Journal of Chromatography A, 1217 (2010) 8001–8008

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Electrochromatography in cyclic olefin copolymer microchips: A step towards field portable analysis

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ARTICLE INFO

Article history:
Available online 11 August 2010

Keywords:
Methacrylate monolith
Cyclic olefin copolymer
Microchip electrochromatography

ABSTRACT

In order to develop a portable and disposable instrument for on-site analysis of neutral compounds, a lauryl methacrylate monolith has been synthesized into a cyclic olefin copolymer microdevice for reversed-phase electrochromatography purposes. This monolith was tested in capillary to evaluate electrochromatographic performances in terms of electroosmotic flow (EOF) mobility, retention and efficiency prior to its transfer into the microfluidic device. The produced monolithic bed exhibited a good run-to-run repeatability, column-to-column reproducibility and batch-to-batch reproducibility, with relative standard deviation (RSD) values below 9% for EOF mobility, retention factors and heights of theoretical plate. The electrochromatographic performances of the monolith were optimized by reducing irradiation time. Photopolymerization time of 10 min was found to be the best process in order to obtain a robust, retentive and efficient system. The on-chip performances of this monolith were evaluated in detail for the reversed-phase electrochromatographic separation of polycyclic aromatic hydrocarbons, with plate heights reaching down to 15 μm when working at optimal velocity. Aiming at the maximum simplification of instrumental fabrication and operation, a direct injection from a 2 μL droplet was compared with more conventional dynamic injection process.

1. Introduction

In the large domain of analytical sciences, the field of action sometimes needs to come out from the labs to meet the sampling point. To carry out an efficient analytical strategy on the field requires a simple analytical method that can be accessed by non-chemists and a portable instrumentation eventually disposable to avoid cross contamination. On-site electrophoresis techniques have already been developed for the separation of charged compounds. Typical examples of such on-site microsystems are the instrument conceived by Crockett et al. [1,2] for the analysis of explosives in soil or the Mars organic analyzer developed by Mathies' group [3,4].

If zone electrophoresis [5] and micellar electrokinetic chromatographic (MEKC) [6,7] are well suited techniques for miniaturisation, the robust separation of neutral solutes requires an interactive stationary phase for chromatographic separation. In literature, two strategies are considered for the implementation of chromatography in microfluidic chip: a "top-down" method consists in the miniaturisation of conventional liquid chromatography (LC) instruments and will use pressure as the liquid driving force (chip-LC) [8,9], while the "bottom-up" idea is the use of a stationary phase in microchip by employing electroosmosis mechanism to bring the fluids into movement (chip electrochromatographic or chip-EC) [10]. One company (Agilent) now markets a chip-LC instrument based on a polyimide device that integrates a trapping column, separation column, and electrospray source [11]. However, the chip-LC system uses conventional LC pumps and a modified injection valve. These features make the overall instrument non-portable and non-disposable.

The chip-EC approach, by implementing both injection and separation within the very same device, is suitable for single use, especially if the device is cost effective. Moreover, the driving force is generated by two electrodes and a power supply, which is easily portable. Therefore, implementing electrochromatography in plastic device seems to be a good strategy to realize an on-site analyzer for neutral solutes.

The advantages of polymer-based microfluidic devices include the reduced costs, relative ease of fabrication and availability of a wide range of plastics with different properties [12,13]. As a rigid polymer, poly(methyl methacrylate) (PMMA) has been particularly useful for microfluidic chips with the features of low price, high optic transparency, and excellent electric and mechanical properties [14]. However, it is not compatible with chromatography organic solvents. Cyclic olefin copolymer (COC) is of particular interest due to its combination of excellent UV transparency, low

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doi:10.1016/j.chroma.2010.07.076

autofluorescence, high mechanical strength and compatibility with a broad range of chemicals and solvents [15,16].

Various stationary phases have been incorporated in microchip for electrochromatography purposes, such as particle [17], collocated monolith support structures (COMOSS) [10,18] and monoliths [19–21]. The porous polymer monolithic materials that have emerged in the early 1990s [22,23], have become popular as stationary phases for LC and capillary electrochromatography (CEC) [24]. One of the advantages of monoliths over packed materials is the ease of preparation and non-requirement of frits [21,25,26]. The first syntheses of organic monoliths for chip-EC purposes were carried out in glass chips [19,27–30]. The main challenge in the synthesis of monolithic columns in COC device is that the hydrophobic nature of the material requires that there is no water in the polymerization mixture. The introduction of aqueous solutions in COC microchannel is difficult and results in non-homogeneous monolith despite all precautions. On the contrary, non-aqueous porogenic solvent leads to highly homogeneous monolithic structure. In a previous paper [31], we demonstrated that an acrylate-based monolith could be synthesized *in situ* in COC microchannels and subsequently used as an efficient stationary phase for electrochromatography of hydrophobic compounds. Separation of non-polar compounds was obtained through a reversed-phase behavior, with relatively good efficiencies. However, reproducibility issues were highlighted, mainly due to the presence of volatile methanol in the polymerization mixture. A formulation based on 1-propanol and 1,4-butanediol as non-aqueous porogens was first introduced by Huo and colleagues in 2007 [32] and developed further on by Herrero-Martinez's group [33–36]. These two solvents were preferred in our work to conventional dodecanol/cyclohexanol system because their low viscosity makes easier electrokinetic rinsing of microchannels after monolith synthesis. The monolith presented here is similar to the one developed by Herrero's group, except that a negative charge is incorporated to generate a cathodic electroosmotic flow. This formulation was tested in capillaries before implementation in the COC microsystem in order to evaluate electrochromatographic performances in terms of EOF mobility, solute retention and efficiency.

2. Experimental

2.1. Materials

Fused-silica capillaries with a UV-transparency coating and an inner diameter of 75 μm were purchased from CIL-Cluzeau Info Labo SA (Sainte-Foy-La-Grande, France). Lauryl methacrylate (LMA), 1,4-butanediol, 2-acrylamido-2-methyl-1-propanesulfonic acid (AMPS), ethylene dimethacrylate (EDMA) and benzoin methyl ether were obtained from Acros Organics (Noisy-Le-Grand, France). Sodium phosphate, lithium dodecyl sulfate (LiDS), various polycyclic aromatic hydrocarbon (PAHs) (naphthalene, anthracene, fluoranthene, pyrene, benzo-a-anthracene and perylene), 3-trimethoxysilyl propyl methacrylate (Bindsilane), ammonium phosphate ($NH_4H_2PO_4$), sodium hydroxide (NaOH) and acetic acid (CH_3COOH) were from Sigma–Aldrich (Isle-d'Abeau, France). Acetonitrile and ethanol were HPLC-grade from SDS (Peypin, France). Concentrations of individual PAHs were chosen according to their fluorescence and absorbance in UV.

The microfluidic devices made of cyclic olefin copolymer (COC) was fabricated by Microfluidic Chipshop GmbH (Jena, Germany). Two types of microfluidic chips were used: the first one has a conventional two cross-intersecting channels and four reservoirs. Injection arms are 6 mm long. The second one is a single channel with two reservoirs only. In both cases, the length of separation channel is 81 mm and the channels are 75 μm deep by 75 μm wide. The chips are available with directly molded reservoirs. According to the literature, reservoirs will be defined as S (sample), SW (sample waste), buffer (B) and buffer waste (BW).

2.2. Instrumentation

The illumination system used to perform photopolymerization was a Bio-link cross-linker (VWR International, Strasbourg, France) equipped with five 8W UV tubes, emitting at 365 nm.

Capillary experiments were performed using Agilent^{3D} CE system equipped with DAD UV detector set up at 214 and 254 nm. An LC pump (Shimadzu LC 10AD, Tokyo, Japan) is used to rinse the capillary after its fabrication. Controls of the electrochromatographic system and data acquisition were carried out by Chemstation system (Agilent Technologies, Waldbronn, Germany).

Microchip analyses were monitored by inverted fluorescence microscopic system (IX-71, Olympus, France) equipped with a 100 W mercury lamp and 330 nm excitation filter (collection above 400 nm) (XF02-2, Omega, USA) for the detection of fluorescent PAH. A CCD camera was combined with NI Vision software (Alliance Vision, France) for detection processing. High-power supply (Micralyne, Canada) was used to apply electric fields to the microchannels through platinum electrodes placed in the reservoirs. All system operations were performed with Labview 7.1 (National Instrument, Austin, TX, USA).

2.3. Preparation of monolithic columns in capillary

Capillary pre-treatment procedure was carried out in order to enhance covalent attachment of the monolith to the capillary walls. The inner wall of the fused-silica capillary was rinsed with 1 M NaOH for 15 min to eliminate any impurities present in the capillary, then rinsed with a mixture of ethanol/water (50/50) and finally flushed with a 0.5% (v/v) solution of 3-(trimethoxysilyl) propyl methacrylate in 6 mM acetic acid for 30 min. Thereafter, the capillary was rinsed with water and dried under nitrogen stream. Polymerization mixture was introduced in pre-treated capillary under gas pressure (2 bar) for 10 min. The capillary ends were then sealed with silicon.

Polymerization mixture was prepared as follows. The porogenic mixture was made up of 55.2% (w/w) of 1-propanol and 4.8% (w/w) of 1,4-butanediol. Porogenic mixture was dissolved in monomer mixture containing 24% (w/w) of LMA and 16% (w/w) of EDMA. The ratio of monomer to porogenic mixtures was 40:60 (w/w). AMPS (0.25% (w/w) of monomers) is added in the polymerization mixture in order to support the electroosmotic flow. Benzoin methyl ether was used as radical photoinitiator (0.5% (w/w) of monomers). Photopolymerization was carried out at 365 nm for different irradiation times. After polymerization, the monolithic column was rinsed with ethanol using a LC pump to remove any remaining reactant. A UV detection window was created by depolymerisation of the monolith under exposure to a deuterium lamp, inside the CE instrument.

2.4. Preparation of monolithic columns in microchip

Unlike capillaries, COC microsystems were used as received without any pre-treatment. The microsystem was simply washed with 1-propanol to remove any impurity. The same polymerization mixture as in capillaries was then introduced in the microchannels by pressure using a syringe. The microchip was submitted to a subsequent irradiation at 365 nm for different irradiation times. Channels were then rinsed with methanol by applying an electric field of 402 V/cm to remove any remaining reactant. Cross-shaped channel chip was fully filled with monolith. Single channel chip was

fully filled and excedent monolith was scrapped off from reservoirs with a thin spatula.

2.5. Electrochromatography procedures in capillary

The electric field is applied over the capillary length (33 cm) and effective length is 8.5 cm. Before CEC runs, each monolithic column was placed in the CEC instrument and equilibrated with mobile phase 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS by applying a stepwise increase in voltage from 5 to 25 kV, until a stable baseline current was observed. Mobile phase was always prepared with a total concentration of 2 mM $NH_4H_2PO_4$ and 5 mM LiDS whatever the percentage of ACN. LiDS was introduced to avoid that the monolith dries when the percentage of water in mobile phase composition is important. The sample solution was electrokinetically injected at 5 kV for 5 s. All CEC runs were performed at 25 °C.

2.6. Electrochromatography procedures in microchip

The microchip was subsequently rinsed with mobile phase 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS for 30 min by applying an electrical field across every channel. The effective length can be modulated following the position of laser beam.

Two types of injection were used in this study: dynamic injection and simple direct injection. Dynamic injection is divided into three steps: pinched sampling, expansion and separation. Pinched injection was realized by applying 1 kV on sample reservoir S, while holding the other reservoirs B and BW respectively at 0.85 and 4.8 kV. SW remained at ground. This step allows the migration of all species into the channel cross-section without diffusion in the separation channel. During expansion, 1 kV is applied through sample reservoir while B, BW and SW were kept at ground in order to increase the injected volume. The injected volume can be modulated by the duration of the expansion step. In our study, we chose an 8 s expansion step at 1 kV, which seemed a good compromise between signal level and efficiency loss. During separation, 5 kV was applied to the buffer reservoir (B) while the buffer waste reservoir (BW) was kept at ground and the injection reservoirs S and SW were placed at 4.5 kV to avoid leakage phenomenon.

In the simple direct injection mode, a single electric field is applied across the separation channel. The configuration is similar to capillary since a single channel with inlet and outlet reservoirs was used. The main advantage of this method is to avoid voltage programming. This injection is composed of four steps: sampling step, injection, rinsing and separation step (Fig. 1). In the sampling step, a droplet of 2 μL of sample mixture is placed in the hole of the inlet reservoir. An electric field of 567 V/cm is then applied during several seconds to inject the sample mixture in the channel (injection step). The inlet reservoir is then fully washed with mobile phase which avoids sample leakage. Finally, an electric field of 567 V/cm is applied during separation step.

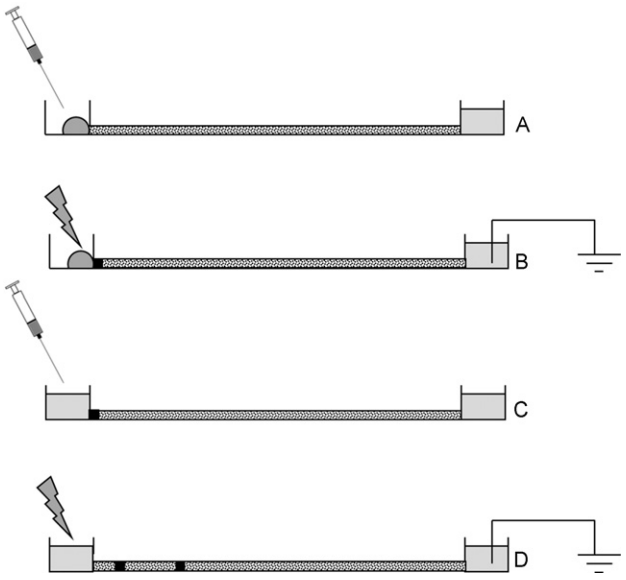

Fig. 1. Scheme of the simple direct injection. (A) Sampling step, (B) injection step, (C) rinsing step and (D) separation step.

3. Results and discussion

3.1. Robustness of synthesis

Robustness of the monolith synthesis in capillary was evaluated in terms of long-term repeatability, capillary-to-capillary and batch-to-batch reproducibilities. This test was carried out on a 30 min photopolymerized monolith, with a standard mixture of 6 alkylbenzenes and a 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS mobile phase. As shown in Fig. 2, the six neutral compounds are effectively separated according to their relative hydrophobicity. Using this standardized test, long-term repeatability was determined by using the very same capillary over 30 days. Capillary-to-capillary reproducibility was determined for five columns prepared with the same polymerization mixture whereas batch-to-batch reproducibility was evaluated for eight columns prepared with different polymerization mixtures having the same composition.

The results obtained for these tests are presented in Table 1. Plate heights were in the range of 9 μm at 757 V/cm corresponding to the highest velocity reachable. Long-term repeatability test showed that analysis could be carried out for up to 30 days without any loss of performances. Indeed, EOF mobility, retention factors and plate heights relative standard deviations (RSD) are less than 8, 5 and 7% respectively. The capillary-to-capillary and batch-to-batch reproducibilities are also satisfactory since the RSD values for the three parameters are between 5 and 9%. Retention factors, EOF mobility and efficiency values indicate a suitable monolith manufactory process. These results prove that the monolith structure is robust over time and its synthesis highly reproducible.

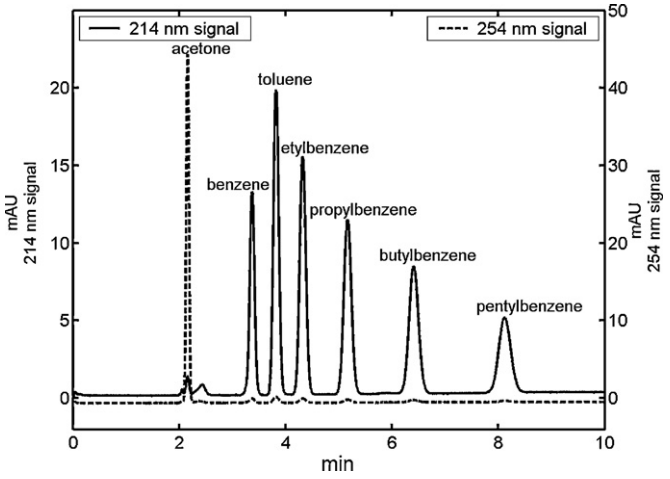

Fig. 2. Reversed-phase electrochromatographic separation of six alkylbenzenes. Separation field strength: 757 V/cm. Mobile phase: 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS. Benzene at 4.5 mM, toluene at 3.7 mM, ethylbenzene at 3.3 mM, propylbenzene at 2.9 mM, butylbenzene at 2.6 mM and pentylbenzene at 19 mM. Effective length: 8.5 cm.

Table 1
Long-term repeatability, capillary-to-capillary reproducibility and batch-to-batch reproducibility expressed in RSD on electroosmotic mobility (μ_{eo}), retention factors (k) and plate heights (H).

	Long-term repeatability		Capillary-to-capillary reproducibility		Batch-to-batch reproducibility	
	Mean	RSD (%)	Mean	RSD (%)	Mean	RSD (%)
μ_{eo} ($cm^2\ V^{-1}\ s^{-1}$)	1.33×10^{-4}	8	1.44×10^{-4}	9	1.47×10^{-4}	9
k	1.58	5	1.59	7	1.53	5
H (μm)	8.8	7	9.5	7	8.9	7

Effective length: 8.5 cm. Mobile phase: 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS. Electrokinetic injection: 5 s and 5 kV. Sample: acetone (non-retained solute) + propylbenzene. Separation field strength: 767 V/cm.

3.2. Influence of irradiation time during the polymerization

Fig. 3A shows the Van Deemter curves of different solutes for 30 min irradiation. The test solutes used, toluene, anthracene and triphenylene, are representative of one, three and five aromatic rings, respectively. It appeared that plate height decreases with decreasing the number of aromatic rings. We think that this issue is due to monolith morphology. Although a number of parameters in the preparation of the monolithic columns affect the porous properties, some key variables such as monomer proportion, composition of the porogen and content of the cross-linker are frequently used to change the average pore size [37]. We studied here the influence of irradiation energy on polymerization process by varying irradiation time.

Several exposure times were evaluated between 5 and 30 min. Irradiation time below 5 min was not used, since it is too short to achieve the polymerization of monolithic beds. Two identical columns were prepared for each energy dose.

The Van Deemter curves of toluene, anthracene and triphenylene obtained for 30, 20, 10 and 5 min irradiation are presented in Fig. 3. It appears that photopolymerization of 5 min results in high efficiency (low plate heights) and no discrepancy between solutes of different numbers of aromatic rings. The monolith synthesis based on non-aqueous porogens provides lower efficiency than monoliths based on aqueous porogens, since the very first attempts provided $H = 20$ μm on PAH [32], while the more recent developments by Herrero-Martinez group improved performances up to $H = 9$–15 μm [34,38] on PAH. In regards to the constraints imposed by our group to realize an efficient transfer from capillary to COC chip, the obtained monolith presents similar performances to the ones found in the literature.

SEM photographs showed significant differences in morphology according to irradiation time. A representative example of porous structure of the monoliths photopolymerized during 5, 10, 20 and 30 min are shown in Fig. 4A, B, C and D respectively. An increase in nodule size is observed at increasing exposure times (size of nodules: 0.9 μm for 5 min, 1.5 μm for 10 min, 2 μm for 20 min and 2.0 μm for 30 min). According to monolithic polymerization process, nodules size increases and pores size decreases when the irradiation time increases [39]. A monolith of small pores (long irradiation time) may result in a steric effect for large solutes, which will generate a loss in efficiency. Monoliths with large pores (small irradiation time) do not exhibit any difference in efficiency for various solute sizes.

But looking closer, at maximum electrical field (757 V/cm), the linear velocity is lower for an irradiation of 5 min. Indeed, the electroosmotic mobility suffers a loss of 35% when irradiation time decreases from 30 to 5 min. In addition, the retention factors for the three test compounds increase slightly from 30

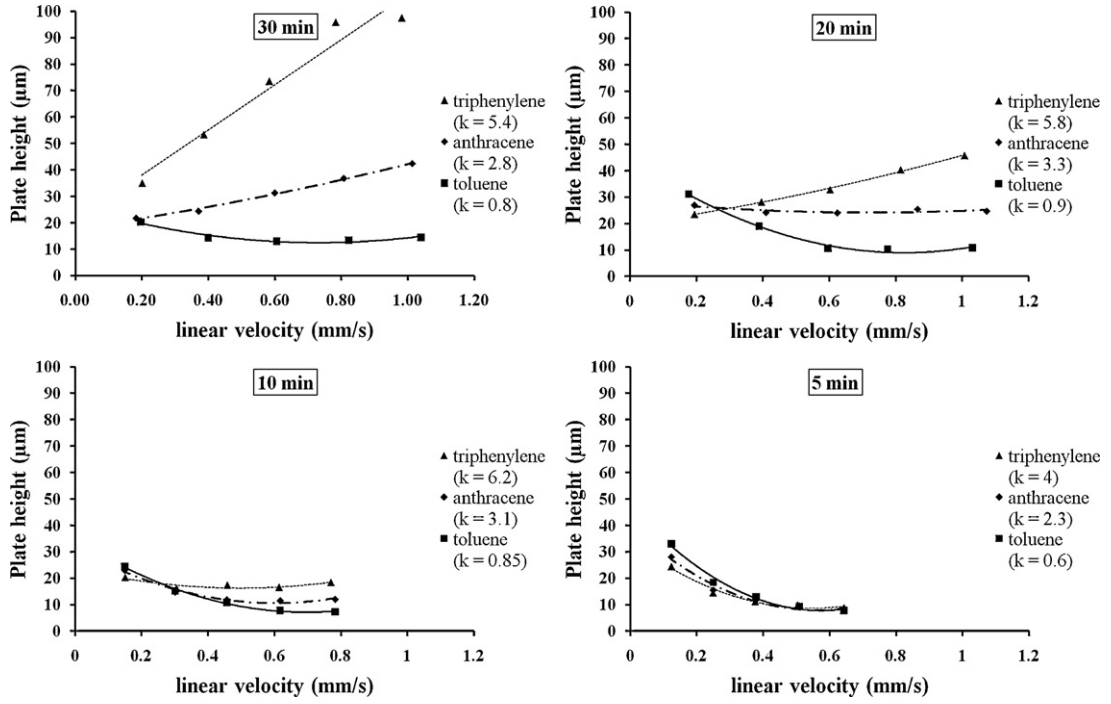

Fig. 3. Van Deemter curves obtained from toluene (3.7 mM), anthracène (0.22 mM) and triphenylene (2 mM) analysis on lauryl methacrylate monolithic for different irradiation time (30, 20, 10 and 5 min). Mobile phase 70/30 (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS.

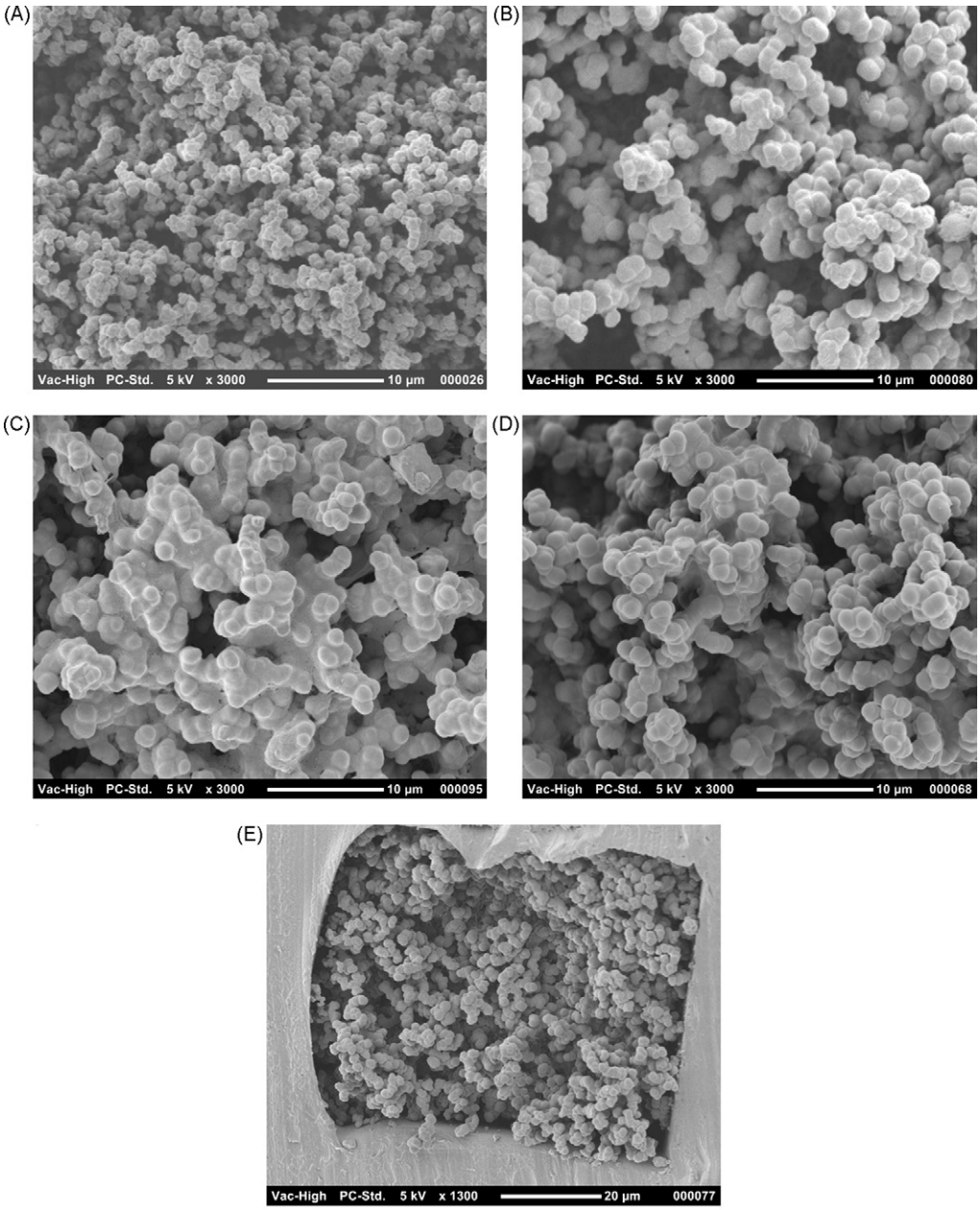

Fig. 4. Scanning electron micrographs of lauryl methacrylate monolith in fused-silica capillary (A–D) and in COC microchannel (E). Polymerization by UV-irradiation at several exposure times: (A) 5 min, (B) 10 min, (C) 20 min and (D) 30 min. (E) Overview of the channel section of a COC microchannel filled with monolith; bar scale of 20 μm.

to 10 min irradiation (RSD less than 2.5%) and decrease by 25% from 10 to 5 min irradiation (see keys of Fig. 3). This is due to the fact that charged monomers (SO_3^- groups) responsible for the EOF generation, and alkyl monomers providing retention, may not be fully incorporated in the polymerization process within 5 min time.

The stability of the capillaries for different time irradiations was also examined. Good long-term repeatability and good capillary-to-capillary reproducibility were exhibited for time irradiation between 10 and 30 min. At 5 min irradiation, however, the long-term repeatability is short (<5 days) due to a weak anchorage of the monolith to the inner wall of the capillary. So, the best compromise between efficiency, retention and stability is to carry out a 10 min synthesis.

3.3. Influence of the mobile phase composition

The monolithic stationary phase obtained for 10 min irradiation was evaluated under a large range of analytical conditions. Fig. 5A shows that the electroosmotic mobility increases when the percentage of ACN in the mobile phase is decreased. This effect could be attributed to the adsorption of LiDS onto the monolith, which becomes more and more important when the percentage of acetonitrile decreases, leading to a larger density of charges adsorbed onto the surface [40]. Without surfactant in the mobile phase, a decrease of EOF should be observed with decreasing acetonitrile content [41].

Fig. 5B shows the dependence of retention factors k of three alkylbenzenes (benzene, toluene and ethylbenzene) on the per-

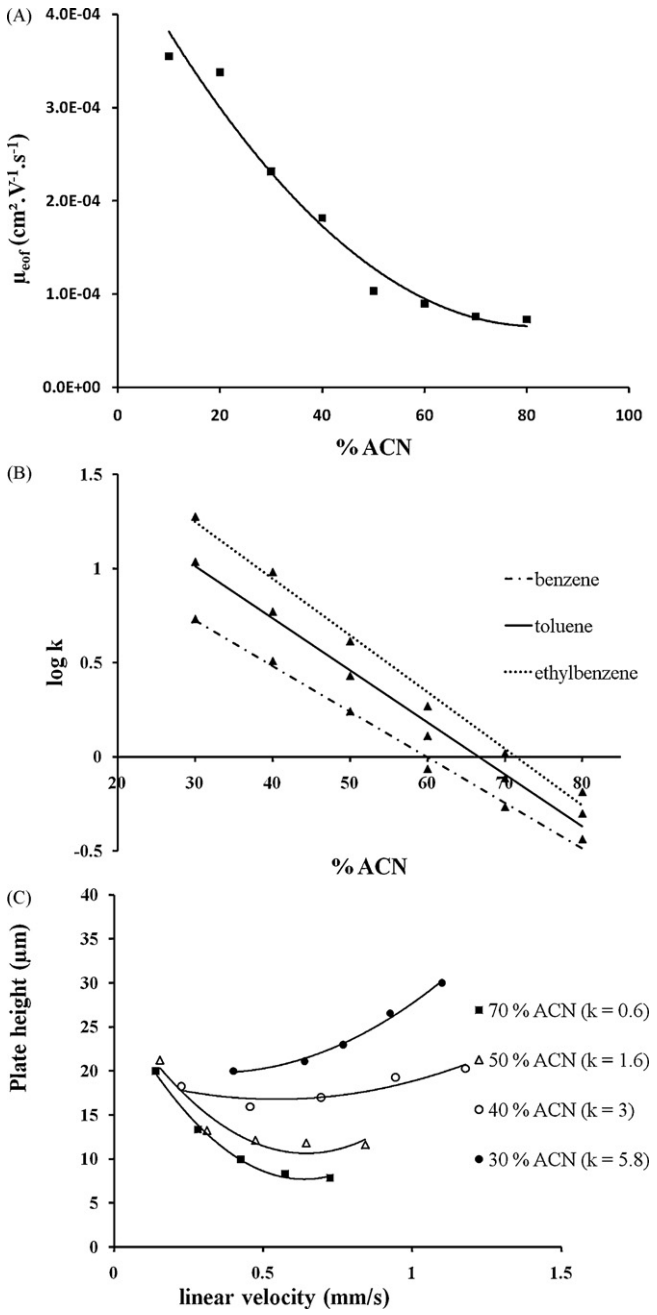

Fig. 5. Evaluation of the monolithic stationary phase obtained for 10 min irradiation. (A) Electroosmotic mobility vs. percentage of acetonitrile in mobile phase. (B) Logarithm of the retention factors for benzene, toluene and ethylbenzene as a function of the mobile phase composition. (C) Van Deemter curve of benzene for different percentage of acetonitrile. Mobile phase: ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS. Solute concentrations: benzene at 4.5 mM, toluene at 3.7 mM and ethylbenzene at 3.3 mM.

centage of ACN (v/v) in the mobile phase. It can be observed that log k linearly decreases with increasing concentration of ACN and the slope of the curve increases with the relative hydrophobicity of the analytes. This is a linear model over a large range of retention ($0.3 < k < 19$). This feature is indicative of true reversed-phase electrochromatography behavior.

The efficiencies obtained for benzene at the different mobile phase compositions are shown in Fig. 5C. An increase of plate height with increasing amount of water and consequently increasing retention was observed. This effect could be attributed to a swelling of monolith in these conditions and is currently studied. Indeed, the factors that affect the relationship between efficiency and retention are numerous and the type of solute and the swelling phenomenon of the stationary phase in the presence of organic modifier appear to be of importance in this issue [36].

3.4. Transfer into COC microsystem

The transfer of monolith synthesis into cyclic olefin copolymer commercial device was straightforward. The polymerization mixture was simply introduced in the channels without any pretreatment. The chip was then irradiated under UV light for 10 min, which is possible due to the good optical properties of COC.

As observed on the SEM imaging (Fig. 4E), the resulting monolith is homogeneous all over the volume of the channel. On the inner wall of the chip, no anchoring was provided. This was not necessary since no pressure is applied across the stationary phase and the ruggedness of the walls appeared sufficient to avoid any movement. The size of nodules is 1.8 μm. Several attempts to decrease nodules sizes have shown that 5 min of photopolymerization is not sufficient because the monolith is heterogeneous over the volume of the channel. Indeed, a large dead volume appears between the chip wall and the monolith, which allows a deformation of plug profile and subsequent band broadening.

Fig. 6A and B compares the separations of five fluorescent PAH obtained respectively in capillary with UV detection and microsystem with fluorescence detection. As the response of PAH in fluorescence detection is different to UV detection, two PAH mixtures with different concentrations were prepared. Dynamic injection on a fully filled chip was used for the microsystem separation. The quality of the two separations seems to be equivalent. However, theoretical plate height for anthracene is in the order of 12 μm in capillary and 17 μm in microsystem. A slight loss in efficiency is therefore noticed due to transfer. Unfortunately, it is impossible to compare EOF mobilities and retention factors between these two systems. Indeed, no EOF marker such as acetone and thiourea could be used in the microchip format because these compounds are not fluorescent.

The repeatability of microsystem separation has been tested for five successive injections. RSD values are less than 4% on retention times and less than 11% on efficiencies. In addition, three chips were made in the same conditions with the same polymerization mixture to evaluate the chip-to-chip reproducibility. Relative standard deviation (RSD) is less than 5% on retention time and less than 8% on efficiencies.

To conclude, the transfer into COC microsystem is completed. Monolith photopolymerized 10 min inside a COC chip is robust and gives good electrochromatographic performances. To the best of our knowledge, the only work on photoinitiated monolith, made exclusively with non-aqueous porogen in microsystem, that could be found in the literature, reports the synthesis of an acrylate monolith for COC with a methanol/2-propanol porogen, providing 12 μm plate heights [42]. If these results may appear to be better than the ones presented here, the batch-to-batch reproducibility was not satisfactory, due to methanol volatility.

3.5. Towards simplicity of injection process

Dynamic injection is a complex process which required several steps (pinched sampling step and expansion step), a cross design, four electrodes and a voltage programming. Towards simplification of use, a very simple direct injection of droplets is introduced here, as described in the experimental section, which requires only a single channel with localized monolith, two electrodes and no voltage programming. This integrated system (reservoirs and separation channel) is advantageous over a single capillary that would require an external reservoir system, which may be expensive and

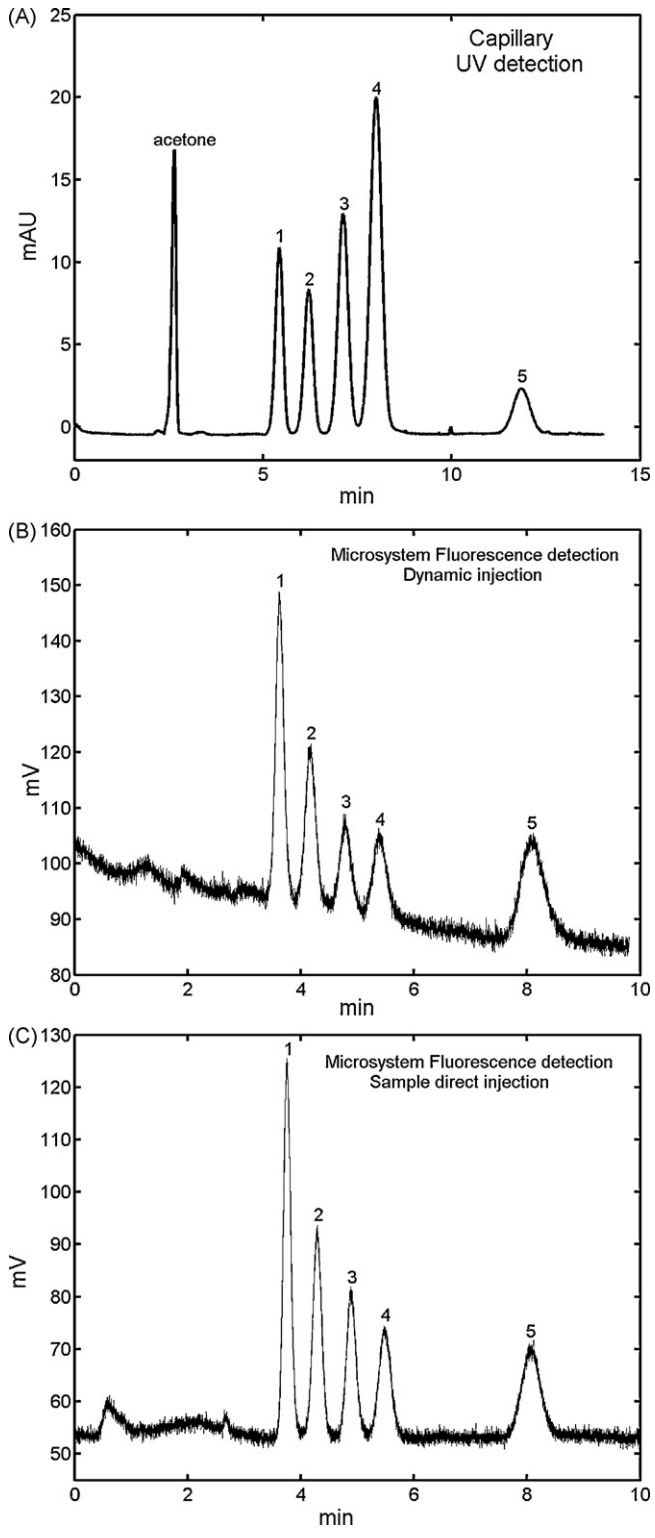

Fig. 6. Reversed-phase electrochromatographic separation of five PAHs, namely (1) anthracene, (2) fluoranthene, (3) pyrene, (4) benzo-[a]-anthracene and (5) perylene. Separation field strength: 560 V/cm. Mobile phase 85/15: (v/v) ACN/water + 2 mM $NH_4H_2PO_4$ + 5 mM LiDS. Effective length for capillary separation: 8.5 cm; for microsystem separation: 6.5 cm. Solute concentration: for capillary separation and UV detection 0.36 mM anthracene, 4.23 mM fluoranthene, 18.6 mM pyrene, 4.2 mM benzo-a-anthracene and 1 mM perylene; for microsystem separation and fluorescence detection: 1.5 mM of anthracene, 0.13 mM of fluoranthene, 1.33 mM of pyrene, 0.6 mM of benzo-a-anthracene and 3 mM of perylene.

non-disposable. Here, the overall electrochromatographic system can be disposed after analysis, due to the low cost of COC material and microsystem fabrication.

Electrochromatograms obtained for dynamic injection and simple direct injection are compared in Fig. 6B and C. Simple direct injection provides the same separation as dynamic injection but leads to a small efficiency loss (17 μm in dynamic injection and 19 μm in simple direct injection for anthracene). This efficiency loss is minimal compared to the gain in simplicity of use.

The repeatability of separation has been tested for six successive direct injections. RSD values are less than 1.5% on retention times and less than 11% on efficiencies. By comparing with the results obtained for dynamic injection, it is concluded that simple direct injection is a viable process for on-site analysis.

4. Conclusion

In this work, we have demonstrated the robust synthesis of monolith, using a non-aqueous formulation, for the efficient implementation of electrochromatography on polymeric microsystem. Separation of non-polar hydrophobic compounds was obtained through reversed-phase behavior, with relatively good efficiencies for a monolith photopolymerized during 10 min. Photopolymerization at this irradiation time is the best compromise to obtain a robust homogenous structure and good electrochromatographic performances. We developed a direct injection process, which contributes to the instrumental simplification towards on-site analysis. Further research is currently being carried out to improve electrochromatography performances of the monolith and to extend its application range to other analytes such as those of biological interest.

Acknowledgement

The authors wish to thank the French National research Agency for funding project ANR-07-PCVI-003601.

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