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Preparation of stationary phases for open-tubular capillary electrochromatography using the sol–gel method

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Abstract

Capillary electrochromatography requires the deposition of a stationary phase inside the capillary. In this paper the sol–gel method is proposed for this purpose. The gels were prepared externally and injected into a fused-silica capillary, where anchorage to the capillary wall was possible through condensation reactions between the silanol groups of the capillary wall and the residual silanol groups the gel. Contrary to a commonly used practice, alkaline pretreatment of the inner capillary wall prior to the introduction and anchoring of the gel was found to be only marginally effective in improving the mechanical stability of the column. The influence of various parameters, such as the pH, the water content, the presence of alcohol (ethanol) on the formation of tetraethoxysilane (TEOS)–n-octyltriethoxysilane (C_8-TEOS) hybrid gels of varied composition is discussed. The pH and the amount of water present were found to be the determining factors in the preparation of a stable gel with the desired mechanical and chromatographic properties. By carrying out the gel formation at 80°C, capillary columns could be produced in 2.5 h. While an acidic pH was required during (external) gel formation, subsequent treatment of the gel inside the capillary with an alkaline solution ('aging') was found to improve separation and stationary phase capacity significantly. The capillary columns were subsequently used to separate a mixture of polycyclic aromatic hydrocarbons in less than 3 min.

Keywords: Electrochromatography; Stationary phases, CEC; Sol–gel method; Polynuclear aromatic hydrocarbons

1. Introduction

Capillary electrochromatography (CEC) has been called the most promising analytical technique for the life sciences. Combining the extraordinary efficiency of capillary electrophoresis with the superior separation characteristics of chromatography, CEC should indeed be capable of separating even the most complex samples. However, the question of how to reproducibly install a mechanically stable stationary phase inside a capillary remains one of the most challenging ones in this area. Several possibilities are discussed in the pertinent literature. The vast majority of CEC separations to date have been carried out with packed bed-type columns. Such columns have shown excellent results. However, they also are known to have some principle drawbacks, such as the necessity for frits, the challenge of packing such beds in general, the fragility of the columns and some difficulties in using such columns for hyphenated techniques such as CEC–MS. Continuous bed-type columns, such as open tubular and porous polymer rod ones (monoliths), have been suggested as alternatives. However, much further work is necessary before such stationary phases can challenge the chromatographic performance of the above-mentioned packed beds.

In this paper the open-tubular approach to capillary electrochromatography (OT-CEC) is investi-

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gated. OT-CEC has the advantage that pressure injection of the sample and rapid wash and column regeneration cycles by pressure driven 'flushes' are possible. The major drawback of this approach is the inherently low stationary phase capacity of such columns [1]. Several methods have been proposed to increase the surface and hence the stationary phase capacity of OT stationary phases, e.g., through etching of the inner capillary wall with ammonium hydrogen difluoride [2] or by cladding it with a thin coat of porous silica [3]. In both cases this initial step has to be followed by a second one, in which the actual retentive layer is fixed to the enlarged surface. An alternative approach consists in the so-called sol–gel technique. In theory, this approach produces a thin coating of a porous (silica hybrid) gel on the capillary wall, which already contains the interactive functions. Consequently, the two goals, i.e. increasing the surface and establishing a retentive phase in the capillary, are achieved simultaneously [4–9].

In the sol–gel approach metalorganic monomers (often alkoxysilanes) are polymerized. At the functional group level, three reactions can be used to describe the process. The first reaction in Fig. 1 corresponds to a hydrolysis of the liquid precursor (monomer), commonly an alkoxysilane. Adding catalytic amounts of acid (such as hydrochloric acid) or base (such as ammonium or sodium hydroxide) can accelerate this reaction. During the reaction alkoxy groups are replaced by hydroxyl ones (formation of silanol groups, SiOH) and some alcohol is released. The silanol groups are highly reactive and condense readily with other alkoxysilanes (reaction 2) or with each other (reaction 3). As a result, a siloxane bond (Si–O–Si) is created and one molecule of alcohol (respectively of water) is released. Through subsequent hydrolysis and condensation reactions first a colloidal solution (sol) and finally after a certain time, t_g, an extended three-dimensional network (gel) is formed [10]. This gel is nor longer fluid, but shows elasticity when under mechanical stress.

Water is critical for gel formation. At the beginning water is required for hydrolysis, later on, water is also produced as a byproduct of the condensation reaction. Depending on the relative reaction rates, water can either be limited and thereby preventing further hydrolysis of the alkoxysilanes, or present in surplus and thereby handicapping the condensation reaction between silanol groups. Both the amount of water initially added and the chosen catalyst are of consequence, the latter because hydrolysis and the condensation reaction are influenced to a differing degree by the catalyst. Under acidic conditions, for example, hydrolysis is known to be faster than condensation and the amount of water needed is increased [11]. If one assumes the hydrolysis/condensation reaction (gel formation) to proceed to its

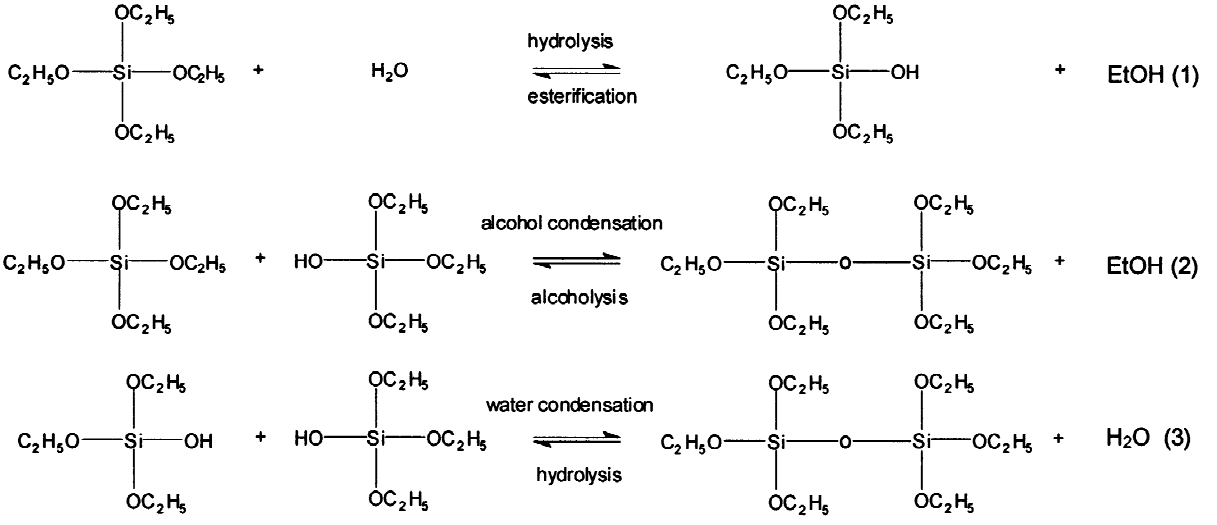

Fig. 1. Reactions involved in forming a three-dimensional polymer network by the sol–gel technique.

theoretical limit, the addition of an amount of water corresponding to $2m + 3/2n$ moles would be required with m corresponding to the number of moles TEOS and n corresponding to the number of moles of C_8-TEOS. However, it has been noted that even the presence of an excess of water does not necessarily drive the reaction to completion [11]. This may be due to the differences between the hydrolysis and condensation rates. Water can also be trapped in the pores of the forming silica network and thus be prevented from reacting.

In this paper a variant of the sol–gel techniques is suggested for the fast production of a reversed-phase type of OT capillary column. Two silanes are copolymerized for that purpose (formation of a hybrid gel). The first monomer is a conventional tetraethoxysilane, the second monomer bears an organic ligand (C_8 group) in addition to three ethoxy groups. The C_8 moities are operative in analyte retention.

2. Experimental

2.1. Instrumentation

All electrochromatography experiments were carried out on a Beckman P/ACE 5500 (Beckman, Fullerton, CA, USA) system using a diode array detection system. Data were processed using the P/ACE station software. For filling the gel into the capillaries a laboratory-made apparatus was used. The system was linked to a pressurized Argon tank (5 bar). The microscope used was an Axiovert 100 (Carl Zeiss, Oberkochen, Germany). Capillaries were dried in a vacuum oven, Heraeus vacutherm model VT 6025 (Heraeus, Hanau, Germany). The oven was connected with a vacuum pump, model MD4C, equipped with a pressure digital controller CVC2, all from Vacuubrand (Wertheim, Germany).

2.2. Material and chemicals

Tetraethoxysilane (TEOS), n-octyltriethoxysilane (C_8-TEOS), acetone (HPLC grade), hexane, polycyclic aromatic hydrocarbons (PAHs) (naphthalene, phenanthrene, pyrene), benzophenone, hydrochloric acid, sodium hydroxide and ammonium hydroxide were from Fluka (Buchs, Switzerland). Before use hexane was dried over metallic sodium using benzophenone as a wetness indicator, then distilled and kept under argon. Acetonitrile (HPLC grade) was purchased from Biosolve (Valkenswaard, The Netherlands). Water was purified using an Elix-3 system (Millipore, Bedford, MA, USA). Fused-silica capillaries were from Bio-Rad (Hercules, CA, USA).

2.3. Capillary pretreatment (optional)

New silica capillaries were flushed with sodium hydroxide (1 *M*, 1 h), followed by HCl (0.1 *M*, 10 min), pure water (10 min), dry argon (10 min), dry hexane (10 min), and finally dry argon (15 min). Afterwards capillaries were dried overnight in vacuum (20 mbar) at 35°C. If capillaries had to be stored for any time prior to use, both ends were plugged with silicon septa.

2.4. Preparation of the gel

Unless mentioned otherwise, the following procedure was used to prepare the gel: 0.5 ml of TEOS was mixed with 0.282 ml C_8-TEOS and 0.2 ml ethanol in an Eppendorf vial. 0.093 ml of water and 0.011 ml of 0.1 *M* HCl were added. The mixture was vortexed for 6 h at 1000 rpm to allow gel formation. The accelerated procedure allowing column production in less than 3 h was as follows: 1.0 ml of TEOS was mixed with 0.488 ml of ethanol, 0.188 ml of water and 0.022 ml of HCl (0.1 *M*) in an Eppendorf vial. The mixture was vortexed for 10 min at 80°C and 800 rpm. Afterwards 0.564 ml C_8-TEOS were added and the mixture was allowed to react for 1 h at 80°C, prior to injection into the capillary.

2.5. Preparation of the gel-filled capillary

The gel was injected into the capillary by pressure using a laboratory-made apparatus (1 min, 5 bar). After 10 min, the capillaries were flushed again with argon (5 min, 5 bar). Capillaries were then completely (overnight, 35°C, 20 mbar) or incompletely dried (2 h, 35°C, 20 mbar) prior to aging (see below). Capillaries prepared by the accelerated protocol were only dried for 30 min, but at 50°C (20 mbar).

2.6. Aging of the gel

For aging, incompletely dried, gel-coated capillaries were flushed (3 bar) for 5 min with 10^{-2} *M* NaOH. The basic solution was allowed to rest within the capillary for an additional 5 min, then the solution was flushed out by applying a pressure of 3 bar for 5 min. Afterwards capillaries were dried overnight as described before (35°C, 20 mbar).

2.7. Electrochromatography

Samples were injected by pressure (1 s, 1.36 bar). For separation a voltage of 15 kV and a detection wavelength of 208 nm were routinely used. Capillary dimensions were 47 cm (40 cm from inlet to the detection window)×50 μm I.D. Acetonitrile–water (1:1, v/v) was used as mobile phase. A mixture of three PAHs, concentration of each 10^{-2} *M*, was used as sample. The sample solvent was acetonitrile–acetone (7:3, v/v). Acetone was used as electoosmotic flow marker.

Before the first run, capillary columns were conditions as follows: a wash with water (5 min) followed by mobile phase (5 min). The capillary was allowed to rest filled with mobile phase for an additional 5 min and then dried in a stream of dry argon (2 min). Then the capillary was dried in the vacuum oven for 2 h (20 mbar) at 35°C. Afterwards, it was washed again with the mobile phase for 4 min and allowed to rest for a further 5 min. A blank run was performed to ensure readiness. Between runs, capillaries were only washed with mobile phase (2 min).

3. Results and discussion

The goal of this work was the quick and reproducible preparation of robust OT stationary phases for RP-CEC. In this context the maximization of the stationary phase capacity is a must. Three methods are commonly suggested in the pertinent literature to achieve this goal. In our hands the sol–gel principle first suggested for this purpose by Guo and Colòn [4] worked best. However, instead of being able to use the established approach, we found it necessary to design the chemistry for stationary phase preparation from the very beginning. The two other methods often suggested for increasing the stationary phase capacity in OT-CEC, namely etching and use of porous silica supports have also been investigated in our laboratory and were found to suffer from two disadvantages. Even after optimization, they did not increase the stationary phase capacity significantly and, since a retentive layer had to be bonded to the support in a subsequent step, their preparation was more time consuming.

3.1. Preparation of the gel

As pointed out above, the preparation of a hybrid silica gel is a delicate procedure, which involves several chemical equilibria. Hence, many factors can affect the outcome of the reaction, amongst them are: amount and type of the monomers, amount of water present, acidic versus basic catalysis (pH of the reaction mixture), the presence of certain additives (for example ethanol), the temperature, and to some extent even the pressure adjusted during the reaction. Depending on these parameters, the resulting polymer may differ in terms of appearance, pore size, and chromatographic ability.

For the application intended here, the most important parameters were found to be the water concentration available during gelation and the type of catalyst used. Both were therefore investigated in detail. The following aspects were used as criteria for quality: (1) appearance of the stationary phase under the optical and scanning electron microscope (homogeneity of the stationary phase, attachment to the capillary wall), (2) mechanical stability of the column (application of pressures up to 5 bar during flushing with mobile phase, occurrence of breakage, plugging of the column), (3) chromatographic performance (separation of a standard mixture of three PAHs — naphthalene, phenanthrene, pyrene) in terms of retention times, resolution, plate height and reproducibility of these parameters.

The amount of water required for hydrolysis/condensation of *m* alkoxysilane and *n* organoalkoxysilane groups corresponds to $2m + 3/2n$. When in our experiments the amount of water was varied between 50 and 200% of this amount, the best results in terms of bed stability and chromatographic performance where observed when working close to the

value of $2m + 3/2n$. Higher and lower water concentrations led to a deterioration of the separation efficiency and resolution under chromatographic conditions. In particular the introduction of higher amounts of water resulted in gels which were less suited to the aging process described below, since for this process low-viscosity gels are preferred for mechanical reasons. Concomitantly, large amounts of water tend to drive the hydrolysis reaction, while incomplete hydrolysis of the gel (i.e. caused by the use of low amounts of water) is required if the gel is to undergo further hydrolysis/condensation during aging.

In the case of alkoxysilanes, the gel forming reactions, hydrolysis and condensation, can be carried out under acid or base catalysis [12]. The dependence of t_g on solution pH has not been investigated in detail, however, according to Yamane et al. [13] the t_g versus pH curve runs through a maximum usually observed at about a pH of 3. When the effect of the pH on the gelation time of the hybrid system under investigation here was studied, true gelation was only observed in the case of acidic catalysis. Under basic conditions droplets or beads were formed instead.

Whatever catalyst is chosen, the gel-forming reaction starts with the formation of dimers, trimers, cyclic oligomers, and finally structures large enough to be called particles. In a secondary phase, these particles link themselves into higher structures (chains, networks), which extend throughout the liquid to form the gel [10]. With time the particles tend to develop a solid (silica) core. Under acidic conditions, the particle size reaches the nanometer range. Since silica is stable under acidic conditions, no further growth occurs. A polymeric network produced with acid catalysis is thus made of interconnected particles having linear and low-branched structure [14]. Under basic conditions, on the other hand, the formed silica is dissolved and the larger particles can grow at the expense of the smaller ones (Ostwald's ripening). No stabilization occurs and the condensation reaction can proceed. In the end beads with average diameters of 100 nm and more are found.

This phenomenon is well known and has in fact been used to predetermine the size of the silica beads produced [15]. In our case, however, the injection of such a suspension into a capillary was obviously not possible. Bead formation under basic conditions was enhanced by high amounts of water and/or catalyst. Dense particles were, for example, seen when 1.5 *M* of catalyst and up to 150% of the water required for complete hydrolysis/condensation were used. Low amounts of C_8-TEOS in the reaction mixture also led to increased bead formation. In most other cases the formation of suspended droplets was observed instead.

In the case of acid catalysis, on the other hand, conditions could be defined for which the formation of a suitable gel took place. The molar ratio between C_8-TEOS and TEOS in the reaction mixture should be below 7:3 and the water amount between 70 and 200% of the amount theoretically needed for complete hydrolysis/condensation. If a molar ratio of more than 7:3 between the two monomers was chosen, the formation of two phases, an aqueous and an organic one (the monomer mixture) was observed, rather than the formation of a gel. In fact, it was found that the formation of a pure C_8-TEOS gel was not possible with either acid or base catalysis, or by varying the amount of water. It is supposed that this phenomenon is related to some steric hindrance effects arising from the organic moieties born by the C_8-TEOS. These steric hindrances impede the condensation reaction.

3.2. Accelerated gel formation

In spite of the fact that the sol–gel method allows the direct production of a gel containing putative interactive moieties, the procedure outlined above is still lengthy. Subsequently, possibilities for speeding up the process without losing performance were investigated. A first result was that the gelation time is proportional to the amount of catalyst (HCl) used. An increase of the catalyst concentration from 10^{-4} to 10^{-3} *M* resulted in a decrease in gelation time by a factor of 4. Reducing the gelation time by this measure did not influence the chromatographic performance of the ensuing column.

Some authors suggest the use of additives to decrease gelation time or improve the solubility of the alkoxysilanes. Ethanol [4], ethyl acetate [6], formamide [9], and some organic solvents [10] have been suggested. We used ethanol as a solubility

enhancer, since it also forms an azeotrop with water. This facilitates the removal of residual water during drying at comparatively low temperatures. It must be recognized, however, that the addition of alcohol may influence both the hydrolysis and the condensation equilibria (reactions 1 and 2 in Fig. 1). Hence the ethanol amount should be high enough for accelerated gelation but still too low for significant alcoholysis. In our case 3.5 *M* (200 μl) were found optimal.

The most important reduction of the gelation time was achieved by carrying out the reaction at elevated temperature. However, at elevated temperatures (50, 100, 150°C), the standard sol–gel reaction (see Experimental) tended to produce beads of inhomogeneous size rather than a gel. A gel could be produced, however, when the reaction was carried out at 80°C, i.e. close to the boiling point of the ethanol in the mixture, when the C_8-TEOS was not present from the beginning but added after the reaction has been allowed to proceed for 10 min. Under these conditions, a suitable gel for is obtained after 1 h.

3.3. Preparation of the stationary phase for reversed-phase OT-CEC

Once prepared, the gel was injected into the capillary where it was allowed to anchor to the capillary wall by a reaction between residual silanol groups of the gel with the silanol groups of the capillary walls. Afterwards, the capillary was flushed and a certain part of the gel removed, leaving a coat of several micrometers thickness on the capillary wall, as was shown by optical microscopy, Fig. 2.

The chemical inertness of fused silica may be a drawback when this material is to be used in the preparation of chemically bonded stationary phases, since only a fairly limited amount of silanol groups ($8–9\cdot10^{-2}/nm^2$ [16]) is available for anchoring of the stationary phase. Alkaline hydrolysis, which causes a partial dissolution of the silica surface, is traditionally used to improve this. Since the base treatment is usually followed by drying at high temperature, this procedure has the additional advantage of removing some of the physically adsorbed water. However, capillary pretreatment adds another step to the preparation of the capillary column and can be quite time consuming, for example, when several washing steps and a lengthy drying period (overnight) are called for. Therefore the extent to which the pretreatment can be simplified (shortened) was investigated and whether it was really required for the preparation of OT stationary phases of the desired quality.

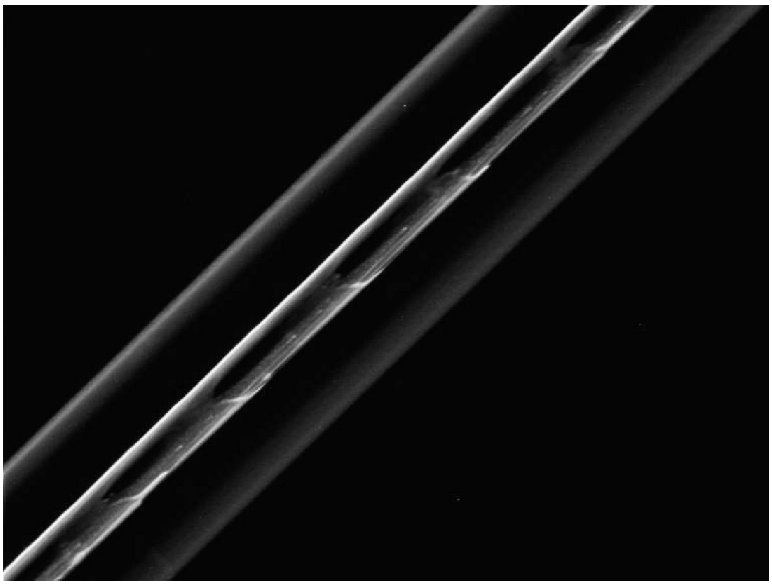

Fig. 2. OT capillary for RP-CEC, I.D. 50 μm. Picture taken with an optical microscope using an UV light source, magnification×50. Stationary phase: hybrid gel containing C_8 moities and 3 m*M* fluorescein.

In order to investigate this, silica capillaries directly filled and bonded with the gel without any pretreatment were prepared and evaluated. Even after several hundred runs and a trial period of months, these capillaries showed a similar performance and stability to either of the pretreated ones. Since it has been shown that just a few silanol groups on the silica surface are sufficient for a stable attachment of a stationary phase [17,18], it can be deduced that the silanol group density of untreated silica capillaries is high enough and pretreatment can be abandoned.

Gels prepared in this manner allowed the separation of the standard PAH mixture — data not shown. However, the chromatographic performance in terms of retention time, plate heights and resolution was far from satisfactory. From the literature it was known that a basic wash can accelerate and improve the ripening of a gel [19]. Hence an aging step was proposed to improve the chromatographic properties of the stationary phase.

A gel prepared by acid catalysis from a monomer mixture containing up to 70% TEOS and more than 50% of the amount of water required for extensive hydrolysis/condensation, exhibits a perfectly smooth surface after drying. However, when a basic wash (NaOH) is performed on such a gel while it is still wet, the result is a pronounced wrinkling of the gel's surface, Fig. 3. The surface wrinkling occurs only if the gel is not yet completely (i.e. overnight) dried, when aging takes place. Best results were observed when the basic wash was used after a brief drying period of 2 h under vacuum (20 mbar) and at 35°C. This improved the mechanical resistance of the gel towards the shear effect caused by the flow of the NaOH solution. The concentration of the NaOH should not exceed 0.01 *M*; higher concentrations of NaOH damage the stationary phase and often cause plugging of the capillary. Alternatively, 30 min of drying at 50°C (20 mbar) may be used with similar results.

The basic wash is assumed to catalyze a second round of hydrolysis/condensation, this time within the already formed gel structure. Since the gel is not yet completely dried, various groups within the gel are still sufficiently mobile for such reactions to occur. Concomitantly, it seems probable that the acid catalysis used for gel formation left a certain number of unhydrolyzed groups ethoxy groups within the network, which can now be activated by the base. The presence of some individual sol particles is also a possibility. The second round of hydrolyzation/condensation also involves groups located within the pores of the silica gel. The size of the pore is thereby reduced and the liquid originally found there is expelled (syneresis). The observed pronounced deformation of the gel network is probably the result of a differential diffusion of OH^-. The diffusion of OH^- is limited as the OH^- penetrates the gel, due to a decrease in the gel pore sizes caused by the formation of new siloxane linkages in the presence of the basic catalyst. Distortive forces are at work between the hard and shrunken surface and the inner part of the gel, which at that time is still soft. The presence of organic modifiers (ethanol) can also influence the structure of the gel, mainly by reducing the internal forces.

3.4. OT-CEC of polycyclic aromatic hydrocarbons

To date OT capillaries tend to exhibited rather long elution times (>10 min). A major goal of the work presented here was to reduce this time. Stationary phases prepared by aging the TEOS–C_8-TEOS hybrid gels, did allow the separation of the standard mixture within 3 min, however, the standard deviation of the retention times was not satisfactory (>20%) and in addition a clear drift towards longer retention times was observed — data not shown. Conditioning of the capillary prior to the first use was found to improve matters considerably.

In order to demonstrate this, a freshly prepared gel-filled capillary was cut in half. One half was used is it was, the other half was conditioned by consecutive washes with water (5 min), mobile phase (5 min flushing followed by 5 min static contact), dry argon (2 min) then dried for 2 h at 35°C and finally again a wash with mobile phase (4 min). The electrochromatograms obtained under identical conditions for the two capillaries are shown in Fig. 4. Clearly, resolution improves considerably with conditioning. In addition, elution times stay stable for conditioned capillaries, Fig. 5. Once the capillary has been prepared, it is sufficient to wash it between runs with pure mobile phase to ensure performance over several hundred runs.

When the influence of various gel parameters on the chromatographic performance was investigated, the best results in terms of resolution and repro-

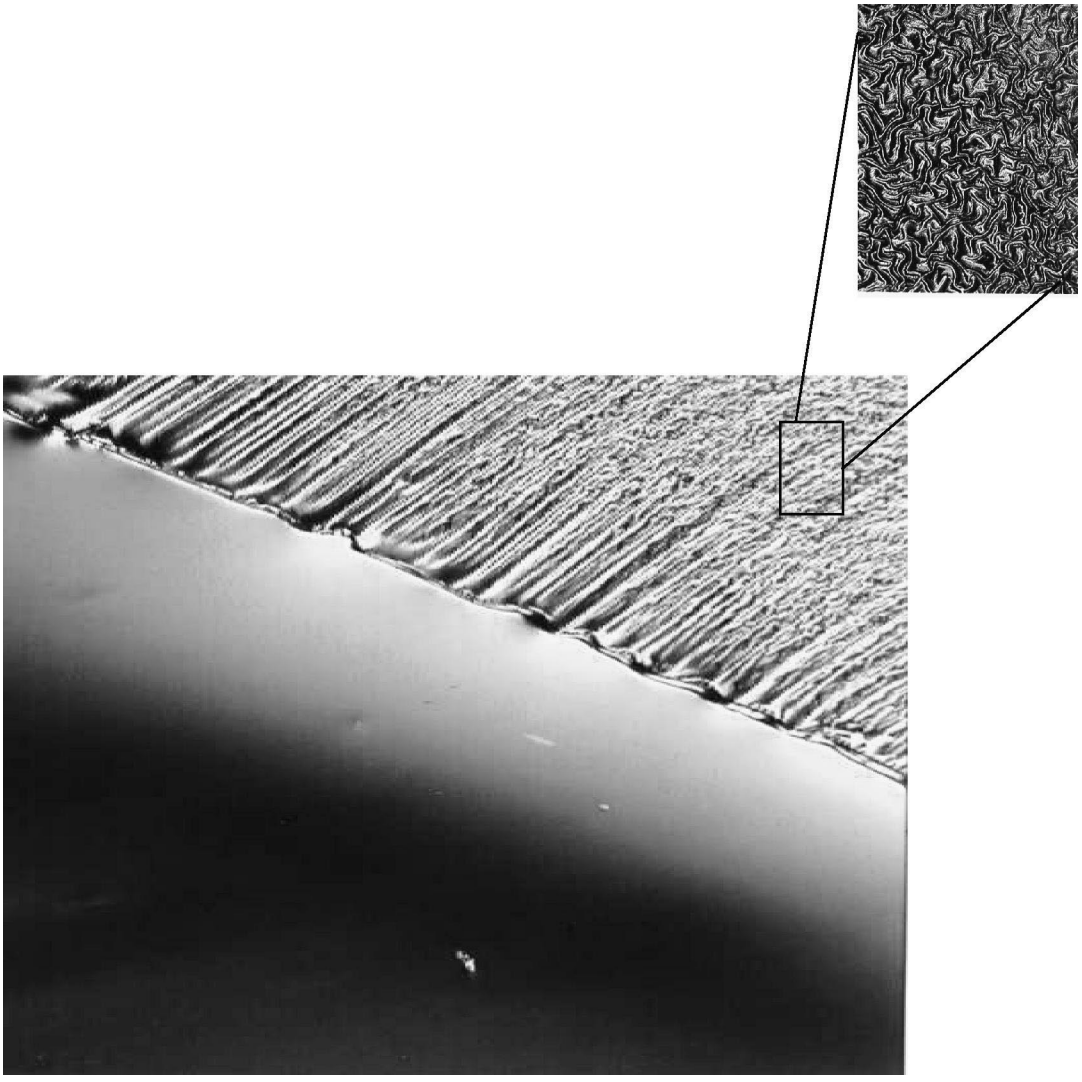

Fig. 3. Picture of a gel where only the upper right half has been treated with a basic wash (10^{-2} *M* NaOH, 5 min) (by optical microscopy, magnification×50, polarized light). Gel: C_8-TEOS–TEOS molar ratio 4:6, water $2m + 3/2n$, ethanol 200 μl, acid catalysis (11 μl, 0.1 *M* HCl). Insert: magnification×100, polarized light, of the part of the gel treated with base.

ducibility were obtained for hybrid gels made from C_8-TEOS–TEOS (40:60, v/v) Table 1. The optimal mobile phase for our purpose was acetonitrile–water (1:1, v/v). Standard deviations for the retention time tended to be below 6% both for different runs in one capillary and between capillaries prepared by the developed procedure either by the long protocol (25°C) or by the accelerated one (80°C). If anything, the accelerated protocol yielded superior capillaries with standard deviations for the retention time of $<1\%$ for a given capillary and $<5\%$ between capillaries. The addition of an internal standard can improve these reproducibilties further.

The calculation of plate numbers was more difficult. Usually plate numbers below 6000 plates/m were calculated for the retained substances and values around 30 000 for the unretained marker. However, as we reduced the absolute retention time,

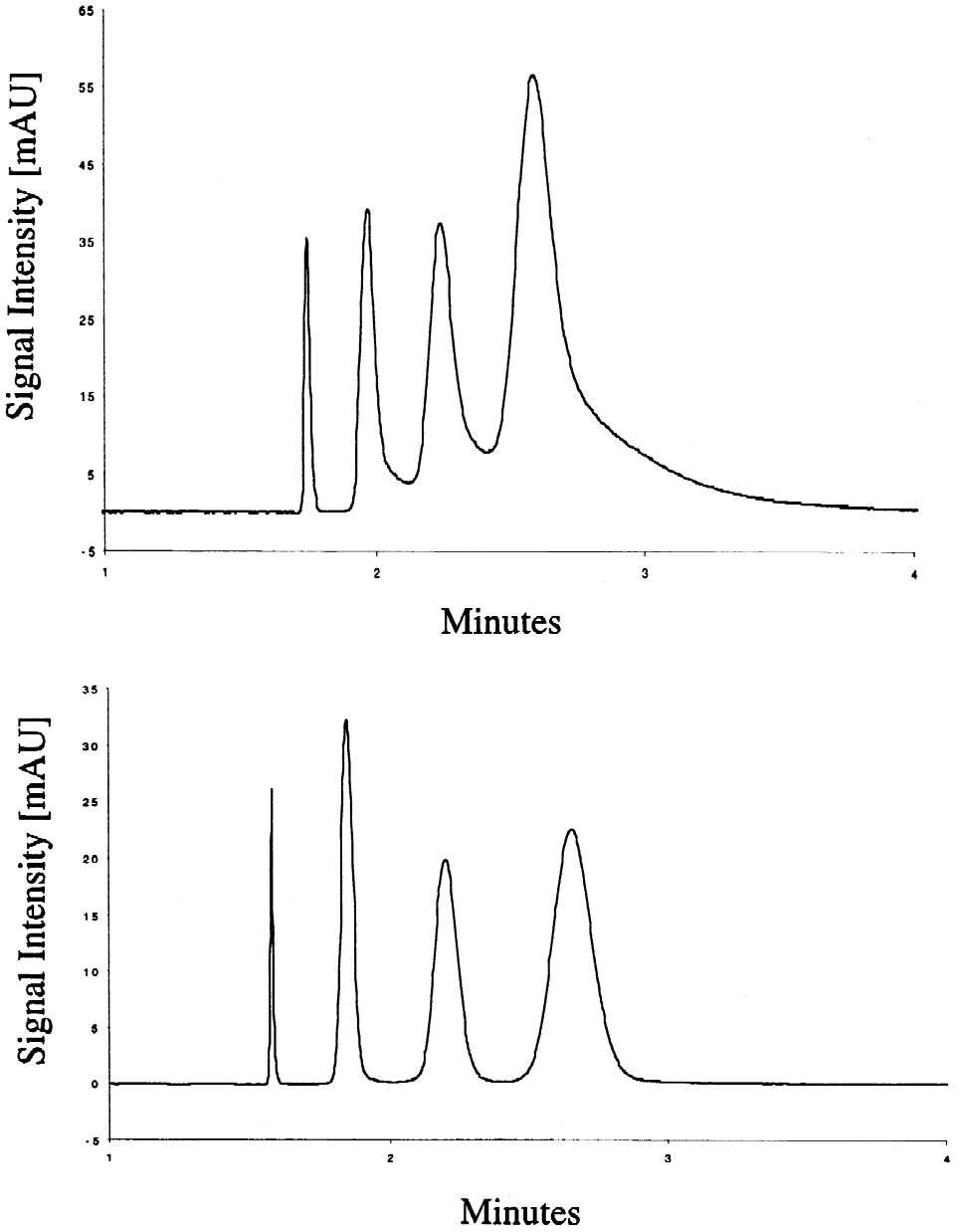

Fig. 4. Electrochromatogram of the standard PAH mixture performed on a capillary with (bottom) and without (top) conditioning. Analytes elute as follows: acetone, naphthalene, phenanthrene, pyrene. Stationary phase: reversed-phase made through the described sol–gel process (molar ratio C_8-TEOS–TEOS, 4:6, water 100% stoichiometric amount required for hydrolysis), mobile phase: ACN–H_2O (1:1, v/v); voltage: 15 kV; hydrodynamic injection: 1 s at 1.36 bar; detection wavelength: 208 nm; temperature: 25°C; capillary: 40 cm (inlet to the detection window)×50 μm I.D.; sample: mixture of naphthalene, phenanthrene, pyrene 10^{-2} *M* in ACN–acetone (7:3, v/v). Acetone serves as electroosmotic flow marker.

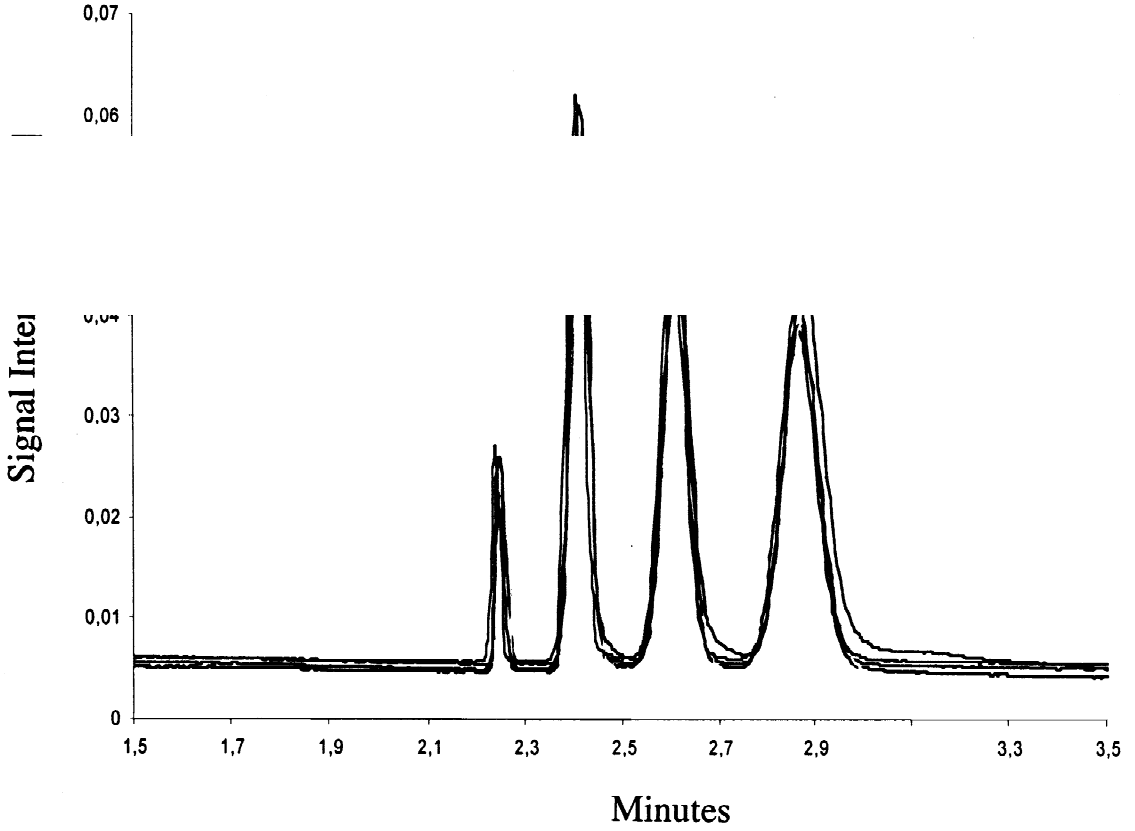

Fig. 5. Reproducibilty of the separation of the standard PAH mixture under optimized conditions using the same conditions as in Fig. 5 (conditioned capillary).

we observed that peak widths tended to stay the same. A similar phenomenon was observed for the differences between retention times. In other words, 'identical' chromatograms were recorded after increasingly shorter operating times. Concomitantly however, the plate number deteriorated due to the decrease in retention time without any real loss in resolution. The plate numbers observed here could therefore be improved considerably by using longer capillaries and hence creating longer retention times.

4. Conclusions

A fast, simple and efficient method was developed to produce reversed-phase stationary phases for OT-CEC. New approaches for the increase of the stationary phase capacity through a modification of the retentive layer surface structure were suggested. The result was a considerable improvement of the chromatographic separation of PAHs. In addition, the preparation of these OT capillaries proved to be less time consuming than the previously published method using the sol–gel technique.

Acknowledgements

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Table 1
Reproducibility of the retention times on a given capillary and between capillaries; capillaries 201, 202. 210, 211, and 212 were prepared at 80°C according to the accelerated protocol, capillaries 167, 169, 173, and 175 according to the protocol at room temperature

Capillary	Retention time[a] (min)	Capillary	Retention time[a] (min)
201	Acetone: 2.26 (2.26%)[b] Naphthalene: 2.54 (2.20%) Phenenthrene: 2.89 (2.03%) Pyrene: 3.34 (1.71%)	167	Acetone: 2.59 (3.52%) Naphthalene: 2.85 (3.93%) Phenenthrene: 3.16 (4.27%) Pyrene: 3.57 (4.55%)
202	Acetone: 2.22 (0,26%) Naphthalene: 2.49 (0.17%) Phenenthrene: 2.84 (0.25%) Pyrene: 3.29 (0.06%)	169	Acetone: 2.69 (2.98%) Naphthalene: 2.94 (3.00%) Phenenthrene: 3.24 (3.10%) Pyrene: 3.64 (3.24%)
210	Acetone: 2.11 (0.57%) Naphthalene: 2.41 (0.50%) Phenenthrene: 2.80 (0.58%) Pyrene: 3.28 (0.69%)	173	Acetone: 2.67 (5.08%) Naphthalene: 2.89 (5.31%) Phenenthrene: 3.12 (5.75%) Pyrene: 3.45 (5.99%)
211	Acetone: 2.11 (0.64%) Naphthalene: 2.42 (0.32%) Phenenthrene: 2.81 (0.08%) Pyrene: 3.30 (0.17%)	174	Acetone: 2.71 (0.65%) Naphthalene: 3.02 (0.68%) Phenenthrene: 3.41 (1.30%) Pyrene: 3.84 (0.76%)
212	Acetone: 2.10 (0.13%) Naphthalene: 2.41 (0.21%) Phenenthrene: 2.81 (0.33%) Pyrene: 3.20 (0.31%)	175	Acetone: 2.91 (2.53%) Naphthalene: 3.18 (3.33%) Phenenthrene: 3.50 (4.20%) Pyrene: 3.94 (5.31%)
[c]	Acetone: 2.19 (4.85%) Naphthalene: 2.48 (3.42%) Phenenthrene: 2.85 (1.98%) Pyrene: 3.32 (0.85%)	[c]	Acetone: 2.65 (3.20%) Naphthalene: 2.94 (4.10%) Phenenthrene: 3.29 (5.38%) Pyrene: 3.71 (5.15%)

[a] Average of six runs.
[b] Relative standard deviation.
[c] Intercapillary values calculated for all capillaries prepared by a given protocol (i.e. room temperature or 80°C).

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