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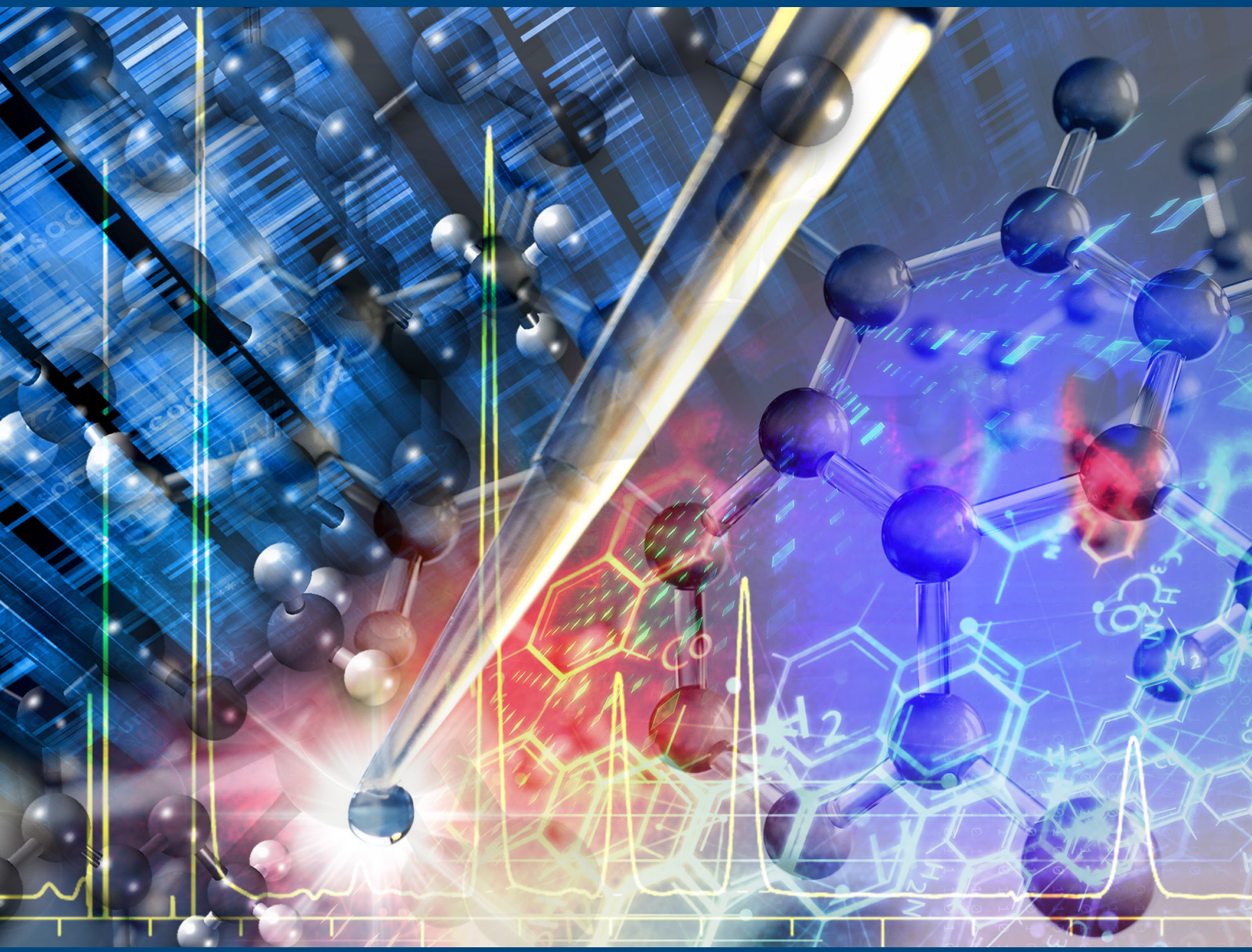

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ORIGINAL ARTICLE

Functionalization of High-Density Polyethylene Capillaries for Open Tubular Ion Chromatography

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ABSTRACT

Ion chromatography is the anion analysis benchmark. A miniature form, Open Tubular Ion Chromatography (OTIC), has attractive attributes for efficient ion separations. Here, we fabricate and characterize high-density polyethylene (HDPE) open tubular anion exchange columns (OTCs). We attach positively charged latex particles onto negatively charged capillary surfaces. For efficient OTIC, column diameters need to be < ~20 μm; functionalizing the bore is challenging. Methods to introduce acid groups to an HDPE capillary bore, e.g., sulfonation using chlorosulfonic or sulfuric acid solutions, with or without grafting of an aromatic ring through photo- or chemical grafting first, are explored. Following quaternary ammonium latex attachment, the ion exchange capacity and separating abilities of each OTC were measured as an index of OTC performance. Gradual loss of capacity was observed for many of these; high-resolution mass spectrometry confirmed the leaching of detached oxidized/sulfonated oligomeric fragments and consequent poisoning of the latex sites. Ways to ameliorate this and/or to rejuvenate the columns are also described.

1 | Introduction

Chromatography in the open tubular mode is generally considered the most efficient; in analytical gas chromatography, it is the only mode in use today. Some four decades ago, Guthrie and Jorgenson [1] showed the power of open tubular liquid chromatography (OTLC) and presciently concluded that *it is detection that will determine if LC in capillaries is going to be a real success*. The use of OTLC is presently limited, perhaps for this reason. There are notable exceptions: Liu's group has repeatedly shown exceptional separations of fluor-tagged analytes and laser-induced fluorescence detection [2, 3] reaching peak capacities of 20/min at slightly super ambient temperatures. With state-of-the-art mass spectrometry (MS) detection, they report the identification of 1000 proteins from 75 pg of tryptic peptides [4]. However, most of these detection schemes are not accessible to most laboratories. Also, fluorescence tagging is often not possible; it is an added step, even when feasible. We have recently reviewed capillary scale detectors in general [5]. Although a limited subclass of OTLC, open tubular ion chromatography (OTIC) may offer the most practical realization: Conductometric detection is easily miniaturized: Admittance measurement has been used with tubes 2 μm in bore [6].

Abbreviations: AEC, anion exchange capacity; AIBN, azobisisobutyronitrile; CEC, cation exchange capacity; COC, cyclic olefin copolymer; COP, cyclic olefin polymer; DCUP, dicumyl peroxide; FSC, fused silica capillary; HDPE, high-density polyethylene; IC, ion chromatography; LOD, limit of detection; MS, mass spectrometry; OTC, open tubular column; OTIC, open tubular ion chromatography; OTLC, open tubular liquid chromatography; PEEK, poly(ether) ether ketone; peq, picoequivalents; PHL, projected half-life; PMMA, poly(methyl) methacrylate; SCIC, single-column ion chromatography.

We devoted much effort over the last decade toward OTIC [7–17]; a review is available [18]. A small portable instrument for reliable extraterrestrial analysis was the goal; perchlorate found in Martian soil was the result of unexpectedly high readings from a potentiometric "nitrate" electrode [19, 20], underscoring the need for a more specific technique like IC [21].

IC is used more for anions than cations; other alternatives exist for metals. Of two conductometric detection modes available in IC, the simpler nonsuppressed mode (also called single column IC, SCIC) does not permit gradient elution or limits of detection (LODs) comparable to the suppressed mode where a membrane device is used to convert the eluent counterion to H^+ in anion IC, which simultaneously decreases the background and enhances the detection sensitivity. On-column admittance detection is applicable to nonsuppressed IC but, thus far, not to suppressed IC at low analyte levels [14]. Connections to a suppressor and a detector in suppressed IC add significantly to band dispersion.

Fused silica capillaries (FSCs) have been the mainstay in capillary chromatography; methods for increasing surface area and functionalizing FS surfaces are well known. Our early OTIC attempts also utilized FSCs [22]. Nevertheless, FSCs are not suited to suppressed IC; the eluents are strongly alkaline; silanols cannot be effectively protected at high pH [23].

We have described OTIC with base-stable custom-extruded polymeric capillaries of relevant dimensions. We described the use of 16–20 μm i.d. (henceforth ϕ) poly(methyl) methacrylate (PMMA) [6, 7], 20 μm ϕ cyclic olefin polymer (COP) [8–12] and nominally 25 μm ϕ poly(ether) ether ketone (PEEK) [16] capillaries (the last now commercially available), all ∼360 μm o.d. (to avail commercial fittings). Capillaries extruded from polyetherimide (Ultem) and cyclic olefin copolymer (COC, TOPAS), were not of usable quality. Performance in OTLC declines linearly with ϕ^2. For ϕ <10 μm, performance may increase in theory, but for us, the dispersion from the injector, suppressor, detector, and connection more than erases any gain.

Geometric considerations are important. Especially with the softer polymer capillaries, near-perfect perimeter circularity [24] is essential to achieve a compression seal without shutting off the bore; in a 20/360 μm i.d./o.d tube, the bore represents only 0.3% of the cross-section. To connect such a tube to another tube/injector/union, there will be no passage unless both bores are highly concentric. Techniques to measure these parameters as well as the bore variance have been described [25]. None of the above capillaries routinely met the above criteria. The commercial PEEK capillary had good circularity but poor concentricity, and i.d. was often up to 50% larger than the stated 25 μm. Only recently have we been able to reproducibly (totaling many kilometers in length over several batches) and consistently obtain capillaries (∼20 μm ϕ , some even ∼10 μm ϕ) made from high-density polyethylene (HDPE) that excel in concentricity and circularity.

Our strategy to make anion exchange OTCs so far has been to create a negatively charged surface first and attach positively charged latex particles to it. PMMA was hydrolyzed to produce -COOH and COP/PEEK was sulfonated to attach $-SO_3H$ in much the same way as Small et al. [26]. The use of these latex particles increases the surface area. Hence, capacity and their known selectivity permit exact prediction of the chromatographic behavior of the OTCs based on available data from packed columns using the same latex [12]. However, HDPE is far more difficult to functionalize compared to the previous polymers. This paper explores different methods to modify the HDPE surface, coat it with cationic latex, and test the separation performance of the OTCs.

2 | Experimental

2.1 | Apparatus

The test system (see Figure S1A) used a high purity 1–5 mM KOH eluent, generated on-line electrodialytically in a macroscale commercial IC system (ICS-2000, www.thermofisher.com) equipped with a 2 μL injector. The injector effluent was split into two streams in a ∼1000:1 ratio, the smaller flow stream proceeding to the OT column, all kept at 30°C in the instrument oven followed by an on-column TraceDec contactless conductivity (admittance) detector (www.istech.at) and a nanoflow mass flow sensor (70–1500 nL/min, MFS-1, www.elveflow.com) both located outside the oven at ∼22°C. Unless otherwise stated, the test sample typically consisted of 0.25 mM each of F^-, Cl^-, NO_2^-, NO_3^- and Br^-. A second test system using a 1 mM sodium benzoate (NaBz) eluent was used in a few experiments, that arrangement is shown in Figure S1B.

Elevated temperature experiments were conducted in a Fisher Isotemp 625 oven (www.fishersci.com), ultraviolet irradiation sources were mercury pen lamps (Analamp, www.BHKinc.com, illuminated length ∼18 cm., one with a quartz housing that allowed both 185 nm and 254 nm output (81-1178-01) and the other without a significant 185 nm output (80-1127-01).

2.2 | Chemicals

Chlorosulfonic acid, concentrated hydrochloric, sulfuric (98%) glacial acetic acid, toluene, acetonitrile, sodium benzoate, benzoic acids and fluoride chloride, nitrate, bromide, and nitrate (all as reagent grade sodium or potassium salts) were from www.fishersci.com. Sodium and potassium persulfate, azobisisobutyronitrile (AIBN), and dicumyl peroxide (DCUP) were from www.sigmaaldrich.com. Reagent-grade styrene, 4-methylstyrene, and *t*-butylstyrene were from www.tcichemicals.com. Ceric ammonium sulfate dihydrate was from www.aquasolutions.org. Quaternary ammonium functionalized AS18 latex nanoparticles (65 nm diameter, anion exchange capacity [AEC] ∼34 zeptoequivalents/particle, ∼20 400 exchange sites of unit charge [7]) were a gift from Thermofisher Scientific.

2.3 | Capillaries

HDPE capillaries (20 μm i.d., 360 μm o.d.) were custom extruded by www.zena-membranes.cz; circularity, concentricity, and bore uniformity of these have been reported [24].

2.4 | Preparation of HDPE Open Tubular Columns. Bore Functionalization

HDPE is resistant to a variety of chemicals and there are temperature limitations; at high temperatures geometrical deformation, even bore collapse occurs. Based on previous experience with sulfonation of COP or PEEK capillaries, we chose sulfonation over oxidation to -COOH groups.

2.4.1 | Direct Sulfonation

Chlorosulfonic and/or sulfuric acid were used with various combinations of concentration, temperature, and time with COP and PEEK [13, 17]. Others have reported sulfonation of PE (polyethylene) with $ClSO_3H$, or H_2SO_4 [27]. (CAUTION: Chlorosulfonic acid is extremely aggressive, explosively reacting with water; it must be used in minimal amounts in a well-vented hood). Dipping an HDPE tube in either conc. $ClSO_3H$ or H_2SO_4 greatly enhanced the binding of a cationic dye like rhodamine B on the exterior. We did not observe geometric deformation or loss of integrity even with high concentrations of either reagent but prolonged exposure to HDPE to conc. $ClSO_3H$ discolored both the capillary and the reagent, eventually leading to blackening, and rendering it rigid and brittle.

Chlorosulfonic acid, 5–20% by volume in glacial CH_3COOH was hence used. The reagent contained in a glass vial within a pneumatic enclosure, was pumped through the capillary at room temperature (rt, 15 min in one direction and 15 min in the reverse direction); this cycle was repeated as desired. Following reagent removal by N_2 pressure (15 min), ~1 M NaOH was pumped through the capillary and left overnight for hydrolysis to sulfonate, then water-washed (≥15 min). With Conc. H_2SO_4, both ends of an acid-filled capillary was sealed with hot-melt polyolefin, then left at 80 or 100°C for 24 h, sequentially flushed with N_2 and water for (≥15 min ea.).

2.4.2 | Sulfonation of a Grafted Aromatic Monomer

Grafting a more reactive monomer on a polymer matrix and then functionalizing the graft is common. Ion exchange groups are introduced to a fluorocarbon matrix by radiation grafting vinylbenzyl chloride and then sulfonating or quaternizing the latter [28]. Grafting an aromatic moiety on HDPE enables the introduction of functional groups under mild conditions [29, 30], preserving the integrity of the capillary. Such grafting on HDPE can be accomplished by photo [26, 31–33], chemical [25, 27, 34], and thermal grafting.

2.4.2.1 | Photografting. HDPE capillaries were soaked in 4-methylstyrene (rt, 1 h), and the tubes swelled perceptibly. An ellipsoidally coiled capillary (long axis ~18 cm, same as the lamp length) of the lamp, was placed parallel to the lamp and irradiated (10 cm away, 2 h). The manufacturer's lamp intensity specifications are given in the Supporting Information. Following a N_2 flush (15 min), 96–98% sulfuric acid was pumped through the capillary (rt, 15 min).

2.4.2.2 | Chemical Grafting. Following Sherazi et al. [34], hydroxylation of the surface was conducted in a sealed capillary (3% (w/v) $K_2S_2O_8$, 80°C, 2 h). Following water and methanol wash, this was latex-coated without sulfonation. Solubility of $K_2S_2O_8$ is limited; we switched to $Na_2S_2O_8$ (30% w/v, pumped @ ~ 250 nL/min, 2 h, @ 65°C in column oven. Following water and methanol wash (rt, 10 min ea.). Following [34], radical generation and polymerization were carried out sequentially. A solution of 0.05 M $Ce(NH_4)_4(SO_4)_4$ was pumped through the capillary (65°C, 5 min) N_2 purged and 4-methylstyrene (50% v/v in toluene) was then pumped (rt, 15 min). After toluene wash and an N_2 purge, the capillary was sulfonated (filled with ~6 M H_2SO_4, rt, 10 min) and then water washed.

As an alternative, simultaneous radical generation and polymerization were carried out by filling the hydroxylated capillary with a solution of 20% v/v styrene and 0.07% w/v AIBN in toluene, sealing both ends with hot-melt polyolefin and allowed to sit for 24 h @ 80°C. After unsealing and a toluene purge (rt, 15 min), N_2 purge, and water rinse (5 min ea), sulfonation was done by pumping 96 % H_2SO_4 (rt, ~2 min).

2.4.2.3 | One-Component one-Step Thermal Grafting. An aromatic substrate can be thermally grafted on HDPE in a single step using a diaryl peroxide; thermal cleavage of the O-O bond leads to highly reactive radicals [35]. DCUP (Figure S2A) is used as an initiator for making PE, linking unsaturated polymers, and modifying the viscoelastic properties of PE [36]. Presently, we used DCUP; the 2-Phenyl-2-propanol radicals produced graft onto HDPE (Figure S2B). The procedure was identical to that used with styrene and AIBN above except the fill solution was 25% w/v DCUP in toluene.

2.4.3 | Latex Coating and Capacity Measurements

AS-18 latex suspension (diluted 10x immediately before use and 0.45 μm membrane filtered) was pneumatically pumped through the (sulfonated) capillary (30 min), then water-washed (10 min). Ion exchange capacity measurements were carried out by conductometric frontal displacement chromatography [7]. For cation exchange capacity (CEC) measurement, alkalimetric on-column conductometric titration was used. The column is rendered to the H^+ form by pumping 1 mM HCl. Water-wash is followed by pumping a known concentration (~5 mM) of electrogenerated KOH at a measured flow rate. At the endpoint, unreacted KOH exits the column, causing a sharp rise in conductance. The CEC is given by $[KOH]*F_{KOH}*$time to the endpoint. AEC is similarly measured by converting the column to OH^- form with 5 mM KOH. Following a water wash, 1 mM HCl is used and the time for the conductivity to rise abruptly is measured.

2.5 | Mass Spectrometric Experiments

An Orbitrap Exploris 120 mass spectrometer was used in this work coupled to a Vanquish binary pump F and a thermostated column oven with a 6-port, 2-position valve (all from Themofisher.com). A sulfonated (H_2SO_4, 80°C, 24 h) and an untreated HDPE capillary were alternately switched (1 h ea.) between the pump and the MS

for 72 h with mass accuracy calibration every 2 h. As eluent, 2 mM NH_4OAc −0.1% v/v HOAc in methanol was used @ 1 μL/min going to a low-flow electrospray source. Negative ion mass spectra were acquired in the m/z 62–400 and 400–3000 ranges (resolution 120 000 at 200 *m/z*). Peak identification was based on the high-accuracy mass data (deviations ≤1. 2 ppm). Further details appear in the Supporting Information.

3 | Results and Discussion

3.1 | Chromatographic Data Format

Anion chromatography is usually conducted in the suppressed form. However, for the present column dimensions it is easier to operate without a suppressor. The added dispersion from the suppressor and differences in each column-suppressor-detector connection would complicate comparisons. Column performance can be judged just as well in the SCIC mode. Initially, we used a manually prepared benzoate eluent directly in the nL/min flow scale. In SCIC the signals arise from the mobility difference of the analyte and the eluent ion. As our test analyte ions are all more mobile than benzoate, all analyte signals are positive. The later experiments were conducted with electrogenerated hydroxide, a slightly weaker eluent ion than benzoate on an AS-18 latex (4 mM KOH behaves similarly to 2.5–2.8 mM benzoate for most monovalent anionic analytes). This required a split-flow setup but was important to do as hydroxide was ultimately intended eluent. Hydroxide is the highest mobility anion; all anionic analytes provide a negative conductance/admittance signal in the SCIC mode. Finally, fluoride is poorly retained on the AS-18 phase; it elutes in the void in most of these chromatograms. Visualizing fluoride as a distinctly separated entity is itself a testament to the capacity and separation ability of the column.

3.2 | Ion Exchange Capacities in Perspective

The topological "polar surface area" of a -SO_3H group is estimated to be 5.44×10^{-13} mm^2 (www.chemicalize.com). While 100% surface coverage is all but impossible (if for no other reason, than electrostatic repulsion), this would amount to ∼3 picoequivalents (peq)/mm^2. While the inner surface area of the capillary is unlikely to be atomically smooth and higher sulfonate group densities are easily attainable, in our experience CECs 3 peq/mm^2 or even less is sufficient for full latex attachment (see below). The projected cross-sectional area of a 65 nm latex particle is 3.3×10^{-9} mm^2. Allowing for a 90% coverage (hexagonal close packing), monolayer coverage implies $\sim 2.7 \times 10^8$ latex particles/mm^2, amounting to an AEC of ∼10 peq/mm^2. We have only used the nominal i.d. of the column to calculate the area above, without accounting for surface roughness; after sulfonation it is high;y unlikely that it is smooth.

Finally, in measuring AEC, HDPE is a linear polymer, and chain breakage is likely during sulfonation. If an oligomeric sulfonate/carboxylate molecule is dislodged, it will attach to the latex, effectively poisoning an anion exchange site directly and thus leading to an underestimation of the overall AEC. If the poisoning moiety is a carboxylate, however, titratable AEC may not be affected but chromatographic performance will suffer. Representative titration plots for both AEC and CEC measurements are shown in Figures S3A and S3B.

3.3 | Summary Results

In reporting capacities, we report the specific capacities (equivalents per unit area) as column lengths were not identical rather than absolute capacities (enumerated in the chromatograms). Similarly, the chromatograms use the retention factor as the x-axis rather than retention time; this normalizes both for differences in length and flow rate. The eluent was 4 mM KOH unless otherwise stated.

3.3.1 | Chlorosulfonation

A sulfonyl chloride is first formed. Hydrolysis of the sulfonate is greatly accelerated by a base. Sulfonation by 5% v/v $ClSO_3H$ in Gl. HOAc (rt, 2 h, OTC - 1) produced a CEC of 1.80 peq/mm^2; CECs were too low to measure well for smaller duration treatment. Latex coating did result in some latex attachment but the separation was poor and fast elution suggested a low overall capacity (see below, Figure 1).

Use of 10% and 20% v/v $ClSO_3H$ in Gl. HOAc (rt, 30 min) respectively produced CECs of 3.9 ± 0.2 and 5.7 ± 0.1 peq/mm^2; measured AECs after latex coating were 31.0 ± 0.9, and 35.2 ± 0.2 peq/mm^2 (OTC-2,3 respectively). As seen hereinbelow, the maximum specific AEC reached is in the 26–51 peq/mm^2 range The AEC is not proportional to the CEC, rather, a minimum CEC of ∼3 peq/mm^2 is necessary to get an AEC in this range. Excessive sulfonation does not increase the AEC and may be deleterious due to subsequent oligomer leaching. Both OTC - 2 and OTC - 3 have about the same total AEC and resolve the analytes, but the specific capacity and *k* values are greater for OTC - 3. Note that because of differences in length and flow rate, column efficiencies cannot be directly compared; OTC - 3 provides a lower plate height) than OTC - 2. As the LODs in SCIC are modest, the absolute amounts injected in these experiments are enough to tax the column capacities for optimum plate heights: higher specific capacities thus also result in a lower plate height.

3.3.2 | Sulfonation With Sulfuric Acid

Sulfonation with H_2SO_4 led to columns quite comparable to those made with the more hazardous $ClSO_3H$ (Figure 2). The latter was not further used.

3.3.3 | Photografting

OTC–6 (HDPE soaked in 4-methylstyrene rt, 12 h; 254 nm exposure 2 h; H_2SO_4, rt, 15 min, latex coated) exhibited an AEC of 46 ± 1 peq/mm^2. OTC–7 differed from OTC–6 only in that the UV source also had the 185 nm line. OTC–8 and 9 were OTC–7 controls. OTC–8 had no 4-methylstyrene present; OTC–9 was OTC–8 without sulfonation.

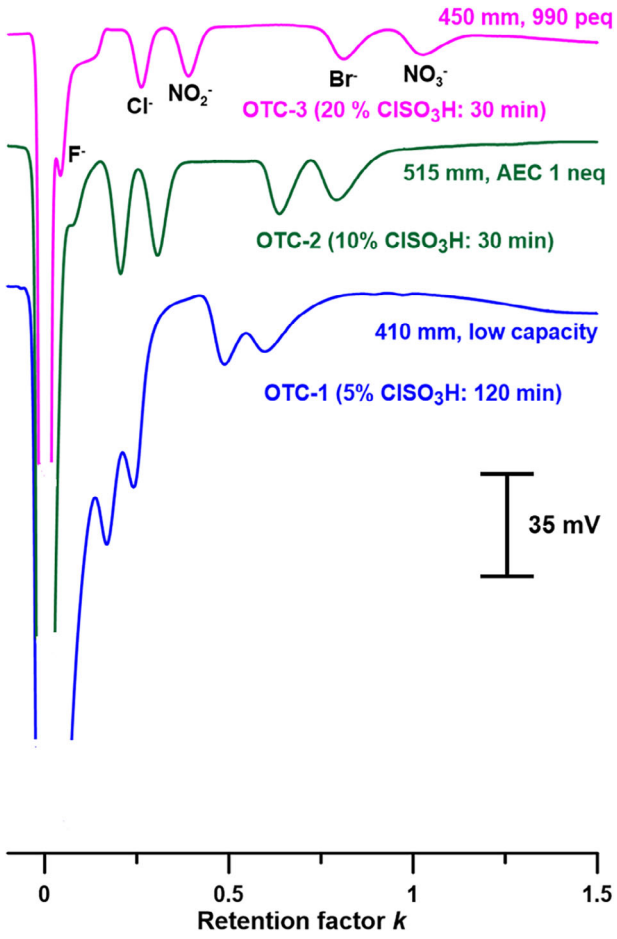

FIGURE 1 | Illustrative chromatograms, F^-, Cl^-, Br^-, NO_2^-, and NO_3^-, 0.25 mm ea., 4.00 mM KOH eluent; capillary columns OTC - 1 through OTC - 3. The capacities given here and in subsequent chromatograms are capacities for the whole column. Specific capacities can be found in Table 1, and/or computed from the length and the total capacity given.

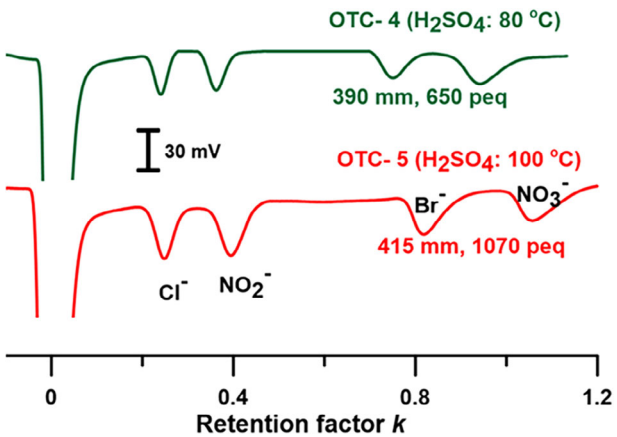

FIGURE 2 | Illustrative chromatograms with OTC - 4 and OTC - 5 where the initial sulfonation was made by elevated temperature treatment with H_2SO_4. The four non-fluoride ions are well separated in both columns.

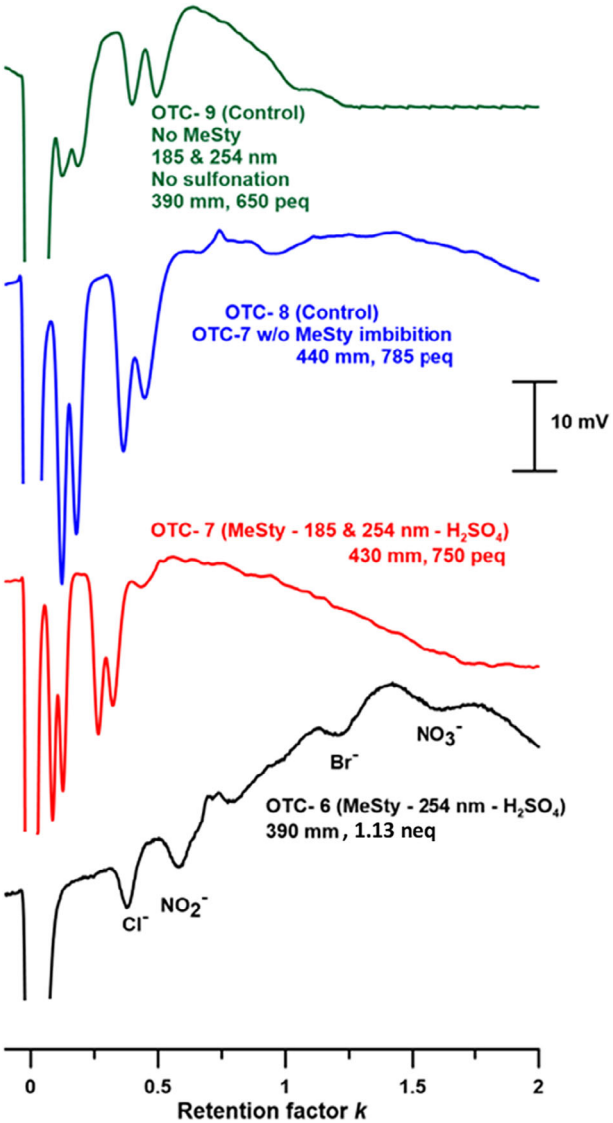

FIGURE 3 | Illustrative chromatograms, column photografted 4-methylstyrene (MeSty) and sulfonated (OTC - 6), others are controls. See text.

While the OTC–6 baseline resolved all four non-fluoride ions, all others had much lower AEC (26.4–28.4 peq/mm^2) and could not resolve the analytes (Figure 3). The low OTC–7 capacity suggests that 185 nm radiation may inhibit grafting, and/or break the grafted C—C bonds. All the 185 nm radiated columns material become more rigid and brittle. Latex attachment in OTC - 9 suggests that the C—C bonds in HDPE itself are broken by UV radiation, and in the presence of ozone epoxidation occurs leading to —COOH groups. Note that the cationic latex attaches poorly to 254 nm irradiated HDPE (not sulfonated) and hardly at all to native HDPE.

3.3.4 | Chemical Grafting and One-Step Homogeneous Thermal Grafting

As a first step to preparing OTC - 10, hot aqueous persulfate (3% $K_2S_2O_8$, 80°C, sealed cap) treatment was used to introduce hydroxyl groups [34]. Whether these behave as weakly acidic

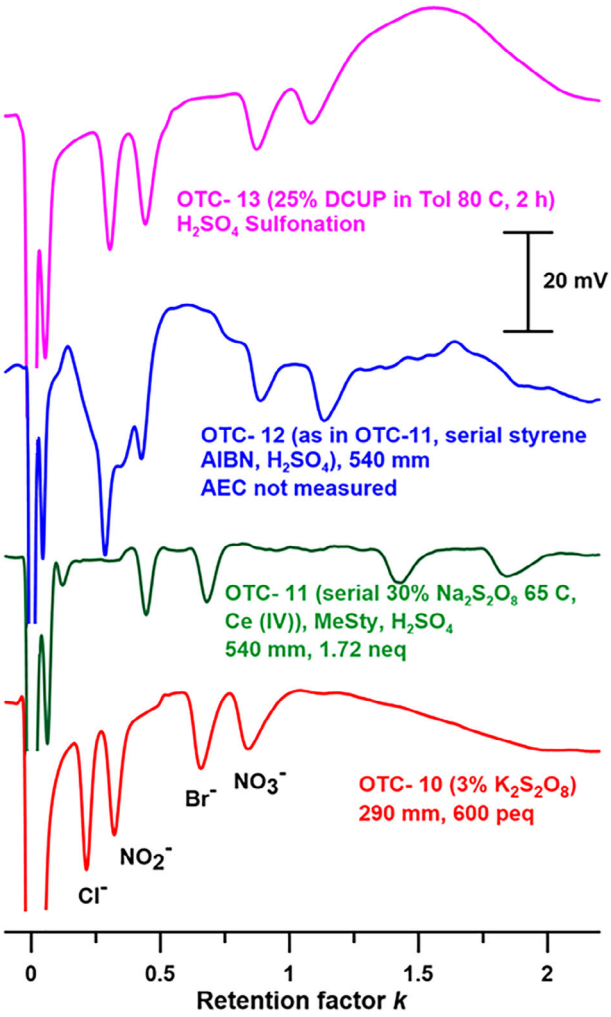

FIGURE 4 | Illustrative chromatograms chemically grafted columns. All of the columns except OTC - 10, the lowest capacity column in this set, not only could baseline resolve the four analytes; fluoride begins to appear as a response separate from the water dip.

groups themselves or are oxidized to —COOH, the measured CEC was significant (9.8 ± 0.9 peq/mm^2), provided an AEC of 33.1 ± 0.7 peq/mm^2 on latex coating, and allowed baseline separation of the non-fluoride anions (Figure 5). OTC - 11 used a similar persulfate-induced hydroxylation step followed by serial treatment with Ce(IV) and 4-methylstyrene. After H_2SO_4 sulfonation and latex coating, the AEC was 50.6 ± 0.4 peq/mm^2, the highest observed in this work. Accordingly, this column exhibited the highest *k* values for all ions, and more than baseline resolved them. Except for OTC - 10, all the columns in Figure 4 could resolve fluoride from the void. OTC - 12 started with the same hydroxylation step as OTC - 11, but styrene was grafted in one step using a toluene solution of styrene and AIBN initiator. Finally, OTC - 13 was grafted in one step using DCUP, the grafted monomer as well as the radical initiator. The AECs of OTC - 12 and 13 were not specifically measured but based on the retention factors, the specific capacities were in the order OTC - 11 > OTC - 13 > OTC - 12 > OTC - 10. Retention factors are generally related to specific capacities; this was found to be so, as demonstrated for nitrate (Figure 5).

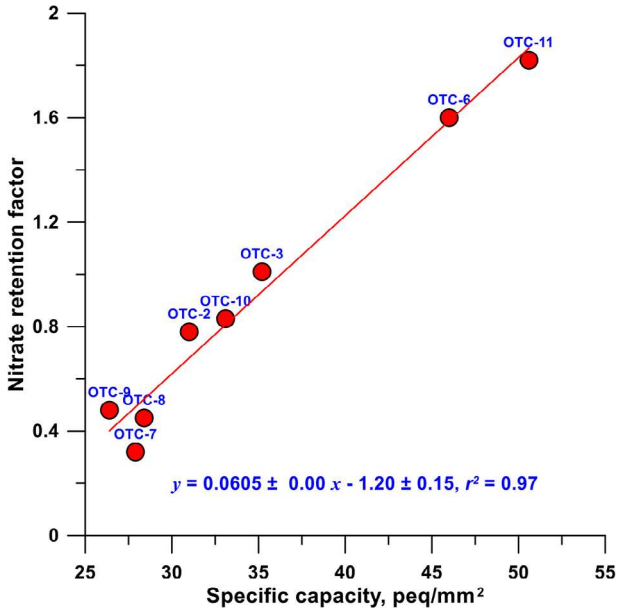

FIGURE 5 | Nitrate retention factor as a function of column-specific capacity.

3.4 | Longevity of Latex-Coated OTCs

While OTCs do not develop a column void, performance may degrade over time from loss of functional group(s) or (quasi)-irreversible sorption of highly retained analytes, present OTCs may also lose capacity via latex detachment. Our present efforts originated with the goal of practical in-situ analysis of soil extracts on extraterrestrial bodies, for example, Mars [21]. Power requirement, size, weight, eluent, and sample volume requirements are among the primary considerations where OTL(I)C systems would be much preferred over their packed column counterparts. Mars and several other extraterrestrial targets also represent sub-freezing environments. Packed columns do not survive freeze-thaw cycles, while it has been demonstrated that a latex-based OTC can be repeatedly dried, frozen, thawed, wetted, and unaffected [8]. In a typical deployment, available power is limited; a given analytical instrument in a multiple-instrument suite sits idle most of the time. As to soil extracts, a representative sample requires a minimum sample size; limitations on how much extractant can be brought aboard limit how many samples can be analyzed. In the last and only time wet analysis had been performed extraterrestrially, the instrument allowed for four sample analyses, including one control.

While longevity was not a major concern for the problem at hand, this is undoubtedly important in many applications. Indeed, it is because the columns produced by direct sulfonation tended to lose capacity quickly that we attempted so many other ways. It is not likely that a C—H bond in HDPE will be simply replaced by a C—SO_3H bond. Extended treatment with H_2SO_4 (and even more with $ClSO_3H$) results in yellow to brown to black discoloration and becomes gradually brittle. Selective oxidation of the hydrogen likely leads to charring/carbonization. Once the C—C chain is broken, oxidation to —COOH, —OH, and/or sulfonation can take place at the terminus. The probability that the other end of the chain would be similarly broken increases with increasing

exposure to these oxidizing acids. Further, if an —OH group is formed, given the reaction conditions, water elimination to form olefinic linkages will be highly likely If the chain is free at both ends. Then only van der Waal's forces remain to keep the chain attached to the polymer. The tendency of such a chain, especially when it contains highly hydrophilic groups at one or both ends, to come off the hydrophobic surface will increase in contact with a (flowing) aqueous eluent. If such an oligomeric fragment does detach, more likely than not, the acidic terminus will attach to a cationic site while the hydrophobic chain will interact with the latex skeleton. Once attached, the presence of the hydrophobic chain may limit analyte ion access to the neighboring ion exchange sites as well.

3.5 | Mass Spectrometric Data

Evidence for acidic fragments (bearing -COOH and/or -SO_3H groups) washing uniquely out of a sulfonated capillary was observed using high-resolution MS (see experimental arrangement in Figure S4 and a representative output in Figure S5). Details are given in Table S2; the m/z values of the six most prevalent and persistent ions observed in the leachate of the directly sulfonated HDPE capillaries were 235.1342, 110.9758, 257.0339, 307.1916, 179.0716, and 291.1968, with the most likely empirical formulae of the negative ions, respectively, based on accurate masses respectively being $C_{14}H_{19}O_3$, $CH_3O_4{}^{32}S$, $C_7H_{13}O_8{}^{32}S$, $C_{18}H_{27}O_4$, $C_{10}H_{11}O_3$, $C_{18}H_{27}O_3$ (Table S3). Possible structures of the 6 ions are provided therein. Table S2 lists the empirical formulae of several ions that were identified in the leachate and how their concentration changed over time. Several ions observed initially disappeared after prolonged washing (Figure S6), confirming the importance of the wash step prior to latex attachment; see Supporting Information for further details. Wash patterns of three persistent ions in the leachate are shown in Figure S7). The second most abundant ion is unambiguously hydroxymethanesulfonate, more commonly referred to as the formaldehyde-bisulfite adduct. No attempt at calibration was made; ion abundance is not always indicative of concentration. Sulfonates, for example, tend to ionize more readily than carboxylates. The presence of hydroxymethanesulfonate confirms that H_2SO_4 is acting at least in part as an oxidizer, producing S(IV) as a byproduct that is reacting with formaldehyde, the likely oxidation product of a terminal olefin. Several of the likely structures involve concatenated unsaturated linkages (which can account for the observed discoloration) that are likely produced by hydroxylation (see for example the proposed structure of the observed fragment $C_7H_{13}O_8{}^{32}S$) and subsequent water elimination. A carboxylic acid functionality is likely produced by the oxidation of a terminal -OH, via an aldehyde intermediate.

3.6 | Column; Longevity

3.6.1 | Longevity and Pre-and Post-Latex Wash Treatments, Rejuvenation

The longevity of the columns prepared in different ways is depicted in Figure 6. The solid lines shown are first-order decay best fits, every 5th or 10th datum is plotted for clarity. Figure S8 shows all the points. In Figure 6, aside from the specific column

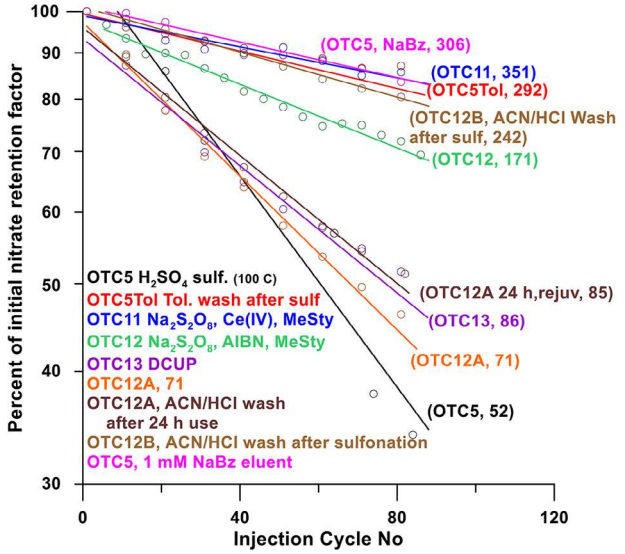

FIGURE 6 | Column longevity for columns prepared in different ways. To avoid excess clutter, the retention data for every 5th or 10th chromatogram is shown. The lines show the best fit to a first-order decay profile. The last number in parenthesis indicates the projected number of injections for the column to lose 50% of the original retention based on a first-order decay projection. Eluent is 4 mm KOH, except as noted. Note logarithmic ordinate.

listed (OTC—*n*, Table 1), also listed is the number of injections projected for 50% retention loss (projected half-life, PHL). These numbers are likely underestimates: retention loss is not exactly first order, the degradation rate gradually slows.

Consider the base case: H_2SO_4 sulfonation @ 100°C, water-washed and latex-coated (OTC - 5, black[1]). The PHL is only ~50 injections. However, if prior to latex coating, the column was thoroughly washed with toluene, which swells the HDPE matrix and is possibly effective in removing the fragments, the longevity is increased by almost 6x (OTC - 5Tol, red). For the next several columns (OTC - 11 [blue], OTC - 12 [green], and OTC - 13 [purple]), no special wash or treatment was carried out. Of these, OTC - 11 displayed the highest longevity with a PHL of >350 injections while OTC - 13 was the worst, with a PHL of 86 injections; OTC - 12 was intermediate with a PHL of 171 injections.

There can, however, be substantial variations in columns, when prepared the same way. Of multiple OTC - 12 type columns prepared, OTC - 12A (orange) showed the least stability with a PHL of 71 injections. After some 88 injections, when <44% of the column capacity remained, the column was washed with 0.2 M HCl in 80% acetonitrile for 1 h. Among many concoctions tried for rejuvenation, this wash solution performed the best. We reasoned that if the poisoning entity is a carboxylate, it will be rendered neutral, and the acetonitrile will decrease any hydrophobic interaction. This treatment restored the initial capacity of the column and in fact slightly improved the PHL to 85 (OTC12A, dark brown). Figure S9 shows the actual chromatograms. Based on this experience, we washed another OTC - 12 column with this wash solution, after sulfonation, and prior to latex coating, this improved the PHL markedly to 242 (Figure S9 shows the chromatogram).

TABLE 1 | Columns prepared in different manners and performance parameters.

Column designation, length[a]	Generic approach	Fabrication details	Sp. CEC peq/mm^2	Sp. AEC peq/mm^2	Brief performance desc. figure reference
OTC - 0, OTC - 00	Chlorosulfonation	5:95 $ClSO_3H$:HOAc, 22°C, 30, 60 min	<LOQ[b]	NM[c]	N/A[d]
OTC - 1, 450 mm	Chlorosulfonation	5:95 $ClSO_3H$:HOAc, 22°C, 120 min	1.8	NM, low	Poor separation, rapid elution, Figure 2
OTC - 2, 515 mm	Chlorosulfonation	10:90 $ClSO_3H$:HOAc, 22°C, 30 min	3.9 ± 0.2	31.0 ± 0.9	Four anions fully resolved, Figure 2
OTC - 3, 450 mm	Chlorosulfonation	20:80 $ClSO_3H$:HOAc, 22°C, 30 min	5.7 ± 0.1	35.2 ± 0.2	Four anions fully resolved, slightly higher capacity relative to OTC-2 leads to slightly slower separation: plate ht for Cl^- 98 µm and 73 µm for OTC-2 versus OTC-3, Figure 2
OTC - 4, 390 mm	Sulfonation w/H_2SO_4	Sealed cap, Conc. H_2SO_4, 80°C, 24 h	NM	26.5 ± 0.5	Four anions fully resolved, Figure 3
OTC - 5, 415 mm	Sulfonation w/H_2SO_4	Sealed cap, Conc. H_2SO_4, 100°C, 24 h	NM	41.0 ± 1.0	Four anions fully resolved, Figure 3
OTC - 6, 390 mm	Photograft	Swell overnight in 4-methylstyrene, 2 h 254 nm irrad., H_2SO_4 sulfonation	NM	46.0 ± 1.0	Four anions fully resolved, Figure 4
OTC - 7, 433 mm	Photograft	Swell ovenight in 4-methylstyrene,184 & 254 nm irrad., H_2SO_4 sulfonation	NM	27.9 ± 1.0	Test anions not fully resolved, Figure 4
OTC - 8, 440 mm	Photograft control-1	As in OTC - 7, but no 4-methylstyrene	NM	28.4 ± 0.8	Test anions not fully resolved, Figure 4
OTC - 9, 390 mm	Photograft control-2	As in OTC - 8, and no sulfonation	NM	26.4 ± 0.5	Test anions not fully resolved, Figure 4
OTC - 10, 290 mm	persulfate hydroxylation	3% Aq. $K_2S_2O_8$, sealed cap., 80°C, 2 h No sulfonation	9.8 ± 0.9	33.1 ± 0.7	Four anions fully resolved, Figure 5
OTC - 11, 540 mm	30% $Na_2S_2O_8$ hydroxylation, Chemigraft	30% $Na_2S_2O_8$ flowed through (65°C, 2 h), Serial Ce(IV), 4-methylstyrene in Tol. H_2SO_4 sulfonation @ 22°C	NM	50.6 ± 0.4	Four anions fully resolved, Figure 5; highest capacity and retention of tested columns
OTC - 12, 540 mm	As OTC–11	80:20 styrene: toluene in sealed cap., AIBN initiator, 80°C, 24 h, 2 min H_2SO_4 sulfonation	NM		Four anions fully resolved, Figure 5, but poor chromatogram, inexplicable reasons
OTC - 13, 270 mm	Direct chemigraft DCUP	25:75 DCUP: toluene in sealed cap. 80°C, 24 h, 2 min H_2SO_4 sulfonation	NM		Four anions fully resolved, Figure 5, this is a very short column.

[a]This is the separation length and injector-detector distance.
[b]Below quantitation limit.
[c]Not measured.
[d]Not applicable.

3.6.2 | Eluent Effect on Longevity

A freshly prepared OTC - 5 column (no special wash protocol after sulfonation, PHLof 50, lowest of all columns) was used with a NaBZ eluent. A 1.00 mM eluent concentration was found optimum for the baseline separation of the four analytes (Figure S10). With this eluent, the PHL was 306 (OTC5, NaBz, pink, Figure S10), a 6x improvement compared to the use of a 4 mM KOH eluent (OTC5, black). Possibly most of the detached fragments contain -OH or -COOH terminations which acquire a negative charge at high pH and make it easier for the fragment to attach to the cationic latex. This is supported by the ability of an HCl- acetonitrile cocktail to rejuvenate the columns. In IC, log k is typically proportional to log [E], [E] being the eluent concentration [11]. Examining a linear relationship based on three data points is admittedly a stretch but based on the retention data for chloride, nitrite, and bromide in Figure S10 and the log [benzoate]—log k plots in Figure S11 with superimposed k values for a 4 mM KOH eluent, the eluting power of the latter is equivalent to that of 2.6 ± 0.1 mM Na-benzoate. While the resolution of the chloride—nitrite pair increases from 1.10 to 1.35 to 2.16 for 2 to 1.5 to 1.0 mM NaBz eluents. Meanwhile, with a 4 mM KOH eluent, the resolution is 1.78, which would be considered baseline resolution. In part the much faster elution while maintaining resolution is possible because the efficiency is much better with the hydroxide eluent, and the plate height is 6+ times lower compared to 1 mM NaBz. Table S1 provides these and other chromatographic parameters for the various columns.

4 | Concluding Remarks

The present goal was to functionalize the bore of an HDPE capillary to be anionic and then electrostatically attach cationic latex nanoparticles to make anion exchange OTCs. Direct sulfonation with $ClSO_3H$ or H_2SO_4 did sulfonate the surface. However, it apparently also leads to oxidative chain scission, making fragments with acidic termini, that later wash off and poison the cationic sites. Although we successfully grafted styrene, 4-methylstyrene, and 2-phenyl isopropanol to HDPE, sulfonation even under mild conditions did not prevent loss of capacity over time. Importantly, we observed that washing the capillary thoroughly prior to latex coating, particularly with acidic acetonitrile, greatly reduced the column capacity loss rate for capillaries regardless of prior grafting. Interestingly, such washing also restored the capacity of columns that lost capacity over a period of use. The observed exchange capacity was the highest and the decay rate lowest for a column grafted with 4-methylstyrene, produced by serial persulfate hydroxylation, radical generation with Ce(IV), and exposure to 4-methylstyrene. Such a process, including a thorough wash (during which ultrasonication has recently been found to be helpful) before latex coating, will merit further study.

Author Contributions

Purnendu K. Dasgupta conceived and directed the work, wrote the final manuscript, and made the final figures, Enas Yousef carried out the experimental work and provided the first draft of the manuscript, including graphics, Seth A. Horn carried out some of the experimental work, and Charles Phillip Shelor provided day-to-day help, supervision in the lab as well as all the MS data.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Any and all data that form the basis of this paper are available from the corresponding author on request.

Consent

Written informed consent for publication was obtained from all participants.

Endnote

[1] All references to color pertain to the web version of the article

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.