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C_{18} silica packed capillary columns with monolithic frits prepared with UV light emitting diode: Usefulness in nano-liquid chromatography and capillary electrochromatography☆

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ABSTRACT

In this paper the potential of fused silica capillaries packed with RP18 silica particles entrapped with monolithic frits using both nano-liquid chromatography (nano-LC) and capillary electrochromatography (CEC) was investigated. Frits were prepared after removing a short part of the polyimide layer on the capillary wall and irradiating the polymerization mixture with an UV-light emitter diode (LED) at 370 nm. The capillary, was rotated during the polymerization procedure in order to obtain a homogeneous monolith. The distance of the LED from the capillary and the exposure time to UV light were studied in order to obtain frits with good porosity and high robustness. A mixture containing five alkylbenzenes was selected as sample and analyzed by both nano-LC and CEC. The standard mixture was baseline separated with good efficiency in the range 78,000–93,000 and 99,000–113,000 plates/m in nano-LC and CEC, respectively. The columns resulted to be very robust and the prepared monolithic frits allowed working with backpressure as high as 400 bar (nano-LC). In addition high voltages were applied in CEC (25–30 kV) without bubbles formation in absence of pressure assistance during runs.

1. Introduction

In the last decades, miniaturization has been successfully introduced in separation science with the aim to improve the performances of recognized analytical techniques widely used, for their features, in various application fields. These modern tools are characterized by their high efficiency, high resolution, short analysis time, easy coupling with non-conventional detectors (e.g., mass spectrometry—MS), etc. Among them, capillary electrochromatography (CEC) and capillary/nano liquid chromatography (CLC/nano-LC) have been developed. This is documented by the large number of publications dealing with theory [1–3], instrumentation [4–6], column and stationary phase development [7–9] and large number of applications [10–13].

CEC and nano-LC make use of capillary columns containing a selected stationary phase. The capillary internal diameter (I.D.) is usually lower than 100 μm with flow rates in the order of hundred nL/min. The main difference between the two techniques is represented by the mobile phase flow generation that in nano-LC is obtained by a μ/nano pump while in CEC by a high electric field causing an electroosomotic flow (EOF). Usually a higher efficiency, typical of electrodriven techniques, is observed due to the plug-flow profile of the EOF. In addition different experimental conditions (mobile and stationary phases composition) could be necessary. In fact in CEC charged/chargeable groups must be present in the structure of the stationary phase for EOF generation.

Recently a unified approach where the same capillary column was applied employing different miniaturized techniques, e.g., CEC, nano-LC, gas chromatography (GC) and super critical fluid chromatography (SFC) [14] has been proposed. Columns so far used in CEC include open-tubular, monolithic and packed capillaries. Although the monolithic columns will soon find a wide applicability, the packed ones seem to offer good performance because some of their features such as (1) higher efficiency, (2) large number of stationary phases available and (3) better performances for small molecules separation [15].

In this type of column, the stationary phase is entrapped into the capillary by using two frits, usually prepared with a heated wire, sintering a small section of the packed material (silica-based).

This approach highlights several problems, e.g. (1) changes of the properties of the stationary phase within the frit itself, (2) frits' porosity and reproducibility can be questionable, (3) increase of band broadening, (4) bubbles formation and unstable EOF, and (5)

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adsorption of polar analytes onto the frits [16–19]. These inconveniences directly affect both column performance and column to column reproducibility.

In order to overcome these drawbacks, alternative approaches have been proposed. Lord et al. inserted at the end of the column a mechanical frit [20,21]. Mayer et al. used end restricted capillaries (tapered) blocking the particles and allowing the mobile phase to elute (fritless columns) [22]; synthesizing frits by macroporous polymer [16].

Packed columns with monolithic frits could be an attractive approach, especially in CEC, because they can offer a higher permeability and reproducibility associated with a good mechanical resistance to the back-pressure. Synthesis can be done *in situ* through a radical polymerization induced by initiator of a mixture containing the monomers, cross linker and porogen solvent.

Although the most common procedure used was the thermal initialization [23,24], in the last 10 years the photopolymerization gained prominence as an alternative method because its advantages over other ones, e.g. (1) faster than thermopolymerization, (2) the reaction is obtained at room temperature allowing the use low boiling point porogenic solvents, and (3) polymer can be prepared at the desired segment of the column [25].

The synthesis of macroporous monolithic columns by photoinitiated polymerization, firstly introduced in 1997 by Svec and co-workers [26], resulted to be successful for some different applications, e.g., for frits preparation in packed columns for CEC [16,27], monolithic frits for solid phase microextraction [28], fritless entrapped column for both CEC analysis [29], SPE extraction [30] and microchip for CEC experiments [31]. In all these cases photo-polymerization was carried out employing special UV lamps, that in many cases resulted to be quite expensive.

In addition to the classical light source used for photopolymerization, recently a cheaper approach utilizing light emitting diodes (LEDs) has been proposed [32–34].

In this study, in order to take advantage of the best properties of silica-based packed stationary phases, e.g., high efficiency, RP18 silica particles were entrapped into capillary columns by means of monolithic frits.

After removing the polyimide layer of the capillary at precise sections, UV-LEDs (370 nm) were used for *in situ* photoinitiation of the methacrylic polymeric mixture in order to prepare a macroporous frit.

A series of capillary columns with such frits were tested in terms of repeatability and reproducibility analyzing a standard mixture of alkylbenzene compounds firstly in nano-LC and then in CEC. The aim was to study the mechanical properties of the frits and the possibility to run CEC experiments without using an assisted pressure during the run.

Following this experimental design, different chromatographic parameters such as linear flow velocity, mobile phase composition, and applied voltage were investigated. Finally eleven NSAIDs were separated in nano-LC and CEC without the assistance of pressure system.

2. Experimental

2.1. Reagents and materials

All chemicals used in this study were analytical reagent grade with no further purification.

Acetonitrile (ACN), ammonia solution (30%), acetic acid glacial (>99%), hydrochloric acid (37%), sodium hydroxide, formic acid (99%), sulphuric acid (96%), acetone, benzene and toluene were from Carlo Erba (Rodano, Milano, Italy). The porogenic solvent: cyclohexanol (≥99%) and 1-decanol (≥99%) were purchased from Fluka (Sigma–Aldrich, St. Louis, MO, USA).

Deonized water was obtained by means of a Milli-Q system (Millipore, Bedford, MA, USA).

The methacrylate monoliths were synthesized using as a main monomer the glycidyl methacrylate (GMA) (purum, ≥97.0%) and ethylene glycol dimethacrylate (ethylene dimethacrylate, EDMA, 98%) (Sigma–Aldrich, St. Louis, MO, USA) as crosslinker. 3-(Trimethoxysilyl)propyl methacrylate (TMSPM) (Fluka, Buchs, Switzerland) was used for coating the capillary.

Butylbenzene, ethylbenzene, fenoprofen, flurbiprofen, ketoprofen ibuprofen, indoprofen, naproxen, propylbenzene and toluene were from Sigma–Aldrich, while cicloprofen was kindly supplied by Dr. Cecilia Bartolucci (Istituto di Cristallografia, C.N.R. Montelibretti, Italy). DF2008Y (2-(3′-carbossiphenyl) propionic acid), DF2107Y (2-(3′-carbossiphenyl) propionitrile), DF1770Y (2-[(4′-benzoyloxy-2′-hydroxy)phenyl]propionic acid) and DF1738Y (2-[(5′-benzoyloxy-2′-hydroxy)phenyl]propionic acid) were kindly provided by Dompè (L'Aquila, Italy) [35,36].

4,4′-Bis (dimethylamino) benzophenone or Michler's keton (MK) (98%) (Sigma–Aldrich) was used as the photoinitiator.

Stock standard solutions (1 mg/mL) were prepared in methanol and daily diluted to the desired concentrations for the analysis.

Capillary columns were packed with RP-C18, Cogent Bidentate C18™ (Microsolv Technology, Eatontown, NJ, USA). These particles were manufactured employing high purity silica with hydride surface (TYPE-C™, 4 μm, pore size 100 Å, carbon load 16.5%).

2.2. Apparatus and software

A FS 100b ultrasonic bath (Decon, Hove, UK) was employed to sonicate solutions and during the packing procedure. An accurate pH measurement was done by a pH meter MicropH 2001 (Crison, Barcelona, Spain).

A model 2209 Multitemp LKB thermostate (Bromma, Sweden) was used for thermostating the capillary during the preparation of monolithic frits. This tool was connected to a lab-made device (aluminum box) to control the capillary temperature.

The column oven (Shimadzu, Kyoto, Japan) was used to perform the coating of fused silica capillary.

A Perkin-Elmer series 10 LC-pump (Palo Alto, CA, USA) was used for packing or flushing the capillary columns.

A UV-LED (light emitting diode) from Roithner LASER Tecnik (Vienna, Austria) (370 nm, NS370L-5RLO, 3.0 mW at 20 mA, 15°, 5 mm clear UV-resistant epoxy) was used for the preparation of monolithic frits. The LED was connected to a laboratory-made electrical device (DC generator, applied voltage 3.3–3.6 V; electrical resistance, 14 ohm). The device was prepared considering the data sheet [37] where optimum conditions were obtained at 20 mA.

2.2.1. Capillary electrochromatography

CEC experiments were carried out with an Agilent Technologies 3D CE system (Waldbronn, Germany) equipped with UV–vis diode array detector (DAD) operating at 200 nm.

The capillary column temperature (25 °C) was controlled by the air thermostating system, while the vial carousel was at room temperature (about 25 °C). A 3D CE Chemstation software (Rev. A. 09.01, Agilent Technologies, Waldbrom, Germany) was used for controlling CEC instrument, data collecting and processing.

2.2.2. Nano-liquid chromatography

Nano-liquid chromatography experiments were carried out by using a lab-made assembled instrument previously described [38].

An Accela® micro pump (Thermo Electronic Corporation, St. Josè, CA, USA) was used to deliver the mobile phase. The flow rate in the range of 100–1000 nL/min was assured using a passive mechanical split. The main component of the split system was a three ports stainless steel T piece (Vici, Valco, Houston, TX) connected to the

injector valve by using a stainless steal tube (5 cm × 500 μm I.D.), to the pump through a peek capillary tube (130 μm I.D. × 50 cm) and the last was tied to the waste by using a fused silica capillary (25 μm I.D. × 20 cm).

Sample injection was done by using a Sepaserve valve (Münster, Germany) equipped with a loop of about 50 μL. The loop was utilized for both injection and mobile phase reservoir.

With such system, the same organic solvent (acetonitrile) was pumped to the injector and recycled from the waste. Injected sample volume was controlled considering both flow velocity and injection time; the procedure has been previously described [39]. Capillary column was directly connected to the injection valve.

The flow rate was estimated by measuring the volume of the mobile phase coming out from the outlet column for a set time. In this respect, a 10 μL syringe (Hamilton, Reno, NV, USA) was connected to the outlet column through a Teflon tube (TF-350, LC-packing, CA, USA).

A on-column Spectra 100 UV detector (Thermo Separation Products, St. Josè, CA, USA) was used to reveal separated compounds. The wavelength was set at 200 nm, the rise time and data acquisition rate were set at 0.2 s and 20 Hz, respectively.

The detection window was prepared directly on the capillary column removing 5 mm polyimide layer by razor. The effective length was 26.2 cm (8.4 cm short packed column).

The micro pump was managed using the Xcalibur™ 1.3 (Thermo-Finnigan) software. The data from detector were acquired by ChromQuest version 3.0 (Thermo Finnigan, St. Josè, CA, USA).

The Van Deemter's plot and A–C-terms were calculated by using the Curve Expert 1.38 from Microsoft Corporation [40].

2.3. Column preparation

For nano-LC and CEC experiments, fused silica capillaries polyimide coated (100 μm I.D. × 375 μm O.D.), from Composite Metal Service (Hallow, Worcestershine, UK) were packed with Cogent Bidentate C18™. The stationary phase was retained into the capillary column by inlet and outlet frits prepared through *in situ* photo radical polymerization.

In this respect the capillary (40 cm long) was pre-treated; the inner wall was chemically modified with TMSPM. Then it was flushed with distilled water for 10 min, 0.1 M HCl for 10 min, 1 M NaOH for 30 min and with distilled water for 10 min. Final flushing was done with acetone for 10 min and dried by vacuum pump. The capillary was filled with the silanization mixture (TMSPM 50% (v/v) in acetone) and both ends were sealed with rubber septum. Afterwards it was left in a HPLC oven for 20 h at 60 °C [32] and at the end washed with acetone, distilled water and dried by vacuum pump.

A typical volume of the polymerization mixture prepared was 400 μL. It consisted of 60 μL EDMA, 60 μL GMA, 1.26–1.41 mg (>1% monomer) MK, 95 μL cyclohexanol and 185 μL of 1-decanol [32]. After sonication (time required for MK solubilization) and purging with nitrogen, the capillary was filled with this mixture for inlet frit preparation. Both ends were sealed with rubber septum.

Prior to photopolimerization, on one side 2 mm of polyimide layer was removed with the razor.

The capillary was placed under the LED array at distance of 5 mm. The exposure time was 15 min at 10 rpm rotation.

The rotation was ensured by using a small electric motor hold in a horizontal position during the polymerization.

This set ensured that all part of the exposed capillary could receive an equal radiation from the light source. The final result was a more homogeneous frit.

As a precaution, the polymerization mixture was stored at −20 °C and kept in the dark resulting stable for 2 weeks.

The polymerization was carried out in the dark in order to avoid any interference of ambient light with the LED initiated polymerization.

After polymerization the residual of monomer and solvent were flushed out with methanol using a 50 μL syringe (Hamilton, Reno, NV, USA). The frit was treated with a solution (80:20, v/v ACN/1 M sulphuric acid), kept at 50 °C for 12 h for the hydrolysis of the epoxy group of the monomer [41]. The open end of the capillary was connected to a HPLC pump, flushed with water for 5 min and then packed with the stationary phase following the procedure previously described [38,42].

Particles were introduced into the capillary for 25 cm and then washed with acetone for 20 min. The stationary phase was dried with nitrogen stream at 5–10 bar for 30 min.

On the opposite side of inlet frit, at the end of the stationary phase, the polyimide layer was removed (about 2 mm). The polymerization mixture was introduced into the empty part of the capillary up to wet the packed material (approximately 5 mm) and was slightly pressurized (1–1.5 bar).

The polymerization was carried out using the same experimental conditions previously reported for inlet frit. The non-reacted compounds and solvents were removed flushing with methanol (20 min).

The outlet frit was also treated with the acidic mixture solution for the epoxy hydrolysis. The solution was introduced into the capillary with the microsyringe making sure that the frit and only a small portion (about 5 mm) of the stationary phase were wet. Conditions for hydrolysis were the same used for the first frit. Afterwards the capillary was connected to the HPLC pump from the inlet side and flushed with water (5 min) in order to eliminate the acidic solution.

The detector window was prepared by removing about 5 mm of polyimide layer with a razor immediately after the outlet frit.

The packed columns were employed for all experiments firstly in nano-LC and thereafter in CEC.

2.4. CEC conditioning

The capillary column was flushed with mobile phase for about 1.5 h at 100 bar by using the LC-pump. After that, the capillary was set in the CE instrument and conditioned increasing the voltage from 5 to 20 kV in 2 h.

Day to day the capillary column was conditioned by increasing the applied voltage (from 5 to 20 kV) until the current and baseline signal were stable (about 1 h).

Any instability/falling of current and bubbles formation were rapidly removed by applying both inlet pressure at 1.0–1.1 bar and voltage.

Although this modality was not standard procedure for Agilent instrument, however the conditioning step was achieved adjusting the external nitrogen pressure.

At the end of working day, the column ends were immersed in an aqueous-organic mixture (1/99 H_2O/MeOH, 5 mM NH_4Ac pH 4).

3. Results and discussion

To prepare capillary columns packed with silica-based stationary phase particles trapped with monolithic frits is necessary, in addition to the problems related to the packing procedure, to also take in mind those related to the frits preparation. In fact, the preparation of a polymer network is influenced by several parameters, e.g., those related to the permeability, mechanical resistance and robustness. In addition, in order to use UV-LED photo-polymerization, attention must be paid to the selection of appropriate mixture components (e.g., monomer) that must be transparent at the UV light used.

Based on data reported in the literature [32] GMA/EDMA (1:1, v/v) was mixed with a porogenic solvent cyclohexanol/1-decanol mixture (2:1, v/v) (monomer/solvent ratio 30/70, v/v) and the polymerization induced by MK with the LED at 370 nm and used for the preparation of monolithic frits.

The inlet and outlet frits were prepared *in situ* after removing about 4–5 mm capillary polyamide layer. The capillary was rotated during the polymerization keeping the temperature around the capillary at about 37 °C. The success of the polymerization was verified by controlling the capillary with the microscope. No homogeneous polymer was obtained without rotating the capillary, while the use of lower temperatures did not allow frits preparation. Concerning the time exposure experiments were carried out at different time exposures in the range 10–20 min. After the preparation of the first frit, capillary was flushed from the empty end with methanol using a 25 μL syringe. Although at the shortest and longest time frits were obtained, their quality was not satisfactory because either it was removed or exhibited low permeability, respectively. Optimum experimental conditions (exposure time) offering good permeability and robustness were found at 15 min. This evidence confirmed that the monolithic porous frit was bonded to the inner surface of the silica capillary wall.

Just to avoid the drawbacks related to the presence of epoxy groups on the stationary phase, e.g. (i) their opening during the use of the column with possibility to generate charged groups influencing EOF and (ii) increasing hydrophilicity even if only in the short portion of the column [11], an hydrolysis step was applied only to the monolithic frits.

Following the procedure reported in the literature [41] and described in Section 2 of this paper, no problems were expected in the treatment of the inlet frit, while attention was paid to reaction at the outlet frit. This approach could be critical because the presence of silica stationary phase. Therefore the acidic solution was introduced in order to completely wet the frit, while only 5 mm of the packed material was wet. To verify the performance of the column prepared employing the described acidic treatment, a standard mixture of akylbenzene compounds was analyzed in the column with or without acidic reaction. The two chromatograms did not show any changes in resolution, efficiency and retention times (reproducibility, RSD 2–4%) (data not shown).

3.1. Column performance

Column performance was studied analyzing an alkylbenzenes mixture (benzene, toluene, ethylbenzene, propylbenzene, butylbenzene) diluted 50% (v/v) and 90% (v/v) of acetonitrile by nano-LC and CEC, respectively. The van Deemter plot was verified in nano-LC changing the linear velocity in the range 0.4–11.0 μm/ms (100–1000 nL/min) with the highest backpressure at 400 bar. CEC experiments without assisted pressure were carried out at different voltages in the range 3–30 kV with the highest current of 2.6 μA.

The plot of current vs. applied voltage and mobile phase flow vs. backpressure, exhibited good linearity with correlation coefficients of 0.9981 and 0.9961 for the nano-LC and CEC, respectively. These results highlighted a stable chromatographic behaviour in a wide range of pressure and current.

Fig. 1 shows the results obtained plotting the linear velocity vs. the heights of theoretical plates for one of analyzed compounds (ethylbenzene) by nano-LC and CEC. As can be observed, the minimum plate heights (10–12 μm and 8.8–10.1 μm) were observed at linear velocity of 1.1 and 0.9 μm/ms in nano-LC and CEC, respectively.

The plug-like flow profile of the electroosmotic flow together with the low contribution of longitudinal diffusion were the main parameters responsible for the higher efficiency of CEC. In addition, at linear velocities higher than 1.5 μm/ms a flatter curve was

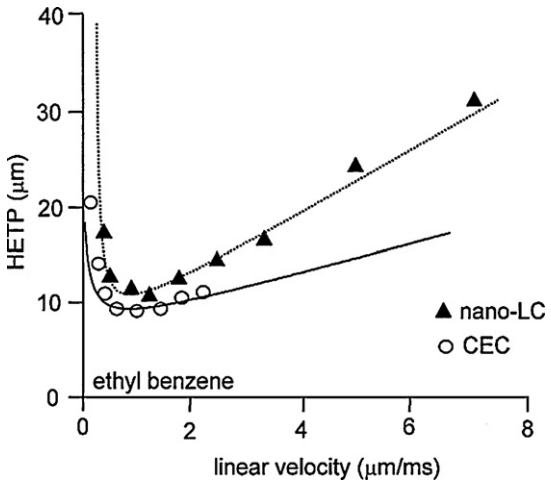

Fig. 1. van Deemter plot in nano-LC and CEC. Capillary column: 100 μm I.D. × 375 μm O.D., packed with Cogent Bidentate C18™ (Lpack = 25.0 cm, Leff = 26.2 cm), monolithic frits (EDMA/GMA, 1/1, v/v). Experimental conditions: nano-LC conditions—mobile phase, 90/10 (v/v) ACN/H_2O; flow rate, 100–1000 nL/min; injection volume, 30 nL. CEC conditions—mobile phase, 90/10 (v/v) ACN/H_2O, 5 mM NH_4Ac pH 4; applied voltage, 3–30 kV, no pressure assistance; injection: 5 kV × 5 s, sample, 0.01% (v/v) ethylbenzene. For frits preparation, see text.

obtained for CEC. Calculated C terms were 2.92–4.21 ms for nano-LC, while 1.68–2.19 ms were obtained for CEC. This can be explained considering the better mass transfer in CEC [43,19]. Employing the minimum values of the linear velocity the standard mixture was separated in less than 12 min (see Fig. 2) with efficiencies of 78,000–93,000 and 99,000–113,000 plates/m for nano-LC and CEC, respectively.

In order to verify the stability of the CEC system (absence of bubbles formation), the mobile phase composition was modified increasing the buffer concentration from 5 to 15 mM. As a result, the EOF mobility was slightly decreased and the current was raised, e.g., 4.2 μA at 20 kV. Also in this case plotting current vs. buffer concentration a satisfactory linear correlation (R^2 = 0.9844) and no bubbles formation were observed.

Changing the mobile phase composition by increasing the water concentration in the range 10–40% (v/v), stable currents were recorded in the range 10–30% (1.8–2.0 μA at 20 kV).

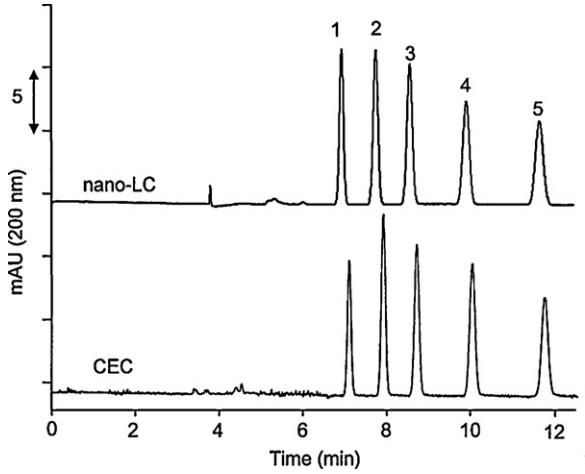

Fig. 2. Comparison of CEC and nano-LC separation of alkyl benzenes mixture. Experimental conditions: applied voltage: 15 kV (CEC), flow rate: 280 nL/min (nano-LC). Sample: 0.01% (v/v) (1) benzene, (2) toluene, (3) ethylbenzene, (4) propylbenzene, and (5) butylbenzene. For other experimental conditions see Fig. 1.

Table 1
Within-day and day-to-day precision (expressed as RSD, %) by using the packed column with monolithic frits.

	Intra-day precision (RSD%, $n = 6$)				Day-to-day precision (RSD%, $n = 15$, day = 4)			
	Retention time		Retention factor		Retention time		Retention factor	
	Nano-LC	CEC	Nano-LC	CEC	Nano-LC	CEC	Nano-LC	CEC
T_o	0.8	0.4	–	–	1.9	1.5	–	–
Benzene	1.0	0.4	0.7	1.8	1.3	0.8	3.1	3.8
Toluene	1.1	0.2	0.8	1.3	1.7	0.7	2.7	4.1
Ethylbenzene	1.1	0.2	0.8	1.1	1.6	0.7	2.6	3.3
Propylbenzene	1.2	0.2	0.8	0.9	1.5	0.8	2.5	2.4
Butylbenzene	1.2	0.2	0.9	0.7	1.5	0.8	1.7	2.5

For experimental conditions, see Figs. 1 and 2. No pressure was applied during CEC runs.

Although with some limitations related to the buffer concentration and water content in the mobile phase, CEC was carried out without pressure assistance. It is worth mentioning that this is particularly useful when electrochromatography must be carried out utilizing those instrumentations that do not have the possibility to apply pressure to both electrode compartments also including the coupling with mass spectrometry.

The packed column with monolithic frits, was tested in order to study repeatability and reproducibility performing the same experiments by CEC (without pressure assistance) and nano-LC. Therefore the standard alkyl benzene mixture was analyzed at linear velocity corresponding to the best chromatographic performance (at the minimum plate height).

Table 1 shows the relative standard deviation (RSD, %) of the retention time and factorcalculated for six ($n = 6$) consecutive analysis for four days ($d = 4$) evaluating the within-day and between day repeatability.

As can be observed good intra-day repeatability of retention time and retention factor was obtained using both CEC and nano-LC (<1% and about 1%, respectively). Concerning day-to-day precision data RSD of retention time, better results were achieved in CEC, while the opposite was observed measuring retention factors.

Excellent peak shape was observed for both CEC and nano-LC experiments: symmetry factors 0.95–0.98 and 1.01–1.03, respectively (results not shown).

Experiments carried out with the same capillary column after 40 and 100 analyses by nano-LC and CEC showed almost the same results concerning retention time/factor, resolution, efficiency and peak symmetry. As an example of the good column performance,

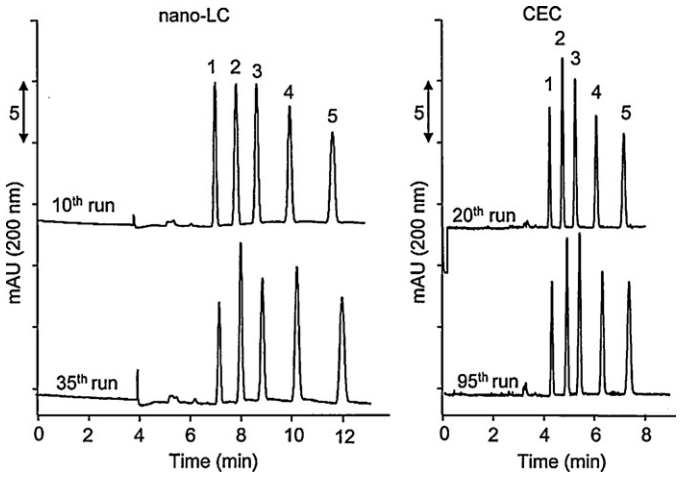

Fig. 3. Comparison of the column performance analyzing a standard mixture by nano-LC after 10 and 35 runs and by CEC after 20 and 95 runs. For experimental conditions see Fig. 2 with the exception of applied voltage in CEC that was 20 kV.

Table 2
Column to column reproducibility (expressed as RSD, %) by using the packed column with monolithic frits.

	Retention time		Retention factor	
	Nano-LC	CEC	Nano-LC	CEC
T_o	2.1	3.8	–	–
Benzene	2.1	2.7	3.3	6.0
Toluene	1.4	2.6	3.1	6.1
Ethylbenzene	1.5	2.9	2.9	6.2
Propylbenzene	1.5	3.1	2.7	4.2
Butylbenzene	1.7	3.3	2.6	4.0

RSD (%), $n = 6$, column = 3. For experimental conditions, see Figs. 1 and 2. No pressure was applied during CEC runs.

Fig. 3 shows the separation of standard compounds at the 10th/35th nano-LC run and 20th/95th CEC run, respectively.

Three columns were prepared following the same experimental conditions and tested in order to verify the reproducibility. The obtained results are reported in Table 2. As can be observed the data are quite satisfactory with an RSD of the retention time <4%, while retention factors were lower than 7% and 3.5% for CEC and nano-LC, respectively.

The better results observed for nano-LC can be due to a slight different frits permeability, that is negligible in nano-LC. Clearly, as a solution to this problem, a better control of the polymerization conditions might improve this aspect.

The results were compared with a column with sintered frits (data not shown). No significant differences were observed in term of efficiency and retention times/factors.

Despite this, columns prepared with monolithic frits are preferable, especially for CEC experiments, as they allow to perform experiments without the need for pressurization of the electrode compartments.

3.2. Capillary liquid chromatography (CLC) at low pressure and fast analysis

In order to verify the possibility to use the commercial instrumentation for capillary liquid chromatography (CLC) experiments, a capillary column was packed for only 8.4 cm (including monolithic frits) and the alkylbenzenes mixture analyzed pumping the column at 8 bar with the same mobile phase used for nano-LC experiments. The base-line separation of the five analytes was achieved in less than 10 min. The results were compared with those achieved in nano-LC utilizing the same column but at a flow rate of 90 nL/min in order to separate analytes in about the same time (<10 min). The two chromatographic systems gave quite similar results concerning resolution and peak efficiency (in the range 60,000–68,000 plates/m).

Analytes mixture was also separated by CEC utilizing the short packed capillary applying 20 kV achieving base-line resolution in less than 2 min. To achieve the separation of the same compounds

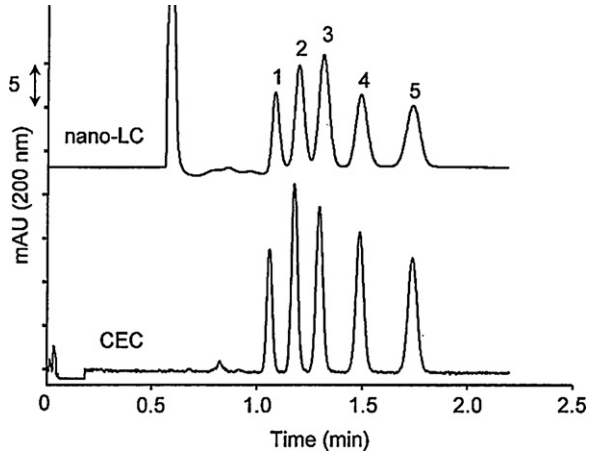

Fig. 4. Comparison of nano-LC and CEC separation of alkyl benzenes mixture. Experimental conditions: capillary column, 100 μm I.D. × 375 μm O.D., packed with Cogent Bidentate C18™ (Lpack = 7.0 cm, Leff = 8.4 cm), monolithic frits (EDMA/GMA, 1/1, v/v). CEC, injection −5 kV × 5 s; applied voltage, −20 kV, no pressure assistance; nano-LC, injected volume, 60 nL; flow rate, 530 nL/min. For the other conditions see Fig. 1.

in the same time by nano-LC, the used flow rate was 530 nL/min (see Fig. 4). These data demonstrate that CEC and nano-LC can offer the possibility to achieve fast alkylbenzenes separation with good resolution, however better results concerning efficiency were obtained employing the electrodriven techniques.

3.3. Nano-LC and CEC separation of acidic compounds of pharmaceutical interest

The chromatographic performance of the packed column (25.0 cm) with the monolithic frits was also tested for the separation of different analytes mixture: eleven acidic compounds including some non-steroidal anti-inflammatory drugs (NSAIDs). The separation was optimized selecting appropriate conditions to achieve the highest resolution in both nano-LC or CEC.

Fig. 5 shows the chromatogram and electrochromatogram obtained employing nano-LC and CEC, respectively. As can

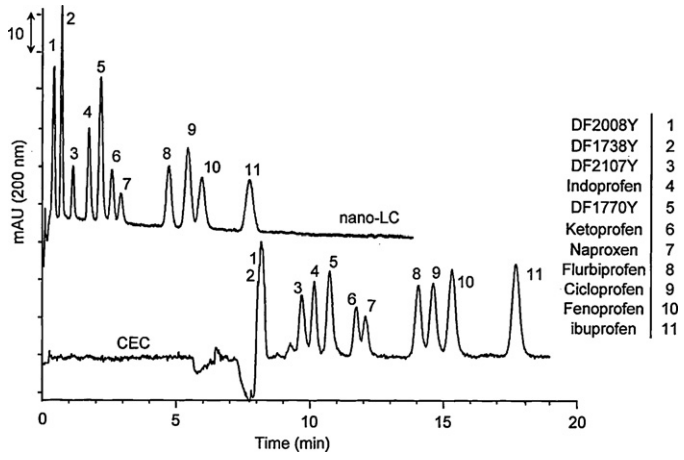

Fig. 5. Nano-LC and CEC separation of compounds of pharmaceutical interest. Experimental conditions: capillary column: 100 μm I.D. × 375 μm O.D., packed with Cogent Bidentate C18™ (Lpack = 25.0 cm, Leff = 26.2 cm), monolithic frits (EDMA/GMA, 1/1, v/v); nano-LC—mobile phase, 25/35 (v/v) MeOH/ACN, 5 mM NH_4Fo pH 2.5; flow rate, 800 nL/min; injected volume, 60 nL. CEC—mobile phase, 70% (v/v) ACN, 20 mM NH_4Fo pH 2.5; applied voltage, 25 kV; no assisted pressure; injection, 10 kV × 20 s. Pharmaceutical compounds sample—nano-LC, 2.5–20 μg/mL in 50% (v/v) MeOH/water; CEC, 20 μg/mL in 60/40 (v/v) ACN/H_2O.

be observed, working at the optimum conditions, complete separation of the eleven acids was obtained utilizing nano-LC, while in CEC analytes #1 and 2 were not resolved. In addition with the electrodriven technique longer analysis time were necessary to achieve comparable resolution. These results clearly show that, at least in the case of the acidic drugs' separation, nano-LC offers better performances in terms of resolution and speed than CEC. This can be explained considering that in nano-LC, for improving the selectivity of the separation, mobile phase composition can be more easily modified than in CEC. In fact in CEC acetonitrile/water (ammonium formate) mixture was selected in order to have enough EOF responsible for the modulation of the flow rate. Even if MeOH could increase the selectivity in CEC separations, in these experiments was not used because its presence would decrease the EOF increasing analysis time.

Although the column was stressed applying 800 nL/min, back-pressure 360 bar and 25 kV, 4 μA in nano-LC and CEC, respectively, the column demonstrated a good robustness.

4. Conclusions

In this work a fused silica capillary column containing C18 stationary phase particles entrapped between two monolithic frits, was studied for the separation of selected standard mixtures of alkylbenzene and acidic compounds of pharmaceutical interest by nano-LC and CEC.

The frits were prepared in about 15 min by photo polymerization of a GMA/EDMA mixture by using the UV-LED as light source controlled by a laboratory made power supply.

The novel capillary was used in CEC without assisted pressure; performing over 100 analyses; no bubbles formation was observed.

Stressing the column by applying relatively high current and high back-pressure, also changing mobile phase composition, good stability and robustness was observed.

From the discussed data it can be concluded that the new column can be easily used also with instrumentation that does not have the possibility to pressurize electrode compartment. Further study is planned in order to couple CEC with mass spectrometry where bubbles formation must be avoided.

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