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Polymer-brush stationary phases for open-tubular capillary electrochromatography

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Abstract

Synthesis of poly(2-hydroxyethyl methacrylate) (PHEMA) brushes from the inside of silica capillaries by surface-initiated atom transfer radical polymerization (ATRP) yields unique stationary phases for open-tubular capillary electrochromatography (OT-CEC). Although PHEMA brushes have only a small effect on the separation of a set of phenols and anilines, derivatization of PHEMA with ethylenediamine (*en*) allows baseline resolution of several anilines that co-elute from bare silica capillaries. Derivatization of PHEMA with octanoyl chloride (C_8-PHEMA films) affords even better resolution in the separation of a series of phenols and anilines. Increasing the thickness of C_8-PHEMA coatings by a factor of 2 enhances resolution for several solute pairs, presumably because of an increase in the effective stationary phase to mobile phase volume ratio. Thus, this work demonstrates that thick polymer brushes provide a tunable stationary phase with a much larger phase ratio than is available from monolayer wall coatings. Through appropriate choice of derivatizing reagents, these polymer brushes should allow separation of a wide range of neutral molecules as well as compounds with similar electrophoretic mobilities.

Keywords: Stationary phases, electrochromatography; Poly(2-hydroxyethyl methacrylate); Phenols; Anilines

1. Introduction

Because of its short analysis time, high efficiency, and small sample size, capillary electrophoresis (CE) is an attractive tool for rapid separation and identification of charged molecules [1]. Unfortunately, unless complexing agents or micelles are added to the mobile phase [2,3], aqueous CE is intrinsically limited to separations involving charged species with different mobilities. This is a significant limitation for analysis of small molecules such as neutral explosives [4,5] and water pollutants [6,7], as well as chiral pharmaceuticals [8,9].

Capillary electrochromatography (CEC) maintains many of the attractive features of CE while offering the potential for separation of a wider range of analytes [10,11]. In CEC, incorporation of a stationary phase into capillary columns allows for separation via selective, chromatographic interactions [10–12]. The most common form of this technique utilizes capillaries filled with functionalized silica [13–15], but formation of bubbles during column operation and challenges in frit production and packing of columns complicate the use of particulate packing materials [12,16]. One way to surmount these difficulties is to employ open-tubular CEC (OT-CEC), where the stationary phase is simply a thin coating that is either tethered to the capillary wall or dynamically formed by adsorption of molecules added to the buffer solution [17–19]. Although OT-CEC is attractive due to the relative ease of coating capillary walls, separations in OT-CEC suffer from a low stationary-phase to mobile-phase volume ratio (phase ratio). This low phase ratio leads to small differences in retention times that often limit resolution [20].

This paper reports the use of "giant" poly(2-hydroxyethyl methacrylate) (PHEMA) brushes as stationary phases in OT-CEC. Relative to typical monolayer OT-CEC coatings [10], these brushes have three principal advantages. First, the swollen thicknesses of PHEMA brushes may reach 1 μm, so phase ratios are relatively high. Second, controlled polymerization from a surface allows variation of brush thickness simply by changing the polymerization time [21], and such control should permit optimization of separations. Finally, every monomer unit in a PHEMA brush is potentially derivatizable, and thus a wide variety of chromatographically active functional groups can be incorporated into these stationary phases to tailor them for specific applications. For

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example, this paper shows that derivatization of PHEMA brushes with octyl side chains facilitates separations based on selective partitioning of non-polar solutes into these films.

To prepare brushes for CEC, we employ atom transfer radical polymerization (ATRP) from a silica capillary (Fig. 1) [21–25]. In this technique, a redox-active metal ion mediates halogen transfer to and from immobilized initiators to produce a low concentration of radicals that participate in polymerization [21,23]. In theory, the low concentration of radicals results in a controlled reaction that yields polymer chains with a low polydispersity index and film thicknesses that often increase linearly with time [21,22,24].

Two groups recently used ATRP to modify capillaries [18,26]. The Wirth group showed that polyacrylamide coatings decrease surface adsorption to enhance protein separations, while Leinweber et al. coated capillaries with PHEMA to reduce electroosmotic flow (EOF) [18]. However, both groups obtained film thicknesses of ~20 nm, which may not be sufficient for substantial chromatographic retention [27]. In this work, we exploit the benefits of ambient-temperature water-accelerated ATRP [28] to produce PHEMA films with thicknesses as high as 600 nm. When derivatized with octanoyl chloride, PHEMA brushes are capable of separating a series of phenols and anilines in OT-CEC. Resolution increases with brush thickness, and such separations are not possible with bare silica capillaries or underivatized PHEMA under similar conditions. Further elaboration of PHEMA should allow tailoring of columns for separation of analytes such as explosives, pollutants, and pharmaceutical enantiomers.

2. Experimental

2.1. Materials

CuCl (99.999%), $CuBr_2$ (99%), 2,2′-bipyridine (bpy, 99%), 1,1′-carbonyldiimidazole (CDI, 98%), ethylene-

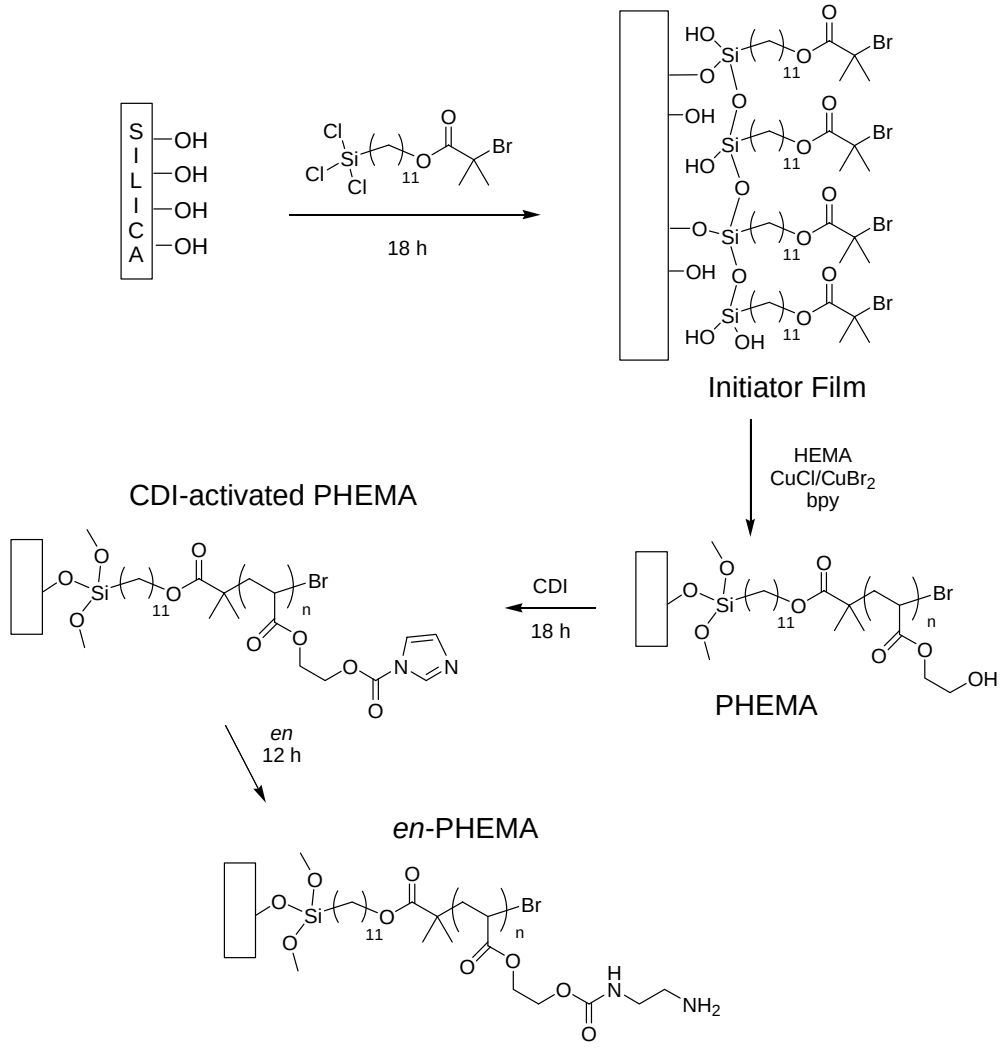

Fig. 1. Attachment of the trichlorosilane initiator to the silica capillary surface, ATRP from the immobilized initiators, activation of PHEMA by CDI, and derivatization with *en*.

diamine (*en*, +99%), octanoyl chloride (99%), aniline (99%), 4-nitroaniline (99.9%), 3,5-dichloroaniline (98%), 3-nitrophenol (99%), 4-fluoroaniline (99%), 4-chloroaniline (99%), 4-bromoaniline (97%), 4-bromophenol (99%), 2,3-dichlorophenol, *N*,*N*-dimethylformamide (DMF, +99.9% HPLC grade) and tetrahydrofuran (THF, anhydrous inhibitor-free, 99.9%) were used as received from Aldrich. Phenol (USP grade) and 4-chlorophenol (98%) were used as received from Spectrum and Fluka, respectively. Deionized water (18 MΩ cm) was prepared with a Millipore Academic water purification system. 2-Hydroxyethyl methacrylate (HEMA, Aldrich, 98%, 300 ppm monomethyl ether hydroquinone inhibitor) was purified by passing it through a bed of basic alumina immediately prior to use.

2.2. Substrate preparation

Fused silica capillary columns (Biotaq.com, 100 μm i.d. × 360 μm o.d.) were initially washed for 1 h with 1 M NaOH, rinsed for 5 min with deionized water, rinsed for 1 h with 1 M HCl, and finally dried in a 110 °C oven overnight. Solutions were passed through the capillary using a pressurized vial and a silicone septum at a flow rate of about 250 μL/min. Silicon wafers [Si(1 0 0), SiliconQuest, Test wafers] and gold-coated silicon wafers [200 nm of gold sputtered on 20 nm of Cr on Si(1 0 0)] were sonicated in toluene for 10 min, then sequentially rinsed with acetone and ethanol, and dried with a nitrogen stream. The wafers were subsequently cleaned in a UV/O_3 cleaner (Boekel Model 135500) for 15 min.

2.3. Initiator attachment

The trichlorosilane initiator, [11-(2-bromo-2-methyl)propionyloxy]undecyltrichlorosilane, was synthesized according to a literature procedure [22]. To attach the initiator to the inside surface of the capillary, a solution containing 10 μL of the initiator in 10 mL of THF (2 μM initiator) was passed through the column for 5 min, and then the column was capped with a silicone septum and allowed to stand for 18 h. The capillary was subsequently rinsed with THF for 10 min and dried with a flow of nitrogen for 10 min. The procedure for attachment of initiators to silicon wafers was similar. The wafers were immersed in a 2 μM solution of the initiator in THF for 18 h, rinsed with THF, sonicated in THF for 1 min, rinsed with acetone and ethanol, and finally dried with nitrogen. The disulfide initiator, $[BrC(CH_3)_2COO(CH_2)_{11}S]_2$, used to modify gold wafers was synthesized according to a literature procedure [29]. Gold wafers were placed in a 1 mM solution of the disulfide initiator in ethanol for 24 h and afterwards rinsed in ethanol and dried with nitrogen.

2.4. Polymerization

Polymerization of HEMA was performed as reported previously [21]. After purification of HEMA with basic alumina, 10 mL of this monomer was added to 10 mL of deionized water in a Schlenk flask, and this solution was degassed via three freeze-pump-thaw cycles. Next, 55 mg (0.55 mmol) of CuCl, 36 mg (0.16 mmol) of $CuBr_2$, and 244 mg (1.56 mmol) of bpy were added to the solution. The flask was then sealed with a septum, purged with nitrogen for 2 min to remove oxygen, and sonicated for 5 min to yield a homogeneous, dark brown mixture. For polymerization in a capillary, this mixture was passed through the column for 10 min, and then the column was sealed with a silicone septum and allowed to stand for times ranging from 1 to 12 h. After the appropriate length of time passed, the column was rinsed with DMF for 2 h and either flushed for 24 h with the buffer to be used in electrophoresis or derivatized (see below).

For HEMA polymerization on silicon and gold-coated wafers, the initiator-modified pieces were placed in 20 mL glass vials and immersed in the polymerization solution described above. The vials were then sealed with septa and purged with nitrogen for 2 min to minimize reaction of the CuCl with oxygen. After the appropriate polymerization time, the wafers were removed from the solution, sonicated in DMF for 5 min, rinsed with ethanol and deionized water, and dried in a nitrogen stream.

2.5. Derivatization of PHEMA films

To functionalize a PHEMA-coated capillary with CDI, the column was flushed with a 0.2 M solution of CDI in DMF for 10 min and then capped with a silicone rubber septum. After 18 h, the column was rinsed with DMF for 1 h to remove excess CDI. CDI-derivatized brushes can subsequently be functionalized with a variety of materials [30,31]. To functionalize CDI-PHEMA films with *en*, the column was flushed with a 0.2 M solution of *en* in DMF for 10 min and left sealed for 12 h. The capillary was then rinsed with DMF for 1 h to remove excess *en*. CDI-PHEMA films on wafers were derivatized in a similar procedure by immersing the wafers in appropriate solutions.

PHEMA films were also derivatized with octyl groups. To do this, a 0.2 M solution of octanoyl chloride in DMF was passed through a PHEMA column for 10 min, and the column was left sealed for 18 h. The capillary was then rinsed with DMF for 1 h to remove the excess octanoyl chloride. PHEMA films on gold-coated wafers were derivatized in a similar fashion.

2.6. Electrophoresis

Before using columns for CEC, they were treated with a conditioning routine. The bare silica columns were flushed with sodium hydroxide for 5 min, allowed to stand for 30 min, rinsed with buffer for 5 min, and finally allowed to equilibrate with buffer for 1 h. Coated capillaries were rinsed with the buffer to be used in electrophoresis for 24 h. With both types of capillaries, approximately 10 chromatograms

were run prior to achieving consistent retention times. The conditioning process may stabilize EOF in coated capillaries by removing residual copper ions that adsorbed to the coatings during polymerization.

CEC was performed with an Agilent G1600A CE instrument that employed a diode array UV-Vis detector. The mobile phase buffer solutions were all prepared from sodium dihydrogenphosphate at concentrations ranging from 10 to 50 mM, and buffer pH was adjusted with 1 M NaOH. In some cases, the mobile phase contained 20% ethanol. Phenols were dissolved in the appropriate phosphate buffer, and the aniline species were dissolved in a 25% solution of acetonitrile in buffer. Mixed solutions of phenols and anilines were dissolved in buffer only. All injections were hydrodynamic, and the vials of phosphate buffer were replaced after every three chromatograms to ensure that separations were performed at a consistent pH. The need for buffer replacement was manifest by changes in the electrophoresis of phenols on bare silica columns as well as literature precedent [26]. Baseline dips, spikes, and oscillations due to detector or environmental effects were common in our operation of this instrument with both bare silica and polymer-coated columns, and although retention times were consistent for different replications, the chromatograms shown here contain some of the best baselines.

2.7. Characterization methods

Reflectance FT-IR spectroscopy was performed with a Nicolet Magna-IR 560 spectrometer containing a PIKE grazing angle (80°) attachment [21], and ellipsometric thicknesses were determined using a J.A. Woollam model M-44 rotating analyzer ellipsometer [21]. Scanning electron microscope (SEM) images were taken with a Hitachi S4700 II field-emission SEM, and prior to imaging, capillaries were scored with a SiC pen, fractured under liquid nitrogen, and sputter-coated (Pelco model SC-7 auto sputter coater) with 15 nm of gold.

3. Results and discussion

3.1. Synthesis and derivatization of PHEMA films

Fig. 1 illustrates the attachment of trichlorosilane-based initiators to silica capillaries and the subsequent ATRP of HEMA from the modified capillary wall. Because of challenges in characterizing the interior of capillaries, ellipsometry studies were carried out with films on silicon wafers, and reflectance FT-IR spectroscopy was performed on gold-coated wafers. The ellipsometric thickness of the initiator layer on silicon wafers was 13 ± 3 Å, which is consistent with the literature value of 16 Å [22]. Polymerization from these initiators using the HEMA/water/CuBr/$CuCl_2$/bpy system for both 2 and 8 h yielded ellipsometric thicknesses of 80 ± 8 and 280 ± 30 nm, respectively. Polymerization of

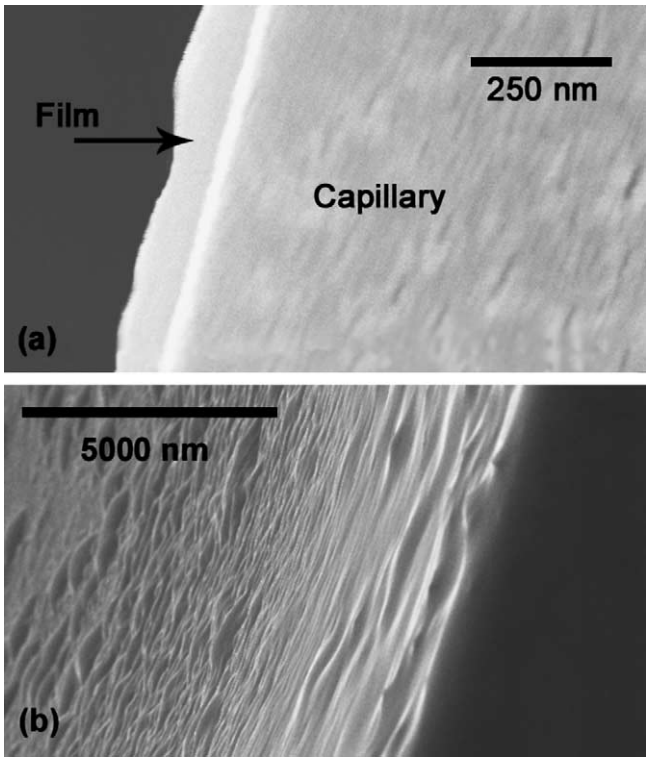

Fig. 2. Cross-sectional SEM image of a PHEMA film grown from the interior of a silica capillary using a 2 h polymerization time (a) and the angled view of a capillary surface coated with PHEMA using a 4 h polymerization time (b).

HEMA in the capillaries resulted in thicknesses of about 100 nm for a 2 h reaction time and 430 nm for an 8 h reaction time, as measured by SEM photomicrographs such as that shown in Fig. 2a.[1] However, the films are rather heterogeneous as shown by the image in Fig. 2b, and SEM thicknesses are approximations at best. In the future, we plan to explore ways to develop more homogenous coatings.

One of the attractive features of PHEMA is that its pendant hydroxyethyl groups can be readily derivatized. To introduce amine groups into these films, we couple PHEMA to *en* using CDI as an activating agent as shown in Fig. 1. This reaction results in a terminal NH_2 group at the end of pendant 2-hydroxyethyl chains. FT-IR spectra of films on gold-coated wafers show that the hydroxyl peak from the PHEMA (3300–3500 cm^{-1}) is not detectable after the CDI attachment step, indicating that the film is essentially quantitatively activated [21]. After reaction with *en*, the CDI carbonyl peak at 1771 cm^{-1} is not present, suggesting that the reaction between the amine and the CDI is also complete. Reaction of PHEMA with octanoyl chloride in DMF also occurs in virtually quantitative yield. Similar to previously published data, the hydroxyl peak of PHEMA vanishes after reaction with the acid chloride, and the ester peak at

[1] Film thicknesses are the average of the minimum and maximum thickness from images of three capillary pieces.

1735 cm^{-1} doubles [21]. After derivatization with octanoyl chloride, ellipsometry shows an ~90% increase in film thickness.

3.2. Capillary electrochromatography

We performed separations with bare capillaries as well as capillaries coated with PHEMA and its *en* and octanoyl chloride derivatives. Each type of column has different separation properties because of unique chemical functionalities. For example, the PHEMA film provides polar hydroxyl groups that are capable of hydrogen bonding to analytes, while the octanoyl chloride-derivatized PHEMA should behave similarly to reversed-phase columns. Below we discuss separations with each type of coated capillary as well as bare silica columns. Additionally, for octanoyl chloride-derivatized columns, we show how increasing film thickness (phase ratio) enhances resolution.

3.2.1. PHEMA-coated columns

We first attempted to separate a series of phenol derivatives with a PHEMA-coated column. We thought that the hydroxyl groups of PHEMA would interact with the phenols via hydrogen bonding or interactions with the phenyl ring, and that the strength of such interactions would be affected by the substituents on the phenyl ring. However, Fig. 3 shows that the separation of phenol, 3-nitrophenol, and 2,3-dichlorophenol is similar on both PHEMA-coated and bare silica columns. The main factor behind this separation is the partial ionization of the solutes. The species with the lowest pK_a, 2,3-dichlorophenol, is ~17% deprotonated at pH 7, while the species with the highest pK_a, phenol, is only 0.1% deprotonated [32]. Because the detector end of the column is the negative pole of our electrophoresis system, the ionized phenols migrate to the injection end of the column, and their movement towards the detector, which is due to electroosmotic flow, is retarded. Phenols with lower pK_a values (more negatively charged) are more affected by electrophoretic forces and have longer retention times. Although electrophoresis dominates these separations, retention time differences among the phenols do increase with polymerization time for PHEMA-coated capillaries (Table 1). (The retention times for phenol differ by less than 20% among the different columns, so increased differences in retention times between analytes are not simply due to changes in EOF.) This implies some interaction between phenols and PHEMA. However, relative to a bare capillary, peaks are about twice as broad with the 12 h PHEMA-coated capillary, so resolution increases by less than 30% compared to bare silica (Table 2).

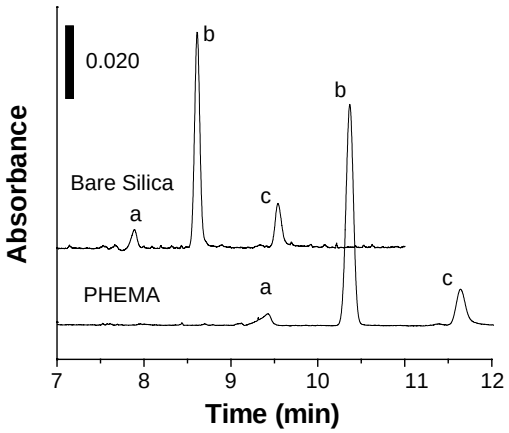

Fig. 3. Electropherograms of a solution containing 225 ppm: (a) phenol, (b) 3-nitrophenol, and (c) 2,3-dichlorophenol. The top electropherogram was obtained with a bare silica capillary, while the lower separation was performed with a capillary coated with PHEMA (12 h polymerization time). Separation conditions: 15 kV; 52 cm column length with 43.5 cm to the detector; pH 7.0, 50 mM phosphate buffer; 5 s hydrodynamic injection at 10 mbar.

To eliminate electrophoretic migration and focus on chromatographic effects, we investigated the separation of a series of aniline derivatives. Like phenols, anilines are environmental pollutants [33], but due to the low pK_a values of protonated anilines, these molecules are essentially neutral at pH 7–8 [34,35]. However, attempts to separate aniline, 4-nitroaniline, and 3,5-dichloroaniline on PHEMA-coated columns at pH 7 resulted in only a single peak with ill-defined shoulders. While it may be possible to find suitable conditions for the separation of anilines with PHEMA, we thought that derivatization of PHEMA would be more productive for increasing interactions with the stationary phase.

3.2.2. en-PHEMA columns

We initially derivatized PHEMA with ethylenediamine (*en*) in an effort to create amine-phenyl ring

Table 1
Average retention time differences (min) for selected phenol species separated on bare silica and PHEMA-coated columns[a]

	Polymerization time			
	Bare silica	1 h[b]	8 h[c]	12 h[d]
3-Nitrophenol/phenol	0.7 ± 0.1	0.6 ± 0.1	0.9 ± 0.1	1.3 ± 0.3
3,5-Dichlorophenol/3-nitrophenol	0.9 ± 0.1	0.8 ± 0.2	1.3 ± 0.3	1.8 ± 0.5

[a] Separations were performed using the conditions listed in Fig. 3.
[b] Estimated SEM film thickness of ~60 nm.
[c] Estimated SEM film thickness of ~430 nm.
[d] Estimated SEM film thickness of ~630 nm.

Table 2
Resolution values from the separation of several analytes using bare silica capillaries, and capillaries coated with PHEMA, *en*-PHEMA, and C_8-PHEMA films

	Phenol/3-nitrophenol[a]	3-Nitrophenol/2,3-dichlorophenol[a]	Aniline/4-nitroaniline[b]	4-Nitroaniline/3,5-dichloroaniline[b]
Bare silica	4.7 ± 0.6	6.6 ± 0.6	Co-elution	Co-elution
PHEMA (12 h polymerization)	6.0 ± 0.9	8 ± 1	Co-elution	Co-elution
en-PHEMA (12 h polymerization)	Not attempted	Not attempted	1.5 ± 0.3	2.2 ± 0.1
C_8-PHEMA (2 h polymerization)	6 ± 3	13 ± 5	2.9 ± 0.9	17 ± 6
C_8-PHEMA (4 h polymerization)	8 ± 1	17 ± 6	4.9 ± 0.8	14 ± 5

[a] Separation of these solutes was performed with the conditions from Fig. 3 for all columns.
[b] Separation of these solutes was performed with the conditions from Fig. 4 for the bare silica, PHEMA and *en*-PHEMA columns, and the conditions of Fig. 3 for both C_8-PHEMA columns.

interactions [36,37]. Using the conditions described in Fig. 4, bare silica columns were unable to resolve aniline, 4-nitroaniline, and 3,5-dichloroaniline. In contrast, when the separation was performed under identical conditions on *en*-PHEMA columns, peaks due to aniline, 4-nitroaniline, and 3,5-dichloroaniline were broad, but fully separated (Fig. 4). However, many anilines do not separate on the *en*-PHEMA columns. Aniline, 4-fluoroaniline, and 4-chloroaniline co-elute, 4-bromoaniline and 4-nitroaniline co-elute, and 3,5-dichloroaniline elutes alone.

The absence of electrophoretic effects with neutral molecules results in co-elution of all the anilines from the bare silica capillary. With the *en*-PHEMA column, however, interactions between $-NH_3^+$ or $-NH_2$ groups and anilines allow a chromatographic separation [36,37]. The pK_a values of protonated anilines correlate with retention times, as the neutral molecules elute in order of their pK_a [35,38]. In contrast with the phenol separations, the correlation between retention times and pK_a reflects susceptibilities to interactions with the stationary phase rather than ionization. One problem with *en*-PHEMA-aniline interactions, however, is that they result in very broad solute peaks, especially for the most retained aniline, 3,5-dichloroaniline. The protonated amine groups of the *en*-PHEMA probably present adsorption sites for the anilines [39], and slow desorption from these sites could lead to peak broadening [40].

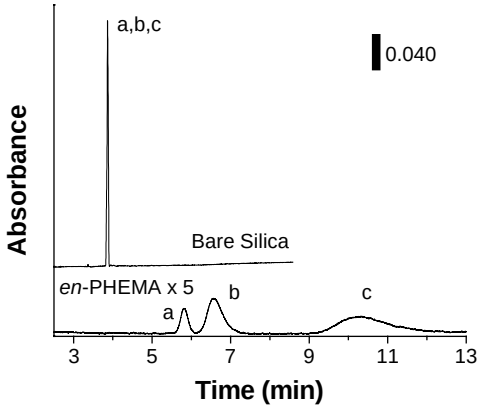

Fig. 4. Electropherograms of a solution containing 225 ppm: (a) aniline, (b) 4-nitroaniline, and (c) 3,5-dichloroaniline. The top electropherogram was obtained with a bare silica column, while the lower separation was performed with a capillary coated with PHEMA (12 h polymerization time) that was derivatized with ethylenediamine. Separation conditions: 30 kV; 46 cm long column (37.5 cm to the detector); pH 8.3 (10 mM phosphate buffer with 20% ethanol); 5 s hydrodynamic injection at 10 mbar.

3.2.3. C_8-PHEMA columns

To overcome problems of co-elution and peak broadening, we derivatized PHEMA with octanoyl chloride to create non-polar, C_8-functionalized films that retain solutes based on reversed-phase partitioning rather than adsorption. We used these C_8-functionalized capillaries to separate the same three-member sets of phenols and anilines that we examined with PHEMA-coated and *en*-PHEMA-coated columns. Table 2 gives resolution values between solutes for all three columns. As shown in Table 2, compared with *en*-PHEMA-coated, PHEMA-coated, and bare capillaries, the C_8-functionalized system either matches or exhibits higher resolution for all solute pairs. Often, resolution increases by a factor of more than 2 compared to PHEMA- and *en*-PHEMA-coated capillaries.

The extra resolution provided by the C_8-PHEMA columns allows for the separation of additional phenols and anilines. As Fig. 5 shows, C_8-PHEMA-coated columns prepared with 2 h and 4 h polymerization times were capable of separating a mixture of 2 phenols and 3 anilines at pH 7. Since several species that co-elute on the PHEMA and *en*-PHEMA columns are now resolved, these data clearly show the enhanced peak capacity of C_8-PHEMA-coated capillaries. Still, several phenols and anilines (phenol, aniline, and 4-fluoroaniline) cannot be resolved by the C_8-PHEMA column.

Not shown in Fig. 5, are peaks due to 2,3-dichlorophenol and 3,5-dichloroaniline. The C_8-PHEMA columns can also separate these compounds from the mixture of phenols and anilines, but for both 2 and 4 h C_8-PHEMA columns, the average retention time for 2,3-dichlorophenol exceeds 35 min and that for 3,5-dichloroaniline exceeds 65 min. Although these excessive retention times lead to the high resolutions shown in Table 2, the long elution times would complicate the use of these columns. Such high resolutions might be

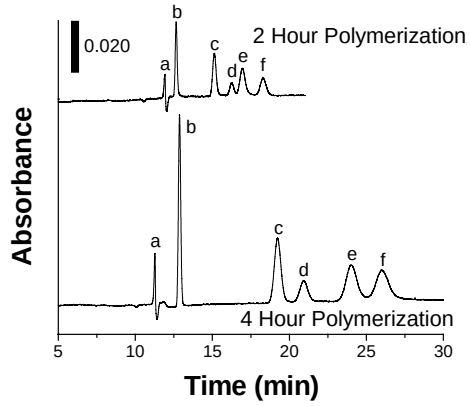

Fig. 5. Electropherograms obtained with C_8-PHEMA-coated capillaries and a solution containing: 10% acetonitrile (a), and 225 ppm (b) 4-fluoroaniline, (c) 4-chloroaniline, (d) 4-chlorophenol, (e) 4-bromoaniline, (f) 4-bromophenol. Separation conditions: 15 kV; 52 cm column length with 43.5 cm to the detector; pH 7.0, 50 mM phosphate buffer; 5 s hydrodynamic injection at 10 mbar.

useful in lab-on-a-chip devices, however, which employ very short columns.

One of the challenges that still must be overcome in working with C_8-PHEMA columns is a relatively high variability in solute retention times and peak widths (Table 3). Even with the same column, run-to-run differences in retention times can reach 30%. These variations in retention times did not show a particular trend towards longer or shorter values with column age and conditioning, but rather varied unpredictably from one run to the next. Typically, columns performed this way for 50–75 injections before breaking down and losing resolution between aniline species completely. Irregularities in the initiator layer thickness, and resulting variations in polymer film thickness, may be one source of the large variations in retention time. Use of a monochlorosilane for initiator attachment may limit formation of initiator multilayers and provide better control over film thickness and retention time. Moreover, incorporation of cross-linkable monomers into films may increase film stability and column longevity [41]. Future work is needed to address these issues. Utilization of columns with smaller diameters should also reduce Joule heating and improve phase ratios.

3.3. Resolution as a function of film thickness

As Table 3 suggests, resolution tends to increase between anilines when using the thicker C_8-PHEMA film. In changing from 2 to 4 h of polymerization time, the 4-fluoroaniline/4-chloroaniline resolution increases from 7 ± 2 to 11 ± 2, the 4-chloroaniline/4-bromoaniline resolution increases from 3.4 ± 0.8 to 4.8 ± 0.7, and the 4-chlorophenol/4-bromophenol resolution increases from 3.2 ± 0.7 to 3.5 ± 0.4. Although, resolution for the anilines increases by 50% on going to the greater polymerization time, there is a negligible increase in resolution for the 4-chlorophenol/4-bromophenol pair. This is a consequence of broader peaks for phenol species on the 4 h column compared to the 2 h column, whereas the aniline species only see a trivial increase in peak width with longer polymerization time. Additionally, as Table 3 shows, this extra aniline resolution of the 4 h column is not achieved at the cost of less EOF. Moreover, if one considers the phenol peak in Fig. 3 to be due to an unretained species, the C_8-PHEMA-coated columns exhibit only a small loss in EOF compared to PHEMA-coated columns.

3.4. Comparison to other methods

Other published reports also describe the separation of phenols and anilines [42,43]. Patsias and Papadopoulou-Mourkidou used solid-phase extraction (SPE) with HPLC to separate a system of 17 halogenated and nitrated phenols and anilines over a span of 30–35 min. They achieved baseline resolution among all of the solutes in their chromatogram (including phenol, aniline, 4-nitroaniline, 4-chloroaniline, and a number of dichlorinated phenols and anilines) as well as relatively narrow peak widths compared to our results with OT-CEC [42]. However, their separation employed a gradient method.

Zhu et al. used liquid–liquid–liquid microextraction (LLLME) and HPLC to separate several haloanilines and nitroanilines. Baseline resolution was attained between several solute pairs in only 15 min [43]. However, their method was unable to completely separate 4-chloroaniline and 4-bromoaniline, while C_8-PHEMA columns show resolutions of three to four for these species. Several groups also stud-

Table 3
Average retention times and peak widths for selected aniline and phenol species separated on columns coated with C_8-PHEMA using two polymerization times

	2 h Polymerization		4 h Polymerization	
	Retention time (min)	Peak width[a] (min)	Retention time (min)	Peak width[a] (min)
Acetonitrile (EOF marker)	12 ± 2	Not attempted	14 ± 2	Not attempted
4-Fluoroaniline	13 ± 2	0.17 ± 0.04	16 ± 3	0.2 ± 0.1
4-Chloroaniline	16 ± 3	0.3 ± 0.2	22 ± 5	0.4 ± 0.1
4-Bromoaniline	19 ± 5	0.5 ± 0.2	26 ± 6	0.6 ± 0.2
4-Chlorophenol	19 ± 4	0.3 ± 0.1	19 ± 3	0.6 ± 0.1
4-Bromophenol	22 ± 5	0.4 ± 0.2	24 ± 5	0.9 ± 0.2

[a] Peak width is the full-width of the peak at the half-maximum of its height. To determine retention times and peak widths a minimum of three capillaries were used with an average of six runs per set of phenols or anilines per column.

ied CE-based separations of anilines or phenols [44–47]. Liu and Pietrzyk separated various anilines and phenols using OT-CEC with a sulfonated-polymer stationary phase and obtained near-baseline resolution for 4-chloroaniline and 4-bromoaniline, but again, resolution is much higher with C_8-PHEMA [48].

All of these methods, including our own polymer-modified capillaries, are able to separate different anilines and phenols. Considering the level of resolution among species in several papers, the C_8-PHEMA brush may provide a competitive separation medium if challenges in reproducibility and longevity can be overcome.

4. Conclusions

The growth of PHEMA brushes via ATRP from the surface of silica capillaries leads to unique stationary phases for capillary electrochromatography. Derivatization of these brushes with *en* or C_8 groups allows for the tailoring of film/solute interactions. *en*-PHEMA-coated columns separate anilines that are unresolvable on a PHEMA-coated or bare silica column, while C_8-PHEMA-coated capillaries are able to resolve a wider spectrum of phenols and anilines than either the *en*-PHEMA-coated or the PHEMA-coated columns. Additionally, comparison of different C_8-PHEMA coatings shows that thicker films produce better-resolved peaks for the majority of the aniline pairs studied, thus allowing for separation of more components. Future work aims at further tailoring of these coatings for specific separations as well as developing a better understanding of electroosmotic flow and capillary surface coverage in these systems. Adaptation of polymer brushes and ATRP to other chromatography systems, such as open-tubular liquid chromatography, should also be possible.

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