length=48 cm (40 cm between the inlet and optical window), ID=75 μm, ED=365 μm, detection at 220 nm.

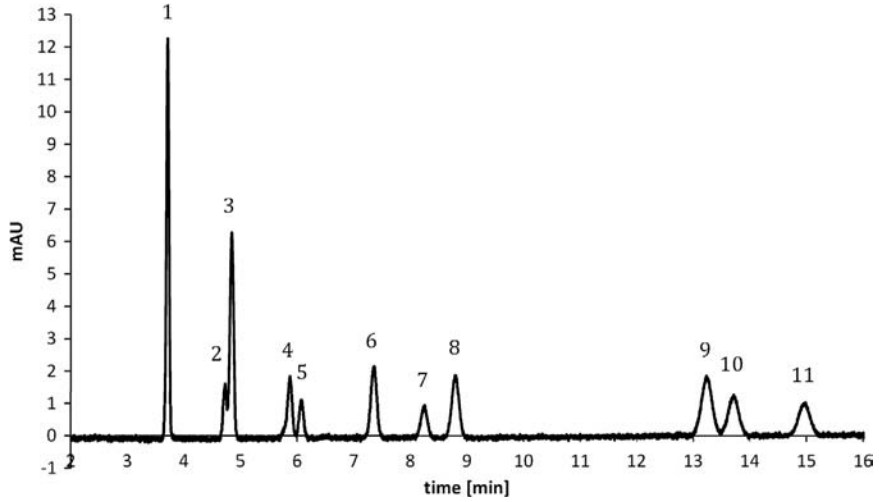

Fig. 4. Example of the separation of eleven PAHs by means of CEC approach. Mobile phase: Hydroxylamine/acetic acid buffer (10 mM; pH 6.8)/MeCN=30/70 (v/v), T=30 °C; injection: 1 s, 10 kV; individual conc=approx. 50 ppm. Separation was conducted with the fixed voltage on the level of 30 kV. **1** – NAP, **2** – FLU, **3** – ACA, **4** – PHE, **5** – ANT, **6** – FLA, **7** – PYR, **8** – HHP, **9** – BBF, **10** – BKF, **11** – BAP. Detection at 220 nm.

capillary total length was 48 cm (40 cm between the inlet and optical window) with 75 μm internal diamenter. Detection was performed at 220 nm. In these conditions, the separation of the eleven PAHs can be observed in Fig. 3.

3.2. Optimization of CEC method

3.2.1. Optimization of monolith formulation

Starting from classical monolith formulation as described in section 2.4 [22] the composition of the polymerization mixture was optimized for the separation of PAHs. In order to study the effect of the stationary phase's polarity, an incorporation of C_{12} group using LMA was tried in 3.65 and 46.05 M proportion in relation to BA keeping the total molar amount of functional monomers unchanged. It was observed that the EOF value dropped proportionally with the growth of LMA contribution in the mixture of monomers. In parallel, a significant drop of the retention factors was noted. It suggests remarkable reduction of the effective surface of the monolithic stationary phase due to enlargement of its pore size. These negative effects surpassed potential benefits deriving from the application of superficial lauryl groups, interacting more strongly with PAHs than butyl ones. Because LMA is more hydrophobic than BA, nuclei of polymerization precipitate earlier than in case of the standard mixture of monomer. It causes the formation of larger pores [29] and consequently decreased the active surface of the monolith. An increase of the C_{12} content could be obtained involving a standard C_{12}-methacrylate monolith procedure using a binary porogenic solvent composed of 1-propanol and 1,4-butane diol at 7:4 volume ratio [30]. The problem in this case would be the high retention of PAH with necessity to use non aqueous phase or optimize the C_{12} content. Finally the polymer with C_4 chains turned out to be the best for the rapid and efficient PAHs separation.

3.2.2. Optimization of CEC mobile phase

Firstly, the mobile phase was optimized using a usual mixture of phosphate buffer (pH 6.8) and MeCN. After optimization a concentration of 5 mM of phosphate buffer and a mixture of equal volumes of buffer and MeCN allowed a baseline separation of all PAHs. Nevertheless this baseline separation was obtained in

65 min leading to a peak broadening deleterious for the detection limit. Since we proved that baseline separation can be achieved by CEC, we decided to focus our attention on the improvement of analysis time and limit of detection (LOD). Moreover, in order to allow possible further mass spectrometry coupling, a volatile buffer was chosen. Finally, best separation was obtained with 10 mM hydroxylamine/acetic acid buffer at the same pH (6.8) and MeCN (30:70; v/v). The final mobile phase was then a mixture of hydroxylamine/acetic acid buffer (10 mM; pH 6.8) and acetonitrile in 30/70 volumic content. Injection was performed elecrokinetically during 1 s by applying 10 kV. Separation was conducted with the fixed voltage on the level of 30 kV. The separation temperature was set to 30 °C and the detection wavelength at 220 nm. These conditions allowed satisfactory separation of eleven PAHs within 16 min with a partial coelution of FLU and ACA (Fig. 4).

3.3. Comparison of CEC and MEKC

3.3.1. Reproducibility and repeatability

Run-to-run repeatability (rRSD) was expressed as a relative standard deviation of chromatographic parameters obtained for given capillary on the basis of a set of 6 repeated injections. Capillary-to-capillary reproducibility relative standard deviation (ccRSD) was obtained from three different capillaries. ccRSD by subtraction of the global relative standard deviation (gRSD) of chromatographic parameters collectively ($n=18$) for all capillaries (inter-capillary) from the average variance within the capillary (intra-capillary). By this method, pure ccRSD was obtained without contribution of run-to-run repeatability.

Concerning MEKC, on the basis of the set of separations, retention factor for each of seven PAHs (NAP, ACA, FLU, ANT, HHP, PHE, BAP) was calculated (Table 1) according to Eq. (1). It better represented the behavior of the analyte than the migration time because it was not affected by the extra-capillary effects, like run-to-run and intra-run EOF variations. The migration time of micelles (t_{mic}) was assigned as benzo [β]fluoranthene (BBF) migration time since this PAH was the most hydrophobic and was expected to migrate with micelles. Values of ccRSD did not differ from rRSD.

$$k' = \frac{t_m - t_0}{t_0(1-(t_m/t_{mic}))} \quad (1)$$

with k' – retention factor calculated for MEKC; t_m – migration time of given compound; t_0 – migration time of bulk solution; t_{mic} – migration time of the micelles (longest migration time).

In order to evaluate ccRSD of CEC technique, three monoliths were prepared according to the protocol described in Section 2.4. All buffers and mixtures of monomers were prepared independently for each capillary. Repeatability of CEC (Table 2) is very satisfying and better or comparable to previous values found in the literature [22,23,31].

Table 1
Retention times, retention factors (k'), separation factors (α) and related rRSD values for the separation of seven PAHs by MEKC, $n=6$ (conditions described under the Fig. 3). No k' value is provided for BBF because it was taken as the micelles' marker (MM).

Compound	Retention time		Retention factor		Separation factor	
	t (min)	rRSD (%)	k'	rRSD (%)	α	rRSD (%)
NAP	**7.01**	1.02	**0.97**	0.64	–	–
ACA	**8.28**	1.15	**1.50**	0.63	**1.55**	0.06
FLU	**10.97**	1.43	**2.97**	0.74	**1.98**	0.21
PHE	**12.21**	1.49	**3.86**	0.65	**1.30**	0.16
ANT	**12.50**	1.49	**4.10**	0.58	**1.06**	0.14
HHP	**19.28**	1.94	**16.89**	0.70	**4.12**	0.34
BAP	**23.63**	2.17	**121.18**	1.65	**7.17**	1.46
BBF	***24.59***	*2.26*	–	–	–	–

Table 2
Average values of retention times, retention factors (k'), separation factors (α) for CEC runs and capillaries, mean run-to-run repeatability (rRSD, $n=18$) within the three capillaries and capillary-to-capillary reproducibility (ccRSD, $n=3$) (conditions described under the Fig. 4).

Compound	Retention time			Retention factor			Separation factor		
	t (min)	rRSD (%)	ccRSD (%)	k'	rRSD (%)	ccRSD (%)	α	rRSD (%)	ccRSD (%)
NAP	**3.78**	0.25	6.28	**1.84**	0.09	2.03	–	–	–
FLU	**4.79**	0.25	6.08	**2.60**	0.09	2.21	**1.41**	0.01	0.20
ACA	**4.85**	0.22	6.12	**2.65**	0.12	2.10	**1.02**	0.04	0.11
PHE	**5.88**	0.24	6.02	**3.42**	0.11	2.13	**1.29**	0.03	0.04
ANT	**6.11**	0.21	6.01	**3.59**	0.12	2.17	**1.05**	0.04	0.07
HHP	**8.82**	0.23	5.89	**5.63**	0.12	2.35	**1.57**	0.03	0.25
BAP	**14.98**	0.23	5.82	**10.26**	0.10	2.25	**1.82**	0.04	0.12

For both CEC and MEKC, rRSD values were at least twice better for retention factor than for retention times. This showed that variations in retention times were due to voltage, capillary or monolith lengths variations and therefore, by taking into account the void volume, micelles volume was correcting these variations. As a consequence it can be seen that the separation parameters were perfectly constant from run to run.

Repeatability of CEC discussed above is better than MEKC for all chromatographic parameters. This effect occurs because micelles, constituting an equivalent of the stationary phase for hydrophobic compounds are subjected to two opposite forces – EOF and electrophoretic mobility. Both of them are sensitive to the voltage/current fluctuations during the analysis. Due to the fact that the mean current value during MEKC separations is approx. 18-times higher than for CEC, it can cause Joule heating and generate bubbles inside capillary and cause uniformity of electrical conditions of the separation. As a result, the longer migration time for a given compound, the more 'interruptions' it collects before passing through optical window, what in turn elevates its rRSD values of all chromatographic parameters (Table 1). In contrast, rRSD values for all PAHs separated by CEC are stable and independent of the residence time inside the capillary. This higher run-to-run repeatability for CEC is finally proved by lower rRSD for separation factors. Nevertheless low values of rRSD values for separation factors obtained for both CEC and MEKC show that the quality of the separation remains identical for repeated injections.

Results of reproducibility studies for CEC were worse than repeatability but remain lower than 0.3%, 2.5% and 6.5% for separation factors, retention factors and retention times respectively. The problem with obtaining satisfying ccRSD parameters for retention time and retention factor belongs to inter-capillary differences in pore size and EOF value that depends on the amount of AMPS exposed on the surface of the monolith. Therefore ccRSD for retention factor values that takes into account the EOF (t_0) variations are much better than ccRSD for retention times.

3.3.2. Capillary capacity

To assess the capacity of capillaries in both CEC and MEKC, higher injection volumes were tested. Injection of twice the optimal amount of sample for MEKC (0.5 s; 50 mbar, Fig. 3) led to a complete deformation of electropherogram in terms of peak symmetry and their separation and greatly affects elution times. Concerning CEC a fivefold excess of sample amount being equivalent to optimal amount for MEKC conserves symmetry of peaks. The only difference is peaks area (width, intensity) and negligible change in retention times. One should then note that the sample

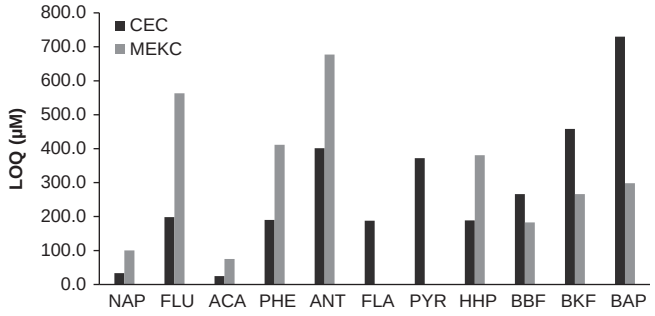

Fig. 5. Comparison of LOQ values for CEC and MEKC.

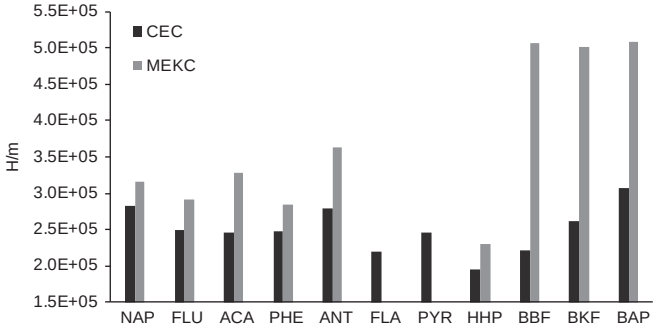

Fig. 6. Comparison of efficiency for CEC and MEKC.

capacity of CEC monolithic capillary is higher than in case of MEKC. One can notice that the peaks symmetry obtained during CEC analysis was very good and better than for MEKC.

3.3.3. *Influence of pH on the separation*

Value of pH was of key importance for MEKC, because the presence of ionized silanol groups on the inner surface of the capillary was determining EOF. Standard operational range of pH should be higher than 6.8. Application of pH = 5.35 or 2.5 leads to a reduction of initial EOF (calculated by the method described by Williams and Vigh [32]) by 30% or 96% respectively.

Due to application of AMPS as EOF generator for the monolithic CEC, influence of pH on the separation was expected to be marginal. Nevertheless, three series of separations were conducted at pH values 2.35, 5.80 and 6.80 to quantify it. Results suggested only mild inhibition of the EOF in the zone of low pH, possibly due to deionization of silanol groups either within the empty part of the capillary or on residual silanol groups related with TMSPA. Indeed at these three pH values the retention times varied from 6.7 min to 6.4 min and 5.7 min for pH 2.35, 5.35 and 6.80, respectively. A very small variation of k parameter (RSD lower than 1.6% between all pH) shows that the EOF mobility was the only factor affecting retention time.

The application of the extreme pH values significantly reduces lifespan of the capillary due to affecting Si–O–Si bonds constituting the covalent attachment of the monolith to the wall of the capillary. It is finally manifested in the complete removal of the stationary phase from the capillary.

3.3.4. *Efficiency and LOQ*

Limit of quantitation (LOQ: 10-fold S/N ratio, Fig. 5) and efficiency, expressed as amount of theoretical plates per meter (Fig. 6), were calculated for each peak separately (with the exception of FLA/PYR in MEKC due to the co-migration of these compounds). For both methods, data were collected at 220 nm, for the reason that this wavelength is the best consensus for all compounds.

LOQ is generally much better for CEC for small PAHs (up to HHP), comparable for BBF and worse for BAP and BKF (Fig. 5). It results from significant growth of the efficiency values for these three latter PAHs in MEKC. Better sensitivity for CEC can be attributed to great increase of electrical resistance in CEC capillary compared to MEKC because of the monolith. Therefore the current corresponding to the given value of voltage was smaller than in MEKC (approx. 18 times). Even if an increase of electrical resistance increase Joule heating, this effect is lower than the one provided by current increase. Indeed excessive Joule heating current in MEKC led to bubbling and was responsible for the growth of the baseline noise (approx. 1.3 times).

Values of efficiency in CEC are stable for all PAHs, in contrast to MEKC, where efficiency grows rapidly for BBF, BKF and BAP (Fig. 6). It results in lack of widening of peaks bases, even for the most retained compounds.

4. Conclusions

This study compared two capillary-profile techniques (MEKC and monolithic CEC) optimized for the separation of uncharged PAHs in the same laboratory. Both techniques were compatible with standard capillary electrophoresis apparatus. The results of this investigation have shown the superiority of monolithic CEC upon MEKC.

In term of protocol optimization the results were mixed. Indeed the overall optimization of mobile phase/running buffer is easier for MEKC but the amplitude of possibilities is restricted because of solubility and miscibility considerations. In contrast the optimization of the mobile phase in CEC is as simple and versatile as in HPLC. The difficulty comes from the synthesis of the monolith and particularly the anchoring step. Preparation of CEC capillary is time-consuming and requires additional laboratory background and experience.

Once these two methods optimized it can be seen that repeatability and reproducibility were in favor of CEC because of simpler mobile phase.

Simple buffers in CEC renders possible the coupling with mass spectrometry, which is not achievable in case of MEKC due to presence of surfactants and MS-incompatible additives. Application of dedicated EOF generator (AMPS) opens possibility of working within low pH range without visible influence on the separation coefficients.

Monolithic CEC is also more versatile thanks to the possibility of changing properties of the stationary phase by modifying composition of the monomer mixtures or by conducting post-polymerization modifications. The choice of an appropriate technique depends on desired applications.

Thanks to the simplicity of the mobile phase, low LOQ values and excellent run-to-run repeatability, CEC technique should be considered better than MEKC for microscale PAHs analysis.

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References

[1] C.E. Searle (Ed.), Chemical Carcinogens, American Chemical Society, Washington, DC, 1976.
[2] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 92, 2010.

[3] IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, vol. 82, 2002.
[4] B. Jiménez, D.G. Patterson, J. Grainger, Z. Liub, M.J. González, M.L. Marina, J. Chromatogr. A 792 (1997) 411–418.
[5] M.-M. Hsieh, Y.-C. Kuo, P.-L. Tsai, H.-T. Chang., J. Chromatogr. A 924 (2001) 397–405.
[6] C.J. Smith, J. Grainger, D.G. Patterson , J. Chromatogr. A 803 (1998) 241–247.
[7] O. Brüggemann, R. Freitag, J. Chromatogr. A 717 (1995) 309–324.
[8] J.M. Treubig , P.R. Brown, J. Chromatogr. A 873 (2000) 257–267.
[9] G. Kavran, F.B. Erim, J. Chromatogr. A 949 (2002) 301–305.
[10] D. Norton, S.A. Shamsi, Anal. Chim. Acta 496 (2003) 165–176.
[11] L. Ferey, N. Delaunay, D.N. Rutledge, C.B.Y. Cordella, H. This, A. Huertas, Y. Raoul, P. Gareil, Talanta, http://dx.doi.org/10.1016/j.talanta.2013.11.062.
[12] F. Svec, Electrophoresis 30 (2009) S68–S82.
[13] B.A. Grimes, A.I. Liapis, J. Coll., Interface Sci. 234 (2001) 223–243.
[14] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. A 218 (1981) 209–216.
[15] Y. Wang, Y. Xiao, T.T.Y. Tan, S.-C. Ng, Electrophoresis 30 (2009) 705–711.
[16] R. Asiaie, X. Huang, D. Farnan, C. Horváth, J. Chromatogr. A 806 (1998) 251–263.
[17] D. Norton, J. Zheng, S.A. Shamsi, J. Chromatogr. A 8 (2003) 205–215.
[18] F.A.S. Vaz, A.D. Moutinho, J.P.R.F.D. Mendonça, R.T.D. Araújo, S.J.L. Ribeiro, F.C. Polachini, Y. Messaddeq, M.A.L.D. Oliveira, Microchem. J. 100 (2012) 21–26.
[19] J. Ou, Z. Zhang, H. Lin, J. Dong, H. Zou, Anal. Chim. Acta 761 (2013) 209–216.
[20] V. Bernabé-Zafón, M. Beneito-Cambra, E.F. Simó-Alfonso, G. Ramis-Ramos, J.M. Herrero-Martınez, Electrophoresis 30 (2009) 3748–3756.
[21] M. Ganzera, I. Nischang, C. Siegl, B. Senzenberger, F. Svec, H. Stuppner, Electrophoresis 30 (2009) 3757–3763.
[22] N. Delaunay-Bertoncini, C. Demesmay, J.L. Rocca, Electrophoresis 25 (2004) 3204–3215.
[23] J. Grafnetter, P. Coufal, E. Tesarova, J. Suchankova, Z. Bosakova, J. Sevcık, J. Chromatogr. A 1049 (2004) 43–49.
[24] D. Hoegger, R. Freitag, J. Chromatogr. A 914 (2001) 211–222.
[25] E.C. Peters, M. Petro, F. Svec, J.M.J. Frechet, Anal. Chem. 70 (1998) 2288–2295.
[26] A.H. Que, T. Konse, A.G. Baker, M.V. Novotny, Anal. Chem. 72 (2000) 2703–2710.
[27] M.R. Buchmeiser, F. Sinner, M. Mupa, K. Wurst, Macromolecules 33 (2000) 32–39.
[28] D. Attwood, A.T. Florence, Surfactant Systems, Chapman and Hall, New York, NY, 1983.
[29] F. Svec, T. Tennikova, Z. Deyl, J. Chromatogr, Library, first ed., Elsevier, 2003.
[30] Y. Ueki, T. Umemura, Y. Iwashita, T. Odake, H. Haraguchi, K. Tsunoda, J. Chromatogr. A 1106 (2006) 106–111.
[31] F. Svec, E.C. Peters, D. Sykora, C. Yu, J.M.J. Frechet, J. High Resol. Chromatogr. 23 (2000) 3–18.
[32] B.A. Williams, G. Vigh, Anal. Chem. 44 (1996) 1174–1180.