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Short communication

Capillary zone electrophoresis in laboratory-made fluorinated ethylene propylene capillaries

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Abstract

Capillaries made of fluorinated ethylene propylene (FEP) with an inner diameter of 50 μm have been employed in capillary zone electrophoresis with UV–Vis absorbance detection. The capillaries were made in the laboratory with a recently developed technique using fluoropolymer heat shrink/melt tubing and a tungsten wire as a template for the channel. An electroosmotic flow was obtained in the channels and it is shown that an FEP capillary is more effective for a cationic test solute than a fused-silica capillary. The compatibility of FEP capillaries with optical detection is evaluated briefly.

Keywords: Capillary columns; Electroosmotic flow; Fluorinated ethylene propylene

1. Introduction

Capillary zone electrophoresis separations are typically performed in capillaries made of fused silica with inner diameters in the range 20–100 μm. The electroosmotic flow normally arises from the presence of deprotonated silanol groups on the wall surface. A common problem with these bare fused-silica capillaries is the adsorption of positively charged compounds onto the negatively charged deprotonated silanol groups resulting in distorted peak shapes for these compounds and in some cases a change in the electroosmotic flow (i.e. a change in the migration time of all analytes). In order to avoid this, different coatings for fused-silica capillaries have been developed [1,2]. However, the coatings typically have a limited lifetime and will never be as stable as the coating material itself. It is therefore of interest to evaluate materials other than fused silica as bulk material for bare capillaries.

Fluoropolymers like fluorinated ethylene propylene (FEP) [also known as poly(tetrafluoroethylene–co-hexafluoropropylene)] and polytetrafluoroethylene (PTFE) have some unique surface characteristics compared to hydrocarbon polymers, e.g. high inertness towards most chemicals (including acids, bases, oxidizing and reducing agents, and polar and nonpolar solvents) [3], low critical surface tensions [4], and low refractive indices [4]. These features make fluoropolymers highly interesting as wall material in capillary electrophoresis separations.

Fluoropolymer capillaries have often been used in isotachophoresis and isoelectric focusing but only rarely in capillary zone electrophoresis. In all cases, the capillary inner diameter has been larger than 100 μm, and hence less suitable for capillary zone

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electrophoresis. Except for PTFE capillaries, fluoropolymer capillaries with small inner diameters (<100 μm) suitable for capillary zone electrophoresis are not commercially available. Compared to PTFE, FEP has a higher optical clarity (due to a lower crystallinity) making it more compatible with optical detection [3]. Here, we describe how capillaries with an inner diameter of 50 μm can be fabricated in FEP. We also demonstrate that an electroosmotic flow is obtained in these capillaries and that these capillaries can be employed in capillary zone electrophoresis separations with UV–Vis absorbance detection.

2. Experimental

2.1. Materials and tools

Fused-silica capillaries (360 μm O.D.×50 μm I.D.) were obtained from Polymicro Technologies (Phoenix, AZ, USA). Heat shrink/melt tubing, consisting of an outer layer of PTFE and an inner layer of FEP, with an inner diameter of 0.036 in. (1 in.=2.54 cm) was purchased from Small Parts (Miami Lakes, FL, USA). Tungsten wires (50 μm diameter) were obtained from Goodfellow (Berwyn, PA, USA). Heating of the heat shrink/melt tubing was performed using a heat gun (with a variable temperature range of 100–600 °C) equipped with a reduction nozzle.

2.2. Chemicals

MOPS [3-(*N*-morpholino)propanesulfonic acid] (99.5%) and methyl viologen (1,1′-dimethyl-4,4′-bipyridinium dichloride) were obtained from Sigma (St. Louis, MO, USA), *p*-methylphenethyl alcohol (99%) was obtained from Aldrich (Milwaukee, WI, USA), and phenol and sodium hydroxide were obtained from EM Science (Cherry Hill, NJ, USA). All solutions were prepared using Milli-Q water from a Millipore system.

2.3. Instrumentation

UV–Vis transmittance spectrum were obtained using a HP 8453 UV–Vis spectrophotometer (Hewlett-Packard).

Capillary electrophoresis with UV–Vis absorbance detection was performed using a Model 3850 capillary electropherograph, Isco (Lincoln, NE, USA). The capillaries were made of FEP or fused silica and had an inner diameter of 50 μm and a total length of 46 cm with the detector located 29 cm from the inlet. All separations were performed in 25 m*M* MOPS buffer, pH 7.20. Injection was performed by applying a small pressure at the inlet reservoir. The signal was filtered with a 4-pole low pass active filter with a rise time (10–90%) of 0.8 s. Data collection was achieved using EZCHROM chromatography data system (Scientific Software, San Ramon, CA, USA).

Some parts in the instrument were modified so that the PTFE–FEP capillary could be properly connected including the waste reservoir and the holder of the capillary at the inlet reservoir. An optical window in the PTFE–FEP capillary was created by cutting two flat surfaces on opposite side of the capillary. In this way, the PTFE layer was removed and the FEP thickness was reduced to 570 μm at the part that was located inside the detector. In order to improve the focusing of the light beam, a buffer-filled fused-silica capillary (360 μm O.D.×50 μm I.D.) without the polyimide coating was located in the groove between the lens and the PTFE–FEP capillary. Alignment of the PTFE–FEP capillary was made under a stereoscope.

The FEP capillary was not pretreated. The fused-silica capillary was flushed prior to use with 1.0 *M* sodium hydroxide for 30 min, stored during a night in 0.10 *M* sodium hydroxide, flushed with water for 5 min and flushed with the running buffer for 5 min. Between measurements the fused-silica capillary was flushed with 0.10 *M* sodium hydroxide for 5 min, water for 5 min and the running buffer for 5 min. In order to facilitate optical detection, the polyimide coating on the fused-silica capillary was removed on the part located in the detector.

3. Results and discussion

3.1. Fabrication technique

The fabrication technique utilizes a dual layer heat

shrink/melt tubing consisting of an outer layer of PTFE and an inner layer of FEP. When heated (>350 °C) the outer PTFE layer shrinks and the inner FEP layer melts. Hence, all empty space inside the tubing will be filled with FEP (while the outer diameter of the tubing decreases somewhat). The capillaries were fabricated by threading a tungsten wire (50 μm diameter) through a heat shrink/melt tubing. A small segment of the tubing (3–4 cm long) was heated using a heat gun until all empty space in that segment was filled with FEP. Then the tubing was removed from the hot air flow and the tubing was allowed to cool to room temperature. During cooling (while still hot), the tungsten wire was pulled a short distance in order to prevent it from getting caught in the FEP. By repeating this procedure for segment after segment, long capillaries could be fabricated. Finally, the tungsten wire was pulled out. Typically, 50-cm long capillaries with an inner diameter of 50 μm could be fabricated after some practice. Recently, fabrication of microchannel structures with integrated objects such as microelectrodes and fused-silica capillaries have been described [5].

3.2. Compatibility of fluorinated ethylene propylene capillaries with optical detection

Fig. 1 shows a transmittance spectrum (using air as 100% transmittance) in the range 200–1100 nm for a 290-μm thick FEP layer. Except at wavelengths close to 200 nm, optical detection e.g. UV–Vis absorbance and fluorescence can be employed in the whole wavelength range. Since the channels fabricated here have an outer layer of PTFE that is less transparent, it is necessary to remove the PTFE layer prior to use. Other spectra for FEP such as IR, Raman and ^{19}F NMR are available in the literature [6].

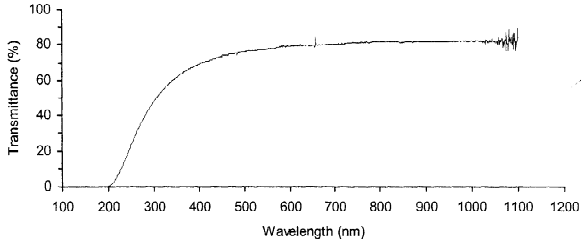

Fig. 1. Transmittance spectrum for a 290-μm thick FEP layer. The PTFE outer layer of a heat-shrink tube was removed. The remaining FEP inner layer was melted between two glass plates using a heat gun. The glass plates were removed from the now flat FEP layer. This layer is placed over the hole defining the light path in a cuvette holder.

3.3. Capillary electrophoresis in FEP channels

It has been shown in the literature that a pH dependent electroosmotic flow (EOF) is obtained in channels made of fluoropolymers due to a negative charge on the surface [7,8]. The EOF at the PTFE surface increased about three times as the pH is increased from 4 to 8, and at pH 4–5 the EOF was almost the same as at a fused-silica surface [7]. It was suggested that the negative surface charge originated from adsorption of hydroxyl and other anions [7] or from the presence of carboxylate groups introduced in the production process [8]. Recently, the zeta potential and the surface conductivity were determined at a Teflon AF surface at different pH values in the presence of potassium chloride [9]. It was found that the surface had an isoelectric point at pH 4.0, the surface charge originated from unequal adsorption of cations and anions, and that adsorption of ions preferentially occurs according to:

$$OH^- > H_3O^+ \gg K^+ = Cl^-$$

In order to avoid joule heating of the solution it is important that the channel material has a high thermal conductivity. The thermal conductivity of FEP is 0.209 Wm/K (23 °C) [10] (compare 0.732 Wm/K for glass and 0.1–0.2 Wm/K for many polymers [11]).

The above suggests that the FEP capillaries should be well suited for capillary zone electrophoretic separations. Methyl viologen and phenol were used here as model compounds since they absorb light in the important wavelength range 200–300 nm, methyl viologen has a charge of 2+ and will likely interact strongly with negatively charged surfaces, and phenol is neutral (at the prevailing pH) and can be used to estimate the electroosmotic flow.

Fig. 2 shows a capillary electrophoresis separation of methyl viologen and phenol performed in a new FEP capillary (50 μm I.D.) (Fig. 2a) and in a

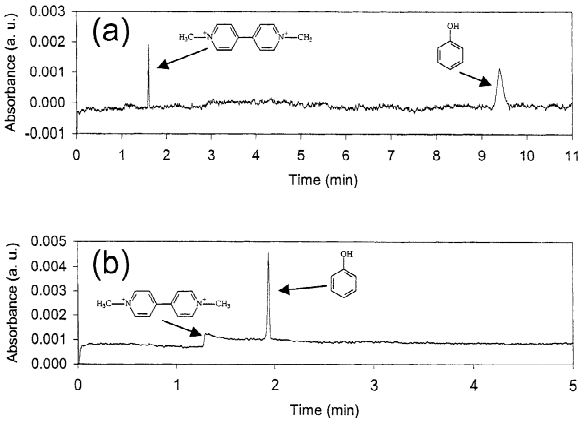

Fig. 2. Capillary electrophoresis separation of (a) 200 μ*M* methyl viologen and 2 m*M* phenol performed in an FEP capillary (50 μm I.D.), and (b) 500 μ*M* methyl viologen and 2 m*M* phenol performed in a fused-silica capillary (50 μm I.D.). Separations were performed in 25 m*M* MOPS, pH 7.20, at a separation voltage of 20 kV with an injection time of (a) 40 s or (b) 2 s and by using absorbance detection at 270 nm. The capillaries had a total length of 46 cm and the detector was located 29 cm from the inlet.

fused-silica capillary (50 μm I.D.) (Fig. 2b) using a separation voltage of 20 kV and UV–Vis absorbance detection at 270 nm. Methyl viologen (with a charge of 2+) gives rise to a sharp peak in the FEP capillary ($N = 55\,300$) while the peak in the fused-silica capillary is severely distorted. The neutral phenol gives rise to a sharp peak in both capillaries ($N = 22\,500$ in the FEP capillary, $N = 63\,800$ in the fused-silica capillary). Phenol may interact with the wall. If it is interacting to any significant degree, then *p*-methylphenethyl alcohol should be 'retained' noticeably longer. No separation of phenol and p-methylphenethyl alcohol was obtained in the FEP capillary indicating no significant interaction between the two neutral compounds and the FEP wall. Separation of methyl viologen and phenol in three additional new FEP capillaries was similar to the separation in the first FEP capillary, although the electroosmotic flow varied between the unconditioned capillaries. Under identical conditions, the initial migration times for phenol in four separately made capillaries were 9.38, 3.32, 1.64 and 2.55 min. Hence, it is clear that the wall in a new FEP capillary initially has a negative charge. The rather large differences in the initially obtained electroosmotic flows might be due to differences in the surface condition between different capillaries. The different surface conditions may influence the adsorption of potential determining ions including the buffer ion, MOPS. These differences might be reduced by a more automatized fabrication procedure or by the development of conditioning procedures.

At high separation voltages (>15 kV for 46-cm long capillaries), gas bubbles were formed in the FEP channels (probably occurring due to degassing of the FEP material) resulting in blockage of the current path. The voltage range where no gas bubble formation occurred was somewhat different from capillary to capillary. In general, if no gas bubble formation had occurred within 5 min at a certain voltage, bubbles would not form for several hours. The upper voltage limit where no gas bubble formation occurred could be extended considerably (to voltages higher than 20 kV for 46-cm long capillaries) by slowly increasing the applied voltage (over 5 min). After having done this once, the voltage could be turned off for a few minutes and then turned back on using the switch without formation of gas bubbles, facilitating injection and separation at a constant voltage in the extended voltage range.

It is not clear why methyl viologen is adsorbed on the fused-silica wall but not on the FEP wall. However, we speculate that anions are adsorbed weakly on the FEP wall and that individual anions exchange rapidly between adsorbed and solution states. Hence, cations like methyl viologen will not be adsorbed for longer times at the surface. On a fused-silica surface on the other hand, the negatively charged surface originates from deprotonated silanol groups fixed on the surface resulting in more permanent adsorption of methyl viologen.

The sensitivity is low but as expected for absorbance detection. A comparison between the FEP capillary and the fused-silica capillary was made by pumping 500 μ*M* methyl viologen through the capillaries with a syringe. At 270 nm, the signal-to-noise ratio for the FEP capillary was 3.5 times lower than for the fused-silica capillary. By using more sensitive detection techniques, such as fluorescence and electrochemical techniques (e.g. amperometry and conductivity), considerably lower detection limits are expected.

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