Polypyrrole-coated capillaries for capillary zone electrophoresis

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Available online 12 April 2005

Abstract

Fused-silica capillaries are permanently coated by silanization with 3-{[3-(*N*-pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane followed by oxidative polymerization of the pyrrole moieties with iron (III) or peroxodisulfate in the presence of chloride, perchlorate, or dextransulfate as anions. This approach allows to modulate the EOF in its magnitude as well as in its direction. With the small anions chloride and perchlorate, the EOF is reversed below pH 5 while with the large dextransulfate polyanions (DS) the EOF is relatively constant over the pH range from 2.5 to 9.4. This can be of advantage at low pH, at which the EOF of uncoated capillaries is close to zero. Application for separation of some herbicides is shown. The lifetime of PP-modified capillaries is satisfactory: the decrease in EOF is less than 3% during 80 analyses (160 min) and less than 5% over three months of storage. The reproducibility of capillary modification is about 5% (RSD of EOF).
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Keywords: Capillary electrophoresis; Polypyrrole-coated capillaries; Electroosmotic flow; Acidic herbicides; Dextransulfate

1. Introduction

Capillary electrophoresis (CE) is potentially a powerful separation technique, especially in environmental monitoring where large numbers of single analyses are necessary in order to arrive at data sets of sufficient statistical significance. The intrinsically high speed of CE at high efficiency facilitates high-throughput analysis. A major disadvantage of CE lies in the fact that with real samples adsorption of matrix components may result in fluctuations of electroosmotic flow (EOF) and of analyte migration times. Therefore, considerable efforts have been devoted to suppress the adsorptivity of the silica surface by use of high or low pH [1], buffers of high ionic strength [2], buffer additives [3], and modification of the fused-silica surface by dynamic [4–7] or permanent coating by physisorption [8,9] or covalent bonding [4,10–13].

Polypyrrole (PP) is well suited for permanent CE-capillary modification as it: (1) carries positive charges (Fig. 1) for reversal of EOF and compensation of siloxanolate charges, (2) is a tenacious polymer insoluble in common solvents [14], and (3) can be modified with various counter anions [15]: with small inorganic anions which are partially mobile within the polymer matrix the net surface charge is positive at neutral pH; with large immobilized [16,17] organic polyanions such as dextransulfate, a negatively charged surface over the whole pH-range is obtained.

The objectives of this work were to investigate whether with covalent PP coating the control of the EOF in both directions is possible and to demonstrate that such PP-modified capillaries are applicable to the analysis of anionic environmental chemicals.

2. Experimental

2.1. Chemicals and materials

3-Aminopropyltriethoxysilane (99%, Fluka, Taufkirchen, Germany) and doubly vacuum-distilled pyrrole (p.a., Fluka) are stored at $-18\,^{\circ}$C under argon in the dark. 3-{[3-(*N*-Pyrrole)-2-hydroxypropyl]amino}propyl-triethoxysilane (SP1, Fig. 1) is synthesized as described [18]. Thiourea (p.a. quality, Fluka) is used as EOF marker at a concentration of $5\,\mathrm{g\,L^{-1}}$. Hexadimethrin dibromide (HMDB, Fluka) at a concentration of 0.001% (w/v) in the running buffer is

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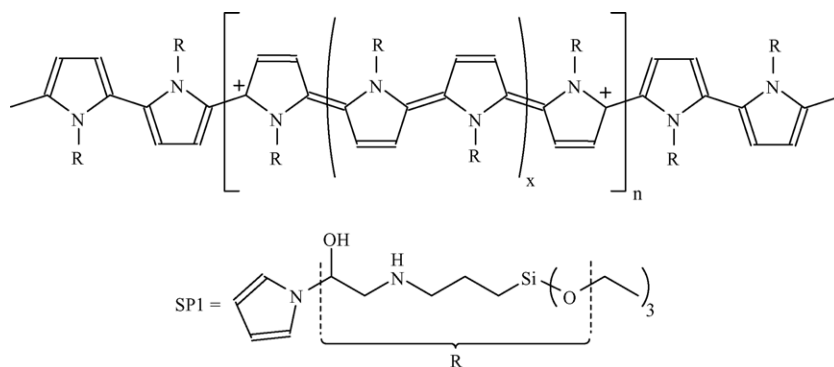*E-mail address:* encetox@uni-bayreuth.de (H. Frank).

Fig. 1. Structure of polypyrrole in the oxidized state, anions not shown; structure of 3-{[3-(N-pyrrole)-2-hydroxypropyl]amino}propyltriethoxysilane (SP1).

used for dynamic coating of capillaries. Dextransulfate sodium salt (DS, from Leuconostoc sep., Fluka), and the herbicide standards bromoxynil, mecoprop, triclopyr, dichlorprop, 2,4-dichlorophenoxyacetic acid (2,4-D), 4-(2,4-dichlorophenoxy)-butanoic acid (2,4-DB), 4-chloro-2-methylphenoxyacetic acid (MCPA), picloram, dicamba, and clopyralid (all Pestanal[TM], Riedel de Haen, Taufkirchen, Germany) are used as supplied. Water is bidistilled (Büchi, Switzerland). Toluene (Fluka) is dried over molecular sieve X5 (Fluka) for one day. All other reagents and solvents are of standard laboratory or analytical grade quality and used as received. Fused-silica capillaries of 50 μm I.D. and 386 μm O.D., 20 μm outer polyimide coating, are used (Polymicro Technologies Inc., Phoenix, USA). Capillary lengths are given as total ones; the distance between optical window and capillary end is 8.5 cm. The following buffers (sodium salts, $50 \, mmol \, L^{-1}$) are used: phosphate (pH 2.5, 6.0, 7.0, 8.0), formate (3.4 and 4.3), acetate (5.0), citrate (6.0), TRIS (8.0), and borate (9.2 and 9.4). SPE cartridges, 60 mg, 3 mL, (Oasis HLB, Waters GmbH, Eschborn, Germany) are employed for enrichment of herbicides from spiked tap water. A peristaltic pump (Minipuls 3, model 312, Gilson, Villiers, France) is used for constant flow of spiked water during analyte extraction by SPE.

2.2. Instrumentation

All measurements are done on a CE-instrument ([3D]CE, Hewlett-Packard, Waldbronn, Germany) equipped with UV–Vis diode array detector. Detection is performed at 200 nm with a band width of ±10 nm. The data are acquired with and stored on a computer (HP Vectra XA PC, Chemstation software version A.05.01 HP).

2.3. Preparation and use of capillaries

All solutions are pushed through the capillaries at a flow of $100 \, \mu L \, h^{-1}$ by applying pressure to the filling vessel. The capillaries (1 m long) are rinsed with a solution of NaOH ($2.5 \, mol \, L^{-1}$) in methanol/water (80/20, v/v) for one hour, with bidistilled water for 1 h, with hydrochloric acid

($1 \, mol \, L^{-1}$) for 1 h at 70 °C [19], and with bidistilled water for 1 h.

2.3.1. Silanization

Pretreated capillaries are dehydrated in a GC oven under nitrogen (99.999% purity, Linde, Wiesbaden, Germany), 0.1 MPa column head pressure, with the following temperature programme: 10 min at 25 °C, $1 \, °C \, min^{-1}$ to 110 °C, hold for 120 min, $1 \, °C \, min^{-1}$ to 140, 160, 180, 200, or 220 °C (two replicates for each temperature), hold for 8 h. The capillaries are cooled to room temperature and flushed for 5 min with a solution of SP1 (10%, v/v) dissolved in dry toluene containing 1% (v/v) glacial acetic acid.

After stopping the flow, about 10% of the capillary volume is emptied by flushing with nitrogen and the capillaries are disconnected from the filling vessel. They are sealed on both ends with a propane/oxygen microtorch and heated in a GC oven to 80, 110, 140, 165, 170, or 200 °C to study the influence of silanization temperature. After 24 h, the capillaries are opened and flushed with dry toluene for 2.5 h and dried with N_2 for one hour at room temperature.

2.3.2. Polymerization

For oxidative polymerization of the pyrrole moieties, the silanized capillaries are rinsed for 24 h with an aqueous solution of iron (III) chloride, $0.25 \, mol \, L^{-1}$, or an aqueous solution of iron (III) chloride, $0.25 \, mol \, L^{-1}$ saturated with potassium perchlorate ($0.59 \, mol \, L^{-1}$ at 20 °C) [20]. For preparation of PP/DS capillaries, an aqueous solution of ammonium peroxodisulfate (APS), $0.04 \, mol \, L^{-1}$ [21], and 3% DS (w/v, corresponding to $0.19 \, mol \, L^{-1} \, SO_3^-$) is used. Finally, the capillaries are rinsed with bidistilled water for 2 h. For each procedure, three capillaries are prepared to determine reproducibility. For the optical window the outer polyimide coating of the modified capillaries is burned off with a spirit lamp over a length of 10 mm, 8.5 cm from the end, with nitrogen flowing through.

Before the first use and after a buffer change, the capillaries are equilibrated with running buffer for 30 min. For analysis they are flushed with running buffer for 5 min and equilibrated at the working voltage for 1 min. Samples are introduced by

hydrodynamic injection at a pressure difference of 50 mbar for 5 s. Capillary cassettes and vials are thermostated at 20° C.

2.3.3. Solid-phase extraction of tap water

Local tap water is spiked with 10 herbicides (listed in Section 2.1) at concentrations of 0.1 μg L^{-1} each. Samples of 1 L volume are adjusted to pH 2 with hydrochloric acid, 10 mol L^{-1}, and loaded on conditioned SPE cartridges (Oasis HLB) at 4 mL min^{-1} by means of a peristaltic pump. After washing with 1 mL bidistilled water (1 mL min^{-1}), the herbicides are eluted with 1 mL acetonitrile (1 mL min^{-1}), the eluate is concentrated to 0.2 mL under a gentle stream of nitrogen and reconstituted to 1 mL with acetonitrile.

3. Results and discussion

A basic characteristic of CE capillaries is the dependence of EOF on pH [22,23]. For uncoated capillaries (Fig. 2, A), the EOF increases steadily with pH since surface-located siloxanolate groups have a wide dissociation range depending on their individual chemical nature, distance to neighbouring groups, purity, or state of hydration of the silica surface. In respect to dehydration temperature of the silica surface prior to silanization, differences are found to be minimal, with a slight optimum at 180 °C (Fig. 3), slightly higher than suggested by Cifuentes et al. [19], likely a reflection of the variability of fused-silica surfaces of different batches [23,24]. The effect of silanization temperature on EOF is also studied; capillaries silanized at 140 °C exhibit highest EOF (Fig. 3). In PP-modified capillaries, the EOF is reduced (Fig. 2, B).

Fig. 3. Dependence of electrophoretic mobility of thiourea on the dehydration and silanization temperature for PP/DS capillaries (length 32.5 cm, field strength 615 V cm^{-1}, sodium formate, 50 mmol L^{-1}, pH 3.8).

There is no difference in the EOF between chloride and chloride/perchlorate as counter anions within precision of measurement, evidence that they escape from the PP [16,17] and do not directly participate in generating the EOF. Reversed EOF (REOF) below pH 5 is evidence of a positive surface charge; above pH 5 it is negative but still lower than of untreated silica. This is the result of the charge balance of siloxanolate anions and of the weakly basic PP. As there are 4–5 siloxanolate groups per nm^2 [22], bonding of SP1 does not entail complete coverage but some siloxanolate groups remain. For comparison, dynamic coating with strongly basic HMDB results in REOF over the whole pH range, with a minimum at pH 6 (Fig. 2, C); below pH 6, REOF is effected by the balance of the positive charges of both the weakly basic PP-coating and the strongly basic HMDB, and of the negative siloxanolate groups; above pH 6, this is mainly by the strongly basic HMDB and the negative siloxanolate groups. Capillaries silanized with 3-aminopropyltriethoxysilane containing a primary amino group showed an REOF only below pH 3 (data not shown). The repeatability of EOF is up to 5% RSD ($N \geq 40$) comparable to that obtained with bare fused-silica capillaries.

Permanent coating with PP as an attachment layer for the strongly acidic DS is a possibility to attain a negative surface charge largely independent of pH. A comparatively high EOF (Fig. 2, D) over the whole pH-range is achieved, evidence that dextransulfate remains immobilized in the PP-matrix. The EOF is largely independent of the buffer anion; the same EOF is obtained when TRIS (pH 8) or citrate (pH 6) is replaced for phosphate.

An pH independent, but lower EOF ($\sim 3 \times 10^{-4}$ cm^2 V^{-1} s^{-1}) has also been observed for capillaries dynamically coated with HMDB and DS [5]. Capillaries
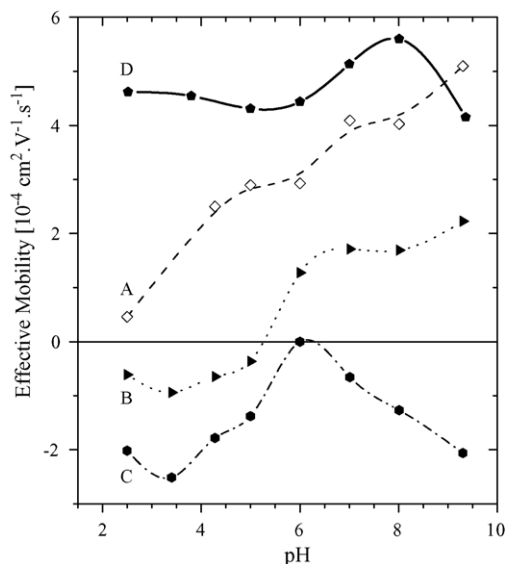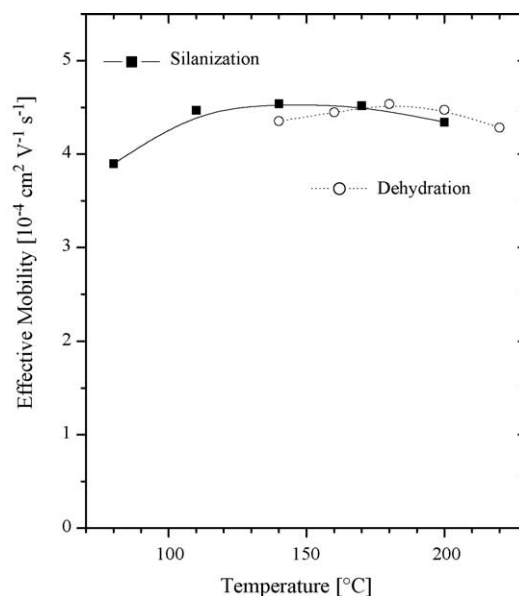

Fig. 2. Dependence of electrophoretic mobility of thiourea on pH; (A) bare capillary, (B) capillary with covalently bonded PP/Cl, (C) the same capillary dynamically coated with HMDB, (D) capillary with covalently bonded PP/DS silanized under optimized conditions. Error bars are not shown as they are smaller than the symbol size of the data points.

dynamically coated with HMDB and poly(vinylsulfonate) with a higher sulfonate fraction (75% (w/w) compared to 51% (w/w) in DS) [6] (EOF 5×10^{-4} cm^2 V^{-1} s^{-1}, pH 2–10) are comparable to those presented here. Graul and Schlenoff [25] suggested coating with alternating layers of poly(diallyldimethylammonium) and poly(styrenesulfonate) resulting in an EOF of similar magnitude (2.2–4.5×10^{-4} cm^2 V^{-1} s^{-1}) but more strongly depending on pH and the number of layers.

To demonstrate the application of PP/DS modified capillaries, the separation of ten acidic herbicides is shown (Figs. 4 and 5). At pH 2.5 (Fig. 4) the electromigration, opposite to the electroosmotic flow, is relatively lower than at pH 9.4 (Fig. 5) since under acidic conditions the dissociation equilibrium is shifted towards the non-charged protonated acid, with the extent depending upon the respective dissociation constants (pK_a) [26]. Various pK_a values for individual pesticides can be found in the literature which makes the explanation of migration order difficult. At pH 2.5 the weakest acids (2,4-DB (**1**), pK_a 4.8; bromoxynil (**2**), pK_a 3.9) migrate slowest against the EOF and appear shortly after the EOF, while the strongest acids (dicamba (**9**), pK_a 1.9; clopyralid (**10**), pK_a 1.8) migrate fastest and appear latest. Notable exception (triclopyr (**5**), pK_a 4.0) demonstrates that structure-specific effects on the hydrodynamic diameter also play an important role beside other effects such as dipol momentum and charge localization in the molecule.
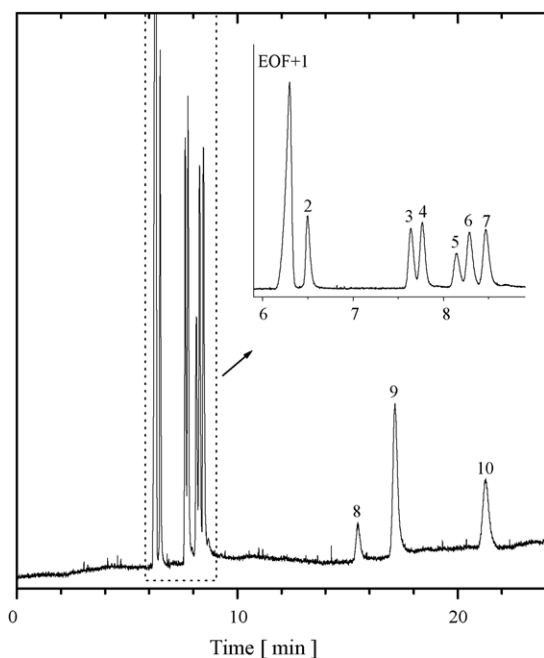

Fig. 4. Separation of herbicides, 10 mg L^{-1}, on PP/DS coated capillary (length 66.4 cm, field strength 452 V cm^{-1}, sodium phosphate, 50 mmol L^{-1}, pH 2.5), **1**: 4-(2,4-dichlorophenoxy)-butanoic acid (2,4-DB, pK_a 4.8) + thiourea, **2**: bromoxynil (3.9), **3**: mecoprop (3.8), **4**: 4-chloro-2-methylphenoxyacetic acid (MCPA, 3.1), **5**: triclopyr (4.0), **6**: dichlorprop (3.0), **7**: 2,4-dichlorophenoxyacetic acid (2,4-D, 2.6), **8**: picloram (2.3), **9**: dicamba(1.9), **10**: clopyralid (1.8).
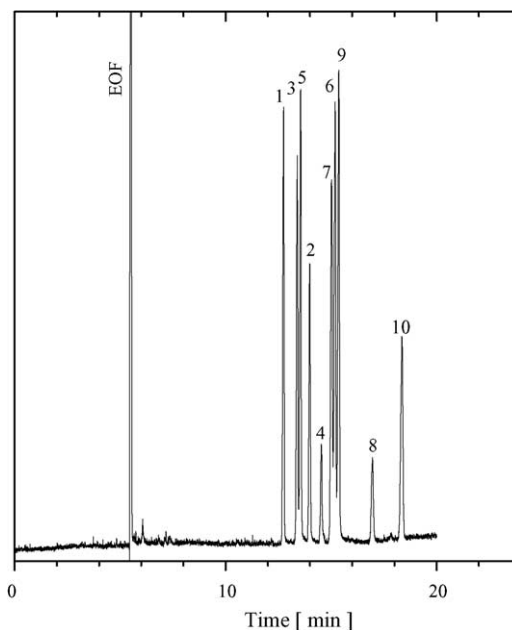

Fig. 5. Separation of the herbicides same as in Fig. 4 at pH 9.3 (sodium borate, 50 mmol L^{-1}).

At pH 9.4, under a condition at which all analytes are fully ionized, no such ordering in respect to dissociation constants is observed and the sequence of analytes is mainly depending upon their hydrodynamic diameters.

The efficiencies of the capillaries are considerably larger at pH 9.4 than at pH 2.5. For example, at pH 9.4 the theoretical plate count for clopyralid (**10**) is 253,000 m^{-1} and for MCPA (**4**) 320,000 m^{-1}, compared to 46,000 m^{-1} and 140,000 m^{-1}, respectively, at pH 2.5. At low pH, interaction of the uncharged forms of the analytes with the wall coating results in slow adsorption/desorption equilibria leading to broadened, tailing peaks. At high pH, the fully ionized analyte anions are repelled from the negatively charged wall surface resulting in a purely electrophoretic separation without adsorption.

Application of a short (32.5 cm) PP/DS capillary for fast separation at pH 2.5 results in elution of the weakly acidic herbicides 2,4-DB (**1**), bromoxynil (**2**), mecoprop (**3**) and MCPA (**4**) with the EOF (data not shown); this is improved by addition of 20% acetonitrile but analysis time increases which is compensated by setting the separation buffer to pH 3.4 (Fig. 6) and using higher field strength (615 V cm^{-1}), as shown for the analysis of tap water spiked with 0.1 μg L^{-1} of the herbicides. In this case 2,4-DB (**1**) is partially, bromoxynil (**2**), mecoprop (**3**) and MCPA (**4**) fully separated from the EOF but dichlorprop (**6**) and 2,4-D (**7**) coelute.

To demonstrate the stability of PP/DS coating, the EOF at pH 2.5 and 9.4 has been determined for 80 runs in a row without conditioning between runs (Fig. 7) but replenishing the buffer after each 10 runs. The relative standard deviation of the EOF is 1.4% in both cases. No decline is noticeable, indicating that dextransulfate is fully immobilized on the PP-
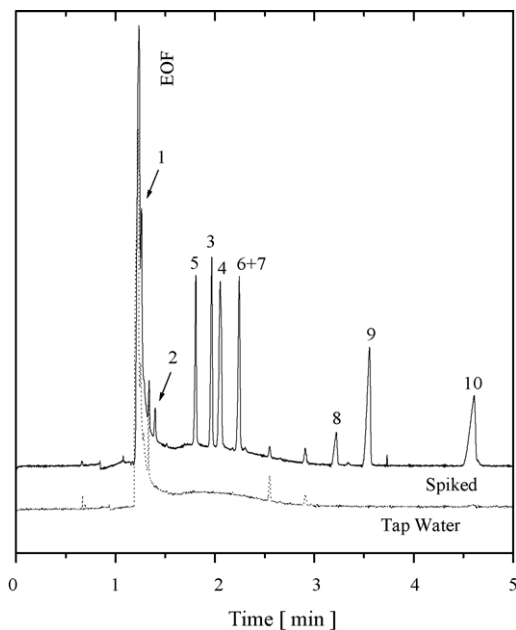
Fig. 6. Separation of herbicides spiked tap water ($0.1\ \mu g\,L^{-1}$) on a short PP/DS coated capillary (length 32.5 cm, field strength $615\,V\,cm^{-1}$, sodium formate, $50\,mmol\,L^{-1}$, pH 3.4, 20% (v/v) acetonitrile).

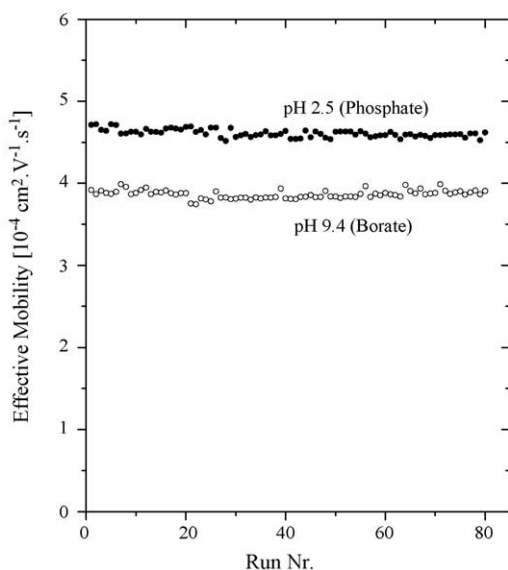Fig. 7. EOF in a PP/DS coated capillary (length 32.5 cm, field strength $615\,V\,cm^{-1}$, sodium salts, $50\,mmol\,L^{-1}$, hydrodynamic injection 1 s, 50 mbars, run time 2 min, no conditioning between runs, buffer replenished after 10 runs).

modified silica surface. Upon storage of the capillaries in ambient air, the EOF is decreased by about 5% over 3 months. The reproducibility of EOF for the PP/DS capillaries is satisfactory, i.e. below 7% (RSD, 6 capillaries, $N = 20$ on each capillary).

4. Conclusions

The reproducible coating of fused-silica capillaries with covalently bonded polypyrrole is presented. The coating conditions are optimized; silanization under non-aqueous conditions followed by oxidative polymerization of the pyrrole units results in stable coating of high efficiency. PP/DS-modified capillaries exhibit largely pH-independent EOF. They exhibit good stability, and their applicability for herbicide trace analysis in water is demonstrated.

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