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Gradient nanopump based suppressed ion chromatography using PEEK open tubular columns

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ABSTRACT

The progress of open tubular suppressed ion chromatography is reviewed from a broad perspective. Then a suppressed open tubular ion chromatography system, composed mostly of commercial components, is described: A splitless gradient-capable nanopump providing eluent delivery in the 10–1000 nL/min regime, a 7-nL injector integral to the pump, an anion exchange column based on a 25 μm i.d. poly(ether ether ketone) (PEEK) capillary, and an on-column admittance detector. The membrane suppressor was custom fabricated. Good gradient separation of seven common anions in 9 min is demonstrated using a manually prepared hydroxide eluent.

1. Introduction

Since its inception in 1975 [1], ion chromatography (IC) has undergone many significant refinements and is presently the benchmark approach to anion analysis. Commercial IC began with 3 mm i.d. packed glass columns [2]. Current practice dominantly uses 2 and 4 mm i.d. packed Poly(ether ether ketone) (PEEK) columns, with some columns available in 3 mm i.d. [2]. For some time now, "capillary" instrumentation that utilize 0.4 mm i.d. PEEK columns have been commercially available. In this laboratory, smaller capillaries have been explored for more than two decades, beginning near-simultaneously both in open tubular (OT) [3] and packed column [4] formats.

Miniaturized analysis systems not only reduce sample and eluent consumption, often it provides higher efficiencies. However, by the time one reaches the OT scale, current optimum practice being columns of ≤ 25 μm i.d., in all of the individual aspects of pumping, eluent generation, injection, column fabrication, suppression, and detection, many hurdles must be overcome. While major strides have been made such that achieving 100,000 plates/m is now more or less routine, virtually all individual components were custom designed. This obviously creates a barrier towards the general pursuit of OTIC. Below the nature of each of these individual elements, and the usability of commercially available components for the purpose, as well as their advantages/disadvantages, are examined. Finally, an OTIC system, comprising a commercially available gradient nanopump with an integrated fixed volume injector, a separation column made from a commercially available PEEK tubing, and a commercial admittance detector, is presented. The preparation of the PEEK column is described in some detail. Overall performance, with a laboratory-made suppressor appropriate for the scale, is described.

1.1. Pumping system

At first sight it may appear that the requirements of relatively little pressure (below 100 psi, 7 atm) and minuscule (sub-μL/min) flow rates are not particularly demanding of a pumping system. In part, this has been true for us: the efforts began with simple pneumatic gas pressure pumping and is still preferred [5,6]. However, a closer examination reveals some limitations. First, the flow rate in a constant pressure pumping system is inversely proportional to the fluid viscosity, which changes 2%/°C for water or aqueous solutions around room temperature. For acetonitrile and methanol, the corresponding values are 1–1.5%/°C but much higher for mixed solvents. Without thermostating, the resulting flow rate changes is tolerable in an airconditioned laboratory setting but would be impractical in an uncontrolled field environment, an important application envisioned for such an instrument. Second, flow rate constancy in isobaric pumping requires constant backpressure. Elastomeric membrane-based devices with small bore passages, such as an eluent generator or the suppressor, represent flow resistors that are susceptible to change over time, especially when flow is discontinued for a while and then the system is restarted. Third, to be really useful, a single constant pneumatic pressure or a manually changed pressure regula-

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tor is not very practical because eluent delivery and injection are often connected. In one major mode, injection volumes are controlled by a combination of pressure applied to the eluent reservoir and the injection period can be as short as 100 ms, requiring sophisticated fast acting digital pressure regulation and appropriate control software.

Of course, there are alternatives to pneumatic pumping. Syringe pumps seem an obvious choice; with a syringe of small enough volume and with high enough resolution of the associated motor drives, achieving the required flow rates is not difficult. Analog, nearly pulseless, motor drives have recently become available (www.cetoni.com). Two practical difficulties arise, however. All commercial small volume syringes are made of glass, susceptible to silicate leaching by an alkaline eluent and the resulting contamination. Smallest non-glass syringes have a relatively large capacity of 3 mL.

The Milligat LF (www.globalfia.com) is a unique 4-barrel syringe pump with rotating barrels designed for especially low flow rates [7]. With a 100-μL syringe capacity and a 22.56× geared down stepper motor capable of 256 μsteps per 0.9 degree step, the theoretical dispense rate is 43 pL/μstep. In practice, while < 200 nL/min flow rates are attainable, large flow rate disruptions occur when the flow source switches from one syringe to another, although it is possible to plan for this in advance.

Peristaltic pumps generally cannot meet OTIC needs: the needed pressures are near the capability limits; the flow rates are too small even for the smallest pump tubes, requiring rotation rates so slow that resulting pump pulsation occur at a low, difficult-to-filter, frequency.

In creating an eluent gradient in OTIC with two pumps, the difficulty is in forming a sufficiently low volume union. In part, this can be circumvented by a split-flow system as has been done repeatedly, e.g. by Liu and co-workers [8,9] for ultra-small OT columns with inner diameters as low as ≤ 2 μm. While it is thus possible to use both the pumping and injection system of a more conventional scale chromatography system and split its output to the OT system, a very large fraction is wasted. Consider the pumping system of a commercial packed capillary IC, that operates at a typical flow rate of 10 μL/min. Some 98% of the flow will be wasted for an OT system operating at ≤ 200 nL/min. The larger problem is that any change in backpressure, fluid viscosity, etc., may change the actual flow rate to the OT system, creating problems in a field usable chromatograph.

In 2015, in conjunction with engineers from Valco/VICI Instruments, scientists from Brigham Young University described a gradient nanopump comprising two integrally joined pumps of 32-μL stroke capacity coupled to a fixed 60-nL volume injector capable of operation at 8000 psi intended for portable HPLC [10]. The pumps use 1.5 mm diameter pistons with 2,048,000 μsteps needed for 1 inch linear movement, from which a resolution of ~28 pL/μstep can be inferred. Subsequent improvements included a 1 mm diameter piston that allowed doubling the pressure capabilities and reducing the minimum microstep size to 10 pL; the gradient dwell time was also reduced in half [11,12]. These and further developments [13,14] have led to a portable HPLC unit, now commercially available [15].

1.2. Electrodialytic eluent generation

On-line high purity eluent generation [16] is a unique attribute of the current IC practice but was not practiced in this work. The reader is referred to a publication that achieves electrodialytic eluent generation through enhanced water dissociation inside a bipolar membrane [17] and an effort to achieve the same in the nanoscale [18].

1.3. Injection

In our efforts, since the earliest no-split OTIC system [5], a time and pneumatic pressure controlled hydrodynamic injection technique has been relied on. This has the advantage of being able to vary the injection volume over a large range. The drawback is potential irreproducibility from changes in temperature and thence flow rate, or variations in sample viscosity. Traditional fixed volume injectors can offer only a single injection volume (albeit time-based partial loop injection is possible), but excellent injection reproducibility is assured. Presently, the lowest volume injector has an internal loop volume of 4 nL (www.vici.com). This volume is too large for poorly retained analytes but overall offers acceptable performance [18].

1.4. Separation columns

By far the most common offering in small diameter tubing are capillaries made out of fused silica. High quality fused silica capillaries are available with excellent consistency of inner and outer diameters over substantial lengths and a high degree of concentricity (www.molex.com). The latter is important if two capillaries ever need to be connected with minimum dead volume. The importance of concentricity can be understood if one considers that in a 25 μm i.d., 375 μm capillary, the bore represents 0.4% of the cross-sectional area. Our OTIC work also started with silica capillaries [3]; others have utilized silica capillaries for OTIC as well [19]. Silica capillaries have surface silanol groups; organic polymer tubes similarly have surface –COOH groups. Such groups promote attachment of positively charged latex particles, facilitating fabrication of anion exchange columns. If synthesis of submicron latex particles are not possible, commercial anion exchange resins can be ground cold (e.g., with small pieces of dry ice) to very fine dimensions. When these are shaken in water and allowed to remain undisturbed, the particles that remain suspended after several hours are of usable dimensions and can be decanted off and passed through the prospective column for attachment to the walls. In applications that do not require strongly alkaline eluents, e.g., in non-suppressed IC, silica columns perform very well.

Suppressed anion chromatography, however, is the preferred mode of operation, and silica-based columns are not stable in strongly alkaline eluents. No stability studies have been conducted with carbonate-bicarbonate eluents, but in flowing hydroxide eluents, any latex coating is removed in a matter of hours. In contrast, such coatings are indefinitely stable with a lower pH eluent like Na-benzoate or Na-salicylate used in the nonsuppressed mode. Polymeric capillaries do not have this problem and can often be chemically functionalized to have hydrolytically stable strongly acidic groups that strongly bind positively charged latex particles.

The quest for polymeric capillaries in our work began in the early 80s, polymeric capillaries in the desired inner diameter (10–25 μm) were not commercially available. A tenth of the laboratory startup funds allocated to the corresponding author, well above his monthly salary, went to custom-extruded polystyrene capillaries. The best that could be made at the time was 125 μm in i.d. (see Fig. S1 in the supporting information (SI)). Nothing chromatographically worthwhile resulted; these diameters are too large to perform efficient chromatography. Also, commercially available conductivity detectors, much less suppressors, of the time were far too large to be used with such columns.

This quest has been rekindled in the last decade. One process of manufacturing polymeric capillaries consists of the following steps: (a) melting the raw polymer material (typically in granular or pellet form) under vacuum and maintaining under vacuum at least overnight to bake a bubble-free “cake”, preferably of a square cross section, (b) machining the “cake” to a cylindrical shape, typically 5–10 cm in diameter, (c) drilling a concentric hole through the cylinder such that this “preform” has the same i.d./o.d. aspect ratio (e.g. 1:15) as that of the eventually desired capillary (e.g., 25/375 μm), and (d) subjecting the preform to extrusion. Except the final step that requires highly specialized extrusion equipment, and depending on what capabilities one has in-house, potentially all of the other steps can be conducted in-house to reduce cost. Even if all but (d) is conducted in-house, the cost is still several thousand US dollars. Further, it is understood that most vendors will only accept such an order on a best effort basis, there are no guarantees

that anything useful will be produced. Obviously, only the future can reveal if such an investment is worthwhile.

In a more recent quest, the first batch of polymethylmethacrylate (PMMA) capillaries were successfully made by a vendor experienced in extruding small diameter PMMA optical fibers. The first lot averaged 340 μm in o.d. (target 365 μm), had an attractive i.d. range of 16–20 μm [20]. But an identically made second lot produced inner diameters averaging ~36 μm. In addition, while it was clearly possible to create –COOH groups by alkaline hydrolysis of the inner ester walls of the capillary, unexpectedly, electrostatically attached anion exchanger latex particles did not survive prolonged use of 5–10 mM KOH as eluent. The key may be that if the base material remains susceptible to attack by the alkaline eluent, much as with silica, a stable attachment is not possible.

Experiments were conducted with polymers that could be sulfonated, with a preference for transparent polymers suitable for visual/microscopic observation. It was found possible to sulfonate a cyclic olefin polymer (COP, Zeonex 330R) with chlorosulfonic acid [21]. These capillaries were also made in two lots; the o.d. in both cases ranged from 360 to 375 μm. The circularity of the cross section was variable, whether because of the soft nature of the polymer or other reasons. The first lot averaged an i.d. of 19 μm, the diametric variation was ≤ 2 μm within 1-m lengths. The second lot exhibited a less desirable average i.d. of 28 μm. The first lot made for very attractive chromatographic performance, ranging up to 160,000 plates/m for chloride at the Van Deemter optimum. There was a further efficiency enhancement when such columns were tightly coiled on 0.8 mm support rods: because of the centrifugal component of the flow, radial mass transfer is enhanced and axial dispersion is reduced [22]. Such coiling is only possible when the column material is sufficiently flexible.

Given the success of the COP material, a polymer of allied composition, a cyclic olefin copolymer (COC), was next tried. Specifically, TOPAS COCs (www.topas.com) have been much used in making microchips for their exceptional optical clarity [23]. The COP capillaries also have a reasonably transmissive UV window around 250 nm, but COC polymers such as TOPAS 8007 × 10 have usable transmission down to 230 nm [24]. It has recently been shown that the dispersion associated with absorbance measurement in a longer path flow cell can be removed computationally in-silico [25], virtually generating shorter cell-path data but with a signal to noise ratio characteristic of the longer path. With a flexible COC polymer, the capillary could be bent near the terminal end, light introduced therethrough and collected at the fluid exit. Unfortunately, two attempts at making this material as capillary tubing failed; not only any tubing of usable length of consistent bore could be produced, the produced capillary was translucent, not optically clear.

Polyetherimide (PEI, Ultem 1000) contains an abundance of aromatic rings; ring sulfonation is expected to be straightforward. PEI is transparent at longer visible wavelengths. But PEI is more rigid than COP or COC material and cannot be spooled as easily. Only ≤ 2 m individual lengths could be produced by a vendor experienced in extruding larger PEI tubes. Target goals were 375 μm o.d., 17–25 μm i.d. tubes. The first attempt resulted in tubes that had far too frequent blockages. The second and final attempt resulted in open tubes that varied in diameter from 20 to 40 μm, often within 1 m, and were relatively poor in concentricity.

Given this experience, when 25 μm i.d., 360 μm o.d. PEEK tubing became commercially available and it was clear that o.d. tolerance, i.d. uniformity, circularity and concentricity were all much better for these than the capillaries thus far tried, the choice was simple. PEEK also has an aromatic skeleton, permitting facile sulfonation [26]. However, any functionalization on the inside walls of a small-bore capillary is always challenging. Aspects of sulfonation for preparing a PEEK column are described in this paper. The rigidity of the material is both a negative and a positive, the rigid nature precludes making a small diameter helix. On the other hand, its rigid nature permits facile connection using compression fittings – which has proven difficult to impossible with smaller i.d. soft COP capillaries: because of their poor circularity and concentricity, getting a seal on the outer periphery involves a degree of compression that shuts off the inner bore and/or misalignment of the central bore to the next stage results in lack of a proper through-passage. The characteristics of the PEEK capillaries obviate these problems. On the downside, there are many occasions in which one would like to use columns longer than the 1.52 m maximum lengths that the manufacturer produces (numerous attempts to persuade to produce longer lengths have not been fruitful). The i.d. is larger than ideally desired. The opacity not only rules out optical interrogation, visual examination is not possible.

1.5. Suppressors

Ion chromatography was originally invented in the suppressed form [1] and the original packed column suppressors were soon replaced by continuously regenerated membrane devices [27]. Non-suppressed IC, based on special low capacity columns and low concentration eluents, was introduced at a later date, declaring that the suppressor column and the use of basic eluents constitute “major disadvantages” [28]. Future historians will have to decide on the veracity of this claim, but once the original suppression patents expired, commercial offerings of nonsuppressed anion chromatography essentially ceased to exist. That OTIC began in the nonsuppressed mode must in contrast be ascribed to the difficulty of making suitably low dispersion suppressors. The first open tubular ion exchange experiments relied on 30–60 μm glass capillaries [29], the analytes were not simple small ions, rather they were nucleosides carrying a pH-induced charge; these could be detected by absorbance measurement. However, this original work did lead to investigations on the separation of small inorganic ions. Far smaller columns (2.3–25 μm in i.d.), were explored with ion selective electrodes of 1 μm tip diameter serving for detection [30–32]. Ion exchange – based suppressors for columns of such diameter, even at the higher 25 μm end, remained unattainable for decades.

The first suppressor for OTIC and the first suppressed open tubular anion chromatogram was described as ongoing work as part of a review [33]; in a later publication, more details were provided on the fabrication of the 80 μm bore suppressor made on a mandrel of 50 μm Pt-wire inserted inside 75 μm bore silica capillaries. The suppressor was fabricated by repeated coating of Nafion® perfluorosulfonate ionomer on the metal wire [34]. Suppression may have been satisfactory but such large bore columns had to sacrifice speed or efficiency. In addition, the great tendency of Nafion to expand substantially when wet, assured poor adhesion.

Huang and Dasgupta [35] took a different approach altogether, mechanically similar to the previous effort in that a short length of the membrane connected two silica capillaries but these were chemically bonded to the silica surface. Monolithic polymeric structures contain an extensively connected porous network. They showed that by controlling the amount of porogen added during the synthesis of such polymers derived from ethylene dimethacrylate – glycidyl methacrylate (EDMA-GMA), which are then converted to an anion exchanger by treatment with trimethylamine, it is possible to obtain rigid ion exchange polymers that behave like anion exchange membranes. They used a 75 μm diameter tungsten wire bridging 100 μm i.d. silica columns as a mandrel, surrounded the entire bridge region with a glass tube and injected the EDMA-GMA-porogen mixture in the annular space to make a rigid polymer. This was followed by removal of the wire and functionalization into an ion exchanger. They demonstrated successful suppressed OT cation chromatography, the only example to date. Subsequently Huang et al. constructed a cation exchanger suppressor based on an acrylic acid-EDMA monolith, to our knowledge, the only weak acid functionality suppressor ever successfully demonstrated for suppressed anion chromatography [36]. They used multilayer chitosan-glutaraldehyde coated silica columns, up to 4.5 m in length, integrally connected to the suppressors. Good plate counts were demonstrated but the large bore

(75 μm) columns required long analysis times to produce efficient separations.

More recently electrodialytic suppressors for anion OTIC have been made using a small block (1 × 5 × 10 mm) of a solid cation exchange polymer like Nafion [37]. In essence, a micro-crack is made in the methanol (or ethanol)-swollen ionomer block using a 0.3 mm dia. hypodermic needle to go through the long axis of the block. The column exit end is honed to a tapered point like a sharpened pencil, and one end of the intended exit tube (similar o.d. and i.d.) is subjected to the same treatment. The tapered end of the column and that of the tube are then inserted from opposite sides of the block until they are 0.5–1 mm apart. While such an arrangement will work as a chemical suppressor if the membrane is bathed externally in an acid regenerant, the original exposition [37] used an electrodialytic suppression configuration: There were two larger (0.3 mm drilled passage) flanking parallel channels adjacent to the micro-crack, into which electrodes were deployed. Water was pumped through these side channels (dilute acid is advantageously used instead in the anode channel) and by insulating the electrodes along their length except in the regions adjacent to the gap between the column and the exit tube, the electric field is focused on the suppression zone. This electrodialytic nanosuppressor was able to exchange 100 mM KOH flowing at 100 nL/min.

The suppression capacity of the above electrodialytic nanosuppressor substantially exceeds present requirements. The ion exchange phase ratio (β_{iex}, see Refs. [20,38]), defined as the ratio of the number of ionic equivalents present in the mobile phase to the stationary phase ion exchange capacity over the same length, is a better index to compare OT and packed columns. The current capacity/unit length of OT columns require much lower eluent concentrations than their packed column counterparts and eluent concentrations approaching 100 mM are not yet needed. While the micro-crack approach results in a passage estimated to have an effective diameter of 40 μm, it does have a tendency to close up/change dimensions over a period of time, resulting in a variable flow resistance that may affects flow rates in a pneumatically driven constant pressure pumping system. If the suppressor is allowed to dry out, flow may be shut off completely when the system is rewetted. More recently, a chemically regenerated suppressor with a 100–200 μm drilled hole in a similar polymer block has been advocated [18]; such devices do not show any blockage even when shut down for some time and restarted; nevertheless, it does introduce more dispersion, as discussed elsewhere [39].

New, easy to make, recently introduced moldable ion exchange polymers will change the horizon of miniature suppressors. It has already been demonstrated that circular channels as small as ~30-35 μm in diameter can be made by casting the monomer solution around a 25 μm wire as mandrel, allowing polymerization to occur, annealing, hydration and removal of the template [40]. Finally, the ultimate goal is to completely integrate the column and the suppressor. This may be possible with polymers which can be sulfonated sufficiently to behave as a membrane.

1.6. Detectors

1.6.1. Conductivity detectors

Conductivity detection has been associated with ion chromatography since its inception. Solution conductivity uniquely identifies an ion in aqueous solution. In the small capillaries presently considered, what is called capacitively coupled contactless conductivity detection (C^4D) [41], very conveniently provides a method for detecting impedance changes of the solution flowing inside the capillary. In essence, such a detector consists of two ring electrodes closely spaced on the capillary, one acting as the transmitter, the other as the receiver. An alternating excitation voltage from the transmitter is coupled through the capillary wall to the solution, travels across the solution and back out through the wall to the other electrode where it is amplified, rectified, and detected. To avoid direct interelectrode crosstalk across the air, it is generally essential to have a grounded shield between the two electrodes. Originally called oscillometry, such detectors, dating back to before 1950 [42], were typically used to conduct titrations with the electrodes outside the titration vessel [43]. By 1980, flow-through detectors were designed for isotachophoresis [44].

It was in late 1990s, however, as the need for simple on-capillary detectors in capillary electrophoresis (CE) arose, these detectors were "born again". They had the added advantage of not contacting a liquid subjected to a high electric field as occurs in CE. Two groups independently and near-simultaneously reported their use in CE [45,46]. Despite the catchy nomenclature, such a detector does not simply measure the conductance of the solution. At best, it produces an output signal that increases with increasing solution conductance. In a typical deployment, the detector output will increase with the overall admittance (reciprocal of impedance) of the system and as such is dependent on a variety of parameters, including capillary material and dimensions, excitation frequency, as well as solution admittance, which is a function of both solution conductivity and permittivity. These detectors are not, strictly speaking, conductivity detectors [47].

Suppressed OTIC systems result in a very low conductivity background, nearly that of pure water. In a very small capillary, even at the lowest excitation frequency available, most commercial C^4D/admittance detectors do not perform as well as they do with a higher background conductance as encountered in CE [42]. Lowering the excitation frequency helps [43], and simple detailed instructions are available through a “Open C4D” project to inexpensively make one’s own admittance detectors that can be tailored [48].

Ultimately, the S/N of any such detector is dependent on the actual amount of energy reaching the receiver electrode. This is controlled not only by the original excitation amplitude, the attenuation is also inversely related to the wall thickness. More energy is coupled into the solution as the dielectric constant of the wall material and the probe frequency increases. However, an increase in the probe frequency also increases the capacitive current through the solution. When the capacitive current is dominant relative to the ohmic current, small changes in conductivity cannot be measured. As such, as judged by (a) the smallest change in conductance from pure water that can really be detected (as opposed to being linearly projecting a limit of detection from higher concentration experiments) or (b) response linearity at low concentrations, there is always an optimum range in the probe frequency. This frequency is also dependent on the dielectric constant of the capillary material. It is important to bear in mind that much of the extant data pertain to fused silica capillaries. Fused silica has a higher dielectric constant than most synthetic polymers. The optimum frequency can be higher in polymeric capillaries.

Admittance/C^4D detectors do offer a reasonable solution to the detection dilemma in suppressed OTIC especially if low or sub-kHz excitation frequencies are available. However, this does extract some SNR penalties and the ultimate in detection performance is not possible in this fashion. Measuring conductivity with electrodes in direct contact with the solution (hereinafter contact conductivity) is not of course difficult; it is difficult is to do this in a small enough volume and without introducing additional dispersion.

Solution contact measurement truly provides the conductivity of a solution. For a review of application in the capillary scale, see [49]; since that time Chouhan and Dasgupta [50] presented attractive results for 37 μm i.d. stainless-steel capillaries facing each other just short of contact, similar to a previously reported design [51], except for the dimensions.

1.6.2. Laser-induced fluorescence (LIF) and electrochemical detection (ECD)

LIF detection [8,9] is exquisitely sensitive. But most molecules have no native fluorescence and must be suitably tagged; this has not been commonly used for small ions. Amino acids, peptides and proteins typically do carry a net charge and is often separated by ion exchange.

However, after fluorescent tag attachment, separations are nearly always based on reverse-phase interactions [8,9]. Usually, there is a strong correlation between fluorescence properties and ECD sensitivity. Underivatized amino acids, peptides and carbohydrates are often separated by ion exchange and measured by ECD. Microfiber electrodes, originally used in neurochemistry, were introduced by Jorgenson and his students [52] for ECD in OT liquid chromatography (OTLC) and studied over many years [53]. Many others have also used end-capillary ECD, often in conjunction with CE. Some general reviews are available [54], but most center on specific analytes, or covers all flow systems regardless of scale [55]. In general, ECDs exhibit response reproducibility problems due to surface fouling. With capillaries, positioning reproducibility creates further issues. In the macroscale, pulsed amperometric detection (PAD), which attempts to renew the electrode surface by a continuously repeated voltage waveform, alleviates the surface alteration problem, but does not eliminate it. Still, ECD, specifically PAD, has been of great utility for bioderived samples separated by ion exchange. There is much potential in the capillary format as well. Potentiometry has been used in OTIC in the past as discussed but not widely used since; the observed response is logarithmic - this complicates quantitation.

1.6.3. Optical absorbance detection

Although less common in IC, in specialized applications, e.g., measurement of nitrate and nitrite, absorbance detection is uniquely useful [56]. Capillary scale postcolumn colorimetric reagent introduction for metal ion chromatography and detection cells have been described [57,58]. For a normal radial path measurement, the permissible illuminated length is relatively small, e.g. 1.1 mm for a 1-m long, 20 μm i.d. column for 5% loss of efficiency. Mathematical strategies have been developed to reduce longer cell path dispersion [25]. Configuring a longer axial path with capillaries of small i.d. is nontrivial: previous attempts were reviewed [25]; since that time, another L-geometry cell of 1-mm effective path length and 0.25 mm i.d. has been described [59]. Although this detector can be configured for simultaneous fluorescence measurement, like several previous other designs, the dimensions are still too large for OTLC.

2. Experimental section

2.1. Materials and reagents

PEEK capillaries (25 μm i.d., 360 μm o.d.) were obtained from IDEX (www.idex-hs.com). Ultrapure water (Milli-Q, www.millipore.com) was used throughout. Stock acid and base solutions were made from concentrated sulfuric and hydrochloric acids, NaOH (50% solution), KOH pellets, and deionized water (DIW). *S*tandardization was carried out by titration, with potassium acid phthalate and sodium carbonate used as primary standards (all from www.fishersci.com). The stock solutions were diluted by DIW and used for column regeneration. They were also used for both cation-exchange (CEX) and anion-exchange (AEX) capacity measurements of the sulfonated and AS18 latex coated PEEK OT columns, respectively, using on-column acid-base titration [6,20]. AS18 latex nanoparticles were a gift from Thermo Fisher Scientific. Analyte anions were prepared in the form of reagent-grade sodium or potassium salts; cations as either chloride or bromide salts (reagent-grade chemicals, used as received). All eluent solutions were prepared in DI water.

2.2. Pumping system

A nanopump in Y-configuration (www.vici.com, Fig. S2) comprising two independent nanoflow pumps (full stroke volume 30 μL each) was controlled by two independently addressable micro stepper motors, thus permitting both isocratic and gradient suppressed open tubular ion chromatography (SOTIC). The overall system is shown in Fig. 1. An internal loop sample injector (7 nL injection volume) is integrally coupled to the two pump heads. This version of the pump/injector system weighed 4 kg and permitted pressures to 8000 psi (~55 MPa), although such pressures are considerably above what is needed for an OTIC system. The pump could reliably pump down to 10 nL/min and up to hundreds of μL/min. The gradient system cable connections, dual pump operation, and load/injection functions are schematically shown in Figs. S3–S5, respectively. For gradient SOTIC, one pump was filled with 10–50 mM KOH and the other pump with DIW, an in-line inverted arrow shaped static mixer (Fig. S6) was used to mix two pump flows. A connection/mixing tubing (straight PEEK capillary, 150 μm i.d., 360 μm o.d., 34 mm in length; volume: 0.6 μL) was connected between the outlet of the mixer and the injector. A gradient sequence can be configured as shown in Fig. S7. When running isocratic elution, the mixer and the connection tubing were bypassed, and either pump A or B was connected directly to the injector.

A pneumatic pump system (PPS) that has been routinely used [5,6] was also used to run isocratic SOTIC and other experiments, including pumping HCl-containing column regeneration solutions. For the initial characterization of PEEK OT columns, largely the PPS was used without any suppressors to eliminate extracolumn broadening.

3. Results and discussion

3.1. Preparation of PEEK tube for creating an OT column

As previously stated, the combination of good circularity, concentricity, rigidity of the polymer that resists deformation upon compressive sealing, and commercial availability, made PEEK a compelling choice. The fact that PEEK has an aromatic skeleton and is easily sulfonated [60,61], is a bonus. In applications such as the present situation where only a sulfonated surface is required, if anything, PEEK may be sulfonated too easily, however.

Either concentrated H_2SO_4 and $ClSO_3H$ attacks PEEK immediately at room temperature. Fig. S8 shows two PEEK filaments momentarily dipped in anhydrous $ClSO_3H$, rinsed by DIW, and then exposed to a solution of rhodamine 6G, and washed. The cationic dye immediately attached to the treated area indicating successful sulfonation. A very similar situation occurs with concentrated H_2SO_4. However, the problem is that following sulfonation of the surface, further sulfonation causes the sulfonated material to simply dissolve in the sulfonating agent [62]. Small pieces of PEEK completely dissolve in Conc. H_2SO_4 at room temperature if allowed to react overnight (Fig. S9). Clearly, neither $ClSO_3H$ nor Conc. H_2SO_4 can be used to functionalize the inner surface of a PEEK capillary as the reaction is too rapid and substantial surface erosion occurs. The latter is important as column efficiency is highly dependent on the column inner diameter and an uncontrolled increase in i.d. is undesirable.

To achieve sulfonation in a more controlled manner, in independent experiments $ClSO_3H$ diluted with glacial acetic acid and H_2SO_4 diluted with DIW were used. As shown in Tables S1 and S2, an 1-h exposure at room temperature is sufficient to sulfonate a PEEK surface (as indicated by subsequent Rhodamine G staining) even when sulfonation reagent concentrations are as low as 46% (w/w) $ClSO_3H$ or 70% (v/v) H_2SO_4. As $ClSO_3H$ is an extremely aggressive reagent (it reacts, violently, indeed explosively, with water [63]), H_2SO_4 was chosen for further work. The H_2SO_4 concentration was reduced to 50% (v/v) but in order to accelerate the reaction that was very slow at room temperature, the temperature was increased to 38 °C. The results show that a 15-h exposure under this condition does result in sulfonation (Fig. S10); the contact angle of a water drop on the PEEK surface also changes dramatically.

3.2. Dynamic sulfonation and column behavior

Based on the above results, a dynamic sulfonation configuration was chosen in which 50% (v/v) H_2SO_4 was pneumatically pumped (50 psi)

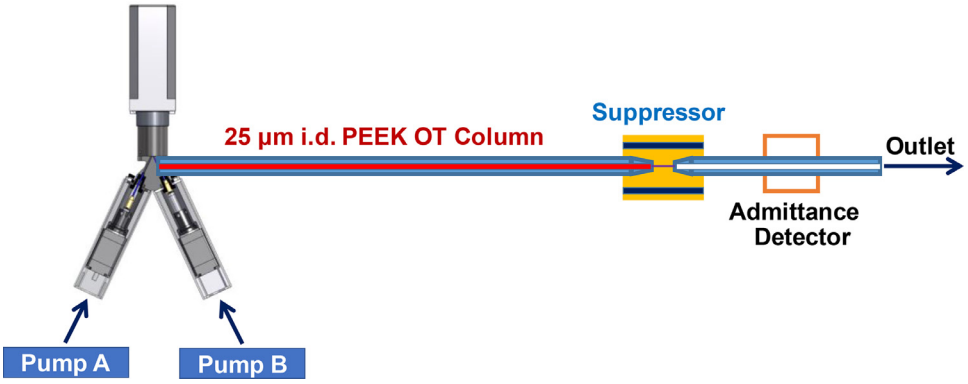

Fig. 1. System setup of SOTIC using nanopump.

through a 25 µm i.d., 334 mm long PEEK capillary for 9 h; both the pressure vessel and the PEEK capillary were kept in an oven maintained at 38 °C (Fig. S11). After the acid solution flowed continuously through the capillary (~100 nL/min) for the indicated period, the acid was flushed out with DIW. As supplied AS18 latex was diluted 10× with water and pumped through the column for 30 min, followed by successive 30 min washes with DIW and then the intended eluent. A standard test mix of five common anions (F^-, Cl^-, NO_2^-, Br^-, and NO_3^-) were fully separated using either 1.0 mM sodium benzoate or 0.50 mM NaOH as the eluent, respectively. However, due to the nature of nonsuppressed detection, fluoride was poorly resolved from the water dip, especially for the NaOH eluent (Fig. S12). Further testing showed that the latex-coated PEEK column suffered no ill effects from being flushed with 50 mM NaOH overnight (14.3 h). While the electrostatic binding between the oppositely charged latex particles and the sulfonated surface of the PEEK inner surface must be the primary factor, the π–π interaction between aromatic rings, present both in the PEEK material and the latex particles, likely augments this binding.

3.3. Limitations of dynamic sulfonation under modestly aggressive conditions for longer columns

A longer PEEK capillary (772 mm) was sulfonated under the same conditions, and then coated with AS18 latex following the same exact procedure above. By simply relocating the position of the admittance detector in multiple but otherwise identical runs (Fig. S13), it was observed that the separation did *not* improve with length as would be expected and as was clearly observed on a comparable COP column (Fig. S14). A plot of the retention factors (Fig. S15) or incremental retention (Fig. S16) as a function of the column length made it clear that only a relatively small initial part of the column was providing any retention. It was thought that the sulfonation procedure under this condition is subject to an equilibrium limitation – sufficient PEEK-sulfonic acid forms and dissolves in the sulfonating agent in the first part of the tube and prevents further functionalization in the latter part of the tube.

Additional dynamic sulfonation in the same direction under the same modestly aggressive conditions was ineffective. Dynamic sulfonation in the reverse direction for the same period and the same sulfonating reagent was attempted, after sulfonation with flow in one direction (hereinafter referred to as the "normal" direction) as conducted above. The column was then washed and latex coated. Separation performance was then checked as a function of the detector location both in the normal (Fig. S17) and the reverse flow direction (Fig. S18). Perusal of these data indicates that in the normal flow direction only the first part of the column is effective, and the latter part of the column contributes little to the overall separation. Conversely, in the reverse flow direction, the analytes are not separated until one nears the end of the column and the first part of the column contributes little. In other words, little was gained in the second sulfonation process with the flow in the reverse direction. These results force one to abandon the equilibrium limitation hypothesis. Unfortunately, it does not necessarily provide a rationale for the observations. It was tentatively concluded that in any case, more aggressive conditions are needed to functionalize the capillary.

3.4. Can static sulfonation work?

A 760 mm long PEEK capillary was filled with 50% (v/v) H_2SO_4, and both ends sealed with hot-melt polyolefin adhesive. It was then kept in an oven @38 °C for 10.5 h; the process is schematically indicated in Fig. S19. After allowing to cool to room temperature, the adhesive blobs were removed, and the sulfonating reagent was then flushed out using pressurized N_2. After prolonged washing with water and NaOH, followed by latex coating, separation of common ions was unsatisfactory. Reasoning that any attached latex will be removed by sulfonation conditions or be sulfonated itself, the same capillary was then subjected to the same sulfonation procedure again and this time left in the heated enclosure for 15.3 h, again followed by washing and latex attachment. This was followed by thoroughly washing with DIW, and coating with AEX latex. The column was characterized the same way as before, using multiple detector locations and flow in both directions. The results of the separations as a function of detector location and the corresponding retention factors are shown in Fig. 2(a) and (b), respectively. The results for another column prepared in a similar manner (sulfonation, latex coating, and testing; repeat sulfonation, washing, latex coating, and testing) are shown for both forward and reverse flow directions in Fig. S20. In both cases, the results indicate that the columns have reasonably uniform capacity along the length.

3.5. Optimization of static sulfonation. Reagent concentration, temperature, and time

Despite successful efforts of static sulfonation above, the fact that the first attempt with shorter duration was not fully effective left one uncertain if the above procedure is going to be reproducible on a repeated basis. The previous benchmark column material, COP, is very inert and cannot be sulfonated by concentrated H_2SO_4, only fuming sulfuric acid, $ClSO_3H$ or gas phase SO_3 is effective. Continued sulfonation results of COP results in increasing CEX capacity, specific capacities of 100 peq/mm^2 are easily attained, up to 300 peq/mm^2 has been recorded [21]. It is presumed that the sulfonated polymer is attached to the base skeleton as tendrils. Certainly, surface area increases; the attained capacities represent far greater than monolayer coverage. PEEK behaves in a completely different manner; while it is readily sulfonated by sulfuric acid, much beyond monolayer sulfonation the sulfonated product dissolves, especially if the H_2SO_4 concentration is high. In reality, a CEX capacity of more than approximately monolayer coverage is unlikely to

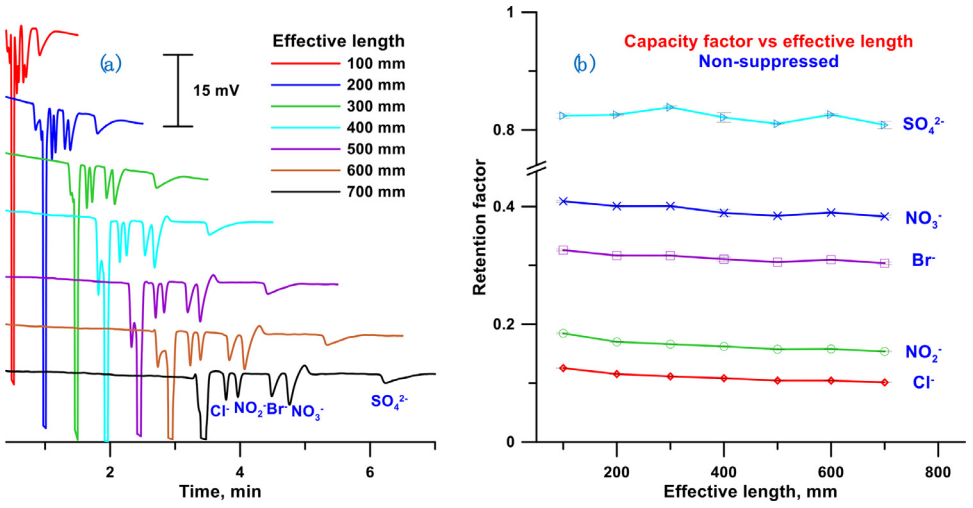

Fig. 2. Uniformity test of AEX latex coated PEEK column. AS18 coated sulfonated PEEK column: 25 μm i.d., 360 μm o.d., 749 mm in length. TraceDec admittance detection: voltage, −12 dB; gain, 50%; frequency, 150 kHz; offset, 0. Various effective length: 100–700 mm, with 100 mm interval. Eluent: 10.0 mM KOH. Sample: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, 0.50 mM each; Injection volume: 2.5 nL. Running pressure: 24 psi.

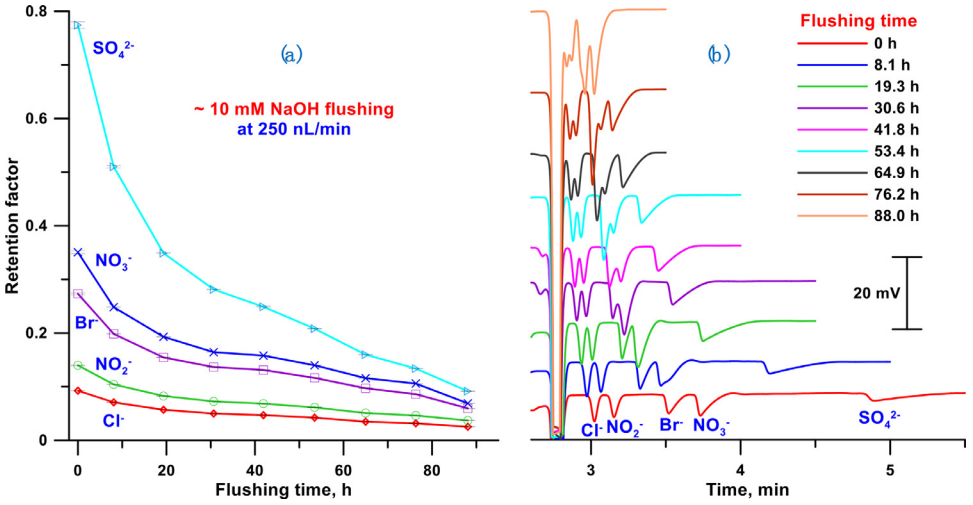

Fig. 3. Decrease of retention as 10.0 mM NaOH is continuously pumped as eluent at 250 nL/min through an AS18 latex-coated, 702 mm long sulfonated PEEK column. Effective length: 650 mm. Nonsuppressed mode, TraceDec admittance detection: voltage, −12 dB; gain, 50%; frequency, 150 kHz; offset, 0. Eluent: ~10.0 mM NaOH. Sample: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, 0.50 mM each.

have much effect on the AEX capacity. Previous experience with various polymers has shown that maximum coverage attained through electrostatic attachment of latex particles also approximately represents monolayer coverage of the latex. For the AS18 latex (65 nm diameter, capacity ~3.4×10^{-8} peq strong and weak base each, per particle), monolayer coverage will lead to an AEX capacity of the order of 20 peq/mm^2. Our goal was to attain an AEX capacity of this order or greater upon sulfonation, washing and latex coating, without perceptibly increasing the column bore (measured by ascertaining the water flow rate through the tube at constant pressure before and after sulfonation). Various combinations of H_2SO_4 concentrations (50%, 60%, 65% and 70% v/v), temperatures (38, 46, 52, 60, and 70 °C), and duration (9 and 15 h, 2, 3, 4, 5, 6, and 8 days) were examined. CEX capacity was measured under each condition. The CEX capacity overall did not markedly change in this entire range of experiments, the vast majority remained within 0.7 ± 0.2 peq/mm^2; as a matter of perspective, the CEX capacity of a blank PEEK tube (due presumably to surface –COOH groups) was measured to be 0.30 ± 0.06 peq/mm^2 (the same for a COP tube is ~1 peq/mm^2). Although the measured CEX capacity of the sulfonated PEEK tubes was comparable to that of an untreated COP tube, when sulfonated under the finally chosen conditions (70% v/v H_2SO_4, 70 °C, 5 d), the columns produced after latex attachment were stable to base, exhibited uniform capacity, and the obtained capacity per unit area with AS18 latex fell within 18 ± 3 peq/mm^2.

3.6. Packed vs. OT columns. What capacity is needed?

A previous publication described capacities of commercially available ion chromatography columns [38]. For 4 × 250 mm columns, most commercially available columns have capacities in the range of 20–200 μeq/column. In real samples, the analyte of interest can often be present in traces in a background of overwhelming amounts of other ions (often chloride and/or sulfate). The situation is particularly troublesome when these major ions are retained more strongly (in preference to) than the analyte ions of interest as they first occupy the column sites, leaving an accordingly smaller portion of the column available to carry out the separation. Let us arbitrarily assume that the maximum amount of the major spectator ion the separation can tolerate is no larger than 50% of the total column capacity; for a 50 μL injection volume typical on such columns, the maximum tolerable major spectator ion concentration would be 0.2–2.0 eq/L for column capacities of 20 and 200 μeq, respectively. The present OT PEEK capillaries are available in a maximum length of 5 ft (1.52 m). For a 1.5 m OT column of 25 μm i.d., a specific capacity of 20 peq/mm^2 will provide a total capacity of 2.4 neq. For 4–7 nL injection volumes, 50% of the capacity will be consumed by 0.17–0.3 eq/L of a spectator ion – which overlaps the tolerance range of available packed columns but only at the low end. Possibilities of improving concentration LODs (and thus injecting less sample volume to improve concentration tolerance) seem limited, the present state of the

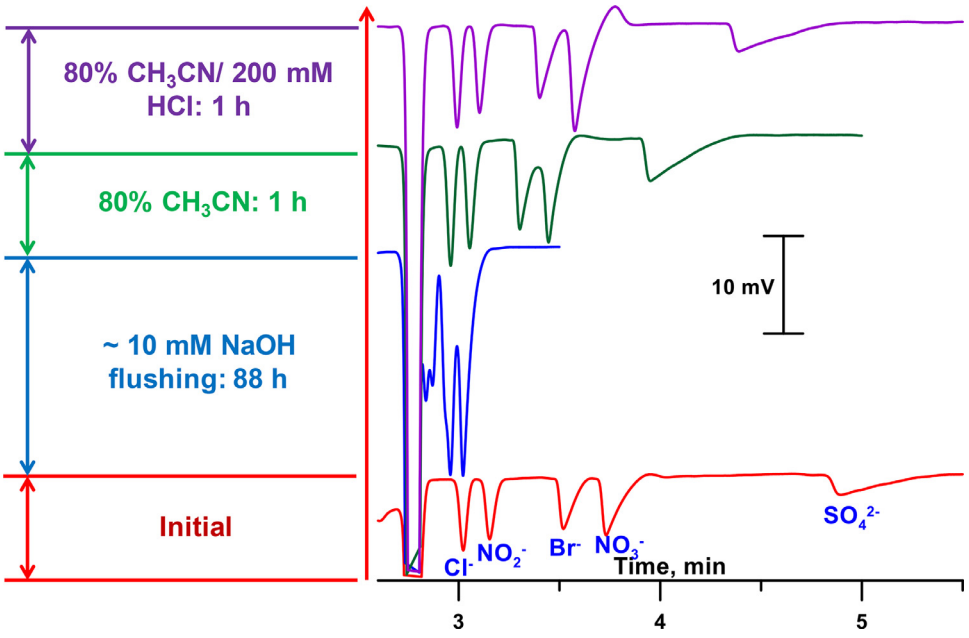

Fig. 4. Column capacity recovery by flushing with column cleanup reagent. AS18 coated sulfonated PEEK column, 702 mm in length. TraceDec admittance detection: voltage, −12 dB; gain, 50%; frequency, 150 kHz; offset, 0. Effective length: 650 mm. Eluent: ~10.0 mM NaOH. Sample: F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, 0.50 mM each.

art conductance noise in macrosystems are limited by the background conductance of water, such that improvements are seen by a reduction of the detector temperature (and thus the extent of autoionization of water) [64]. The practical utility of OTIC can thus only be improved by methods that improve column capacities.

3.7. Methods to increase column capacity

Although methods to dramatically increase column capacities have not yet emerged, several modest but meaningful steps are possible. From the synthesis process, latex suspensions contain a nonionic surfactant that may inhibit latex attachment to some degree by adsorbing on the PEEK substrate, masking the charged site and thus decreasing latex attachment. The detergent can be dialyzed out of the latex suspension; but this is a time-consuming process. Regardless, after coating the column, during washing and normal use, the surfactant will wash off. Recoating the column again may then lead to further latex attachment. A 765 mm capillary was thus tested: the capacity of the column was determined three times by on-column titrations, the column performance was checked as well with six common anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, and SO_4^{2-}) with 10.0 mM KOH as eluent. After that, the column was coated with latex again and the whole procedure was repeated. This entire cycle was repeated twice more. After the 1st through the 4th latex coating, the capacity/unit area increased as 18.3 ± 0.2, 20.8 ± 0.6, 22.8 ± 0.6 and 23.1 ± 0.9 peq/mm^2, representing an overall increase of 26%. The increase between the 3rd and the 4th coating not being statistically significant, the process was not further repeated. Fig. S21 in the SI shows the retention factors after each latex coating.

While the sulfonation procedure adopted (70% v/v H_2SO_4, 70 °C, 5 d) is reliable and not particularly onerous, it takes time. A single shorter duration leads to both lower CEX and eventually lower AEX capacities, but such capillaries can be re-sulfonated. The eventual AEX capacities are typically higher if latex has been attached prior to the repeat sulfonation step. Two identical capillaries were sulfonated with 65% v/v H_2SO_4 at 52 °C for 15 h. One was coated with latex, washed, and re-sulfonated using identical conditions and the process repeated (total three sulfonations, with latex coating after each sulfonation). For the control, the capillary was sulfonated three times identically, but latex coated only once, after the final sulfonation step. Both processes and the resulting final capacities are shown schematically in Fig. S22. For the control case, the final CEX capacity was 0.32 ± 0.04 peq/mm^2, and the AEX capacity was 10.5 ± 0.3 peq/mm^2. For the column where the latex provided an opportunity to be sulfonated itself, the final CEX capacity was 1.11 ± 0.02 peq/mm^2 (an increase of ~250%), and the AEX capacity was 19.2 ± 0.3 peq/mm^2 (increased 83%). Although we are giving the results in terms of the nominal surface area, it is more than likely that attachment of the latex particles is effectively increasing the surface area. It is expected that both AEX and the underlying CEX sites may be accessible in such columns much as is observed in certain commercial packed columns [65].

It is possible that the PEEK capillaries, as obtained, may contain residual contamination from the extrusion process. Does such contamination exist and/or do they affect the sulfonation and/or the latex attachment step? To avoid any softening and deformation of the capillary if pure acetonitrile is used, 80% v/v CH_3CN (balance water) was used for prewashing two 750 mm long capillaries @80 psi for 24 h. Two other capillaries of the same length from the same lot were used as control and underwent no washing. One prewashed and one control capillary were sulfonated by 70% v/v H_2SO_4 at 70 °C for 3 days, and the other two capillaries were sulfonated under the same condition, but for 6 days. Note that all experiments reported on in this paragraph uses capillaries sulfonated only once. For each PEEK column, both CEX and AEX capacities were measured, respectively before and after latex coating, each in triplicate. For the CEX capacity data in Table S3, whether prewashed or not, sulfonation for 6 days vs 3 days exhibited different variances. On the other hand, whether the 3-day or the 6-day sulfonation data were individually considered, variances for the washed vs unwashed capillaries were comparable. There was no statistical difference in the CEX capacity in the 3-day vs 6-day sulfonation for the prewashed capillaries but for the unwashed capillaries the longer sulfonation period did result in increased CEX capacity to a very high degree of significance. A comparison of the CEX capacity data in Table S3 with the AEX capacity data in Table S4 indicates poor correlation ($r = 0.76$). After coating with latex, however, all of the variances become comparable, whether one looks at 3-day vs 6-day sulfonation or prewashed vs. unwashed capillaries. The effect of sulfonation duration on the ultimate AEX capacity was significant at the 99.9+% level for both washed and unwashed capillaries but whereas it increased with sulfonation duration for the washed capillaries, it decreased with sulfonation duration for the unwashed capillaries, even though the CEX capacity in the latter case increased greatly.

Whether the capillaries were sulfonated for 3 days or 6 days, prewashing increased the AEX capacity at the 99.9+% confidence level. Considering both sulfonation periods as a group, at 27.7 ± 1.7 peq/mm^2, the prewashed capillary capacities were higher (by ~50%) at the 99.99% confidence level compared to the control (18.2 ± 2.9 peq/mm^2).

3.8. Recovering lost capacity

The downside of a miniaturized analysis system is that the increased surface to volume ratio. Any contamination in upstream components has to be washed out and it is easy to contaminate downstream components, notably the column, which manifests itself by loss of retention, the underlying cause being loss of capacity. Of course, if the electrostatically bound latex is somehow washed off, the symptoms will be the same. The degree to which a newly machined pump requires thorough cleaning was not initially realized and the low flow rates make such cleaning a long process. Thus, initially, the loss of column capacity was thought at the beginning to arise from the latex being lost from the column altogether instead of contamination. The loss of retention of a ~70 cm column that was continuously flushed with ~10 mM NaOH (@ 250 nL/min) is shown in Fig. 3. An acetonitrile wash, or preferably an acetonitrile-HCl wash ([66], from a pneumatic pump, as the HCl in the wash reagent is not compatible with a metallic pump) allowed substantial recovery of the lost capacity, as shown in Fig. 4. The column capacity can also be fully recovered, without any cleaning, by recoating the column with latex. Fig. S23 shows that the retention gradually decreased. The column capacity in this case could be fully restored by washing with the column cleanup reagent (Fig. S24). However, the fact that the column capacity can be recovered also by re-coating with latex, suggests that the latex suspension may be acting as a cleaning reagent, as it contains both latex particles and surfactants. It seems possible that this combination might be removing the contaminants through a partition equilibrium transfer of the contaminants from the electrostatically bound latex particles to the suspended latex particles passing through the capillary.

3.9. Retention factor and column capacity

The retention factor *k*, especially when it is compared between several analytes, is essentially a measure of the selectivity of the stationary phase for the different ions. At constant column capacities, log *k* is linearly related to log [*E*] where [*E*] is the eluent concentration (strictly, activity but at the low mM concentrations presently used, activity is tantamount to concentration) with a slope that is equal to the ratio of the charge on the sample ion to that on the eluent ion [67].

Such a log *k* – log [*E*] relationship has been verified in numerous papers, including in OTIC [6,38]. The dependence of *k* on the capacity *C* has been rarely studied, if ever. One source [67] states that log *k* will have the exact same dependence on log C, as it does on log [*E*]. This is clearly fallacious if we increase capacity by increasing column length. Here the retention factor is a measure of selectivity and this does not change by using, e.g., a 200 mm long column packed with the same stationary phase as a 100 mm column (actual experimental measurements will often show a small change because of void volumes that are extraneous to the packed bed, but this difference is very small). The aforementioned dependence on capacity as argued in [67] could be true if the capacity can be changed at the microscopic level. e.g., by changing each functionalization site on a latex particle to have two active ion exchange sites instead of one, but this is usually far beyond the ability of an average user to experimentally study. Indeed, this has never been so verified. Ingenious procedures for making a column, especially photoinitiated functionalization that readily allows different capacities, have been described [68,69]. But thus far, the focus in these attempts has been on creating a longitudinal gradient in capacity along a column. The multiple variables studied during this work for column fabrication led to columns that varied in the specific capacities significantly, nearly

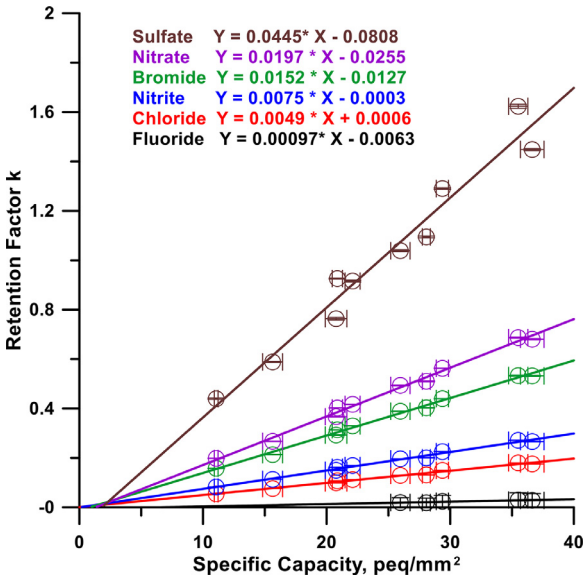

Fig. 5. Relationship between retention factor and column capacity. AS18 coated sulfonated PEEK columns: 25 μm i.d., 360 μm o.d., ~700 mm each in active length. TraceDec admittance detection: voltage, −12 dB; gain, 50%; frequency, 150 kHz; offset, 0. Effective length: 700 mm. AEX capacity determined by on-column acid-base titration. Eluent: 10.0 mM KOH. Error bars represent ±1 SD (*n*=3).

by a factor of four from ~10 to ~40 peq/mm^2. Further, this data set represented a unique example where the column size (strictly, the void volume) remains the same but the capacity changes because the degree of surface coverage by the latex changes. Although the individual columns might vary in length, the ability to locate the admittance detector anywhere on a column allowed the detector to be placed at the same distance from the injector in all cases, as long as we utilized the nonsuppressed detection mode.

The results for 10 different columns representing a range of capacities (the capacity of each having been measured by on-column acidimetric titrations), and all monitored at an effective length of 700 mm, are shown for six analytes in Fig. 5. The absolute retention as well as the retention factor varied linearly with the capacity for all analytes, regardless of their charge. It will be appreciated that if *k* and *C* are linearly related with a zero intercept, log *k* will be related to log *C* with a slope of unity, regardless of the charge on the analyte ion.

3.10. Isocratic/gradient elution suppressed OTIC by the nanopump system

Compared to a pneumatically pumped SOTIC system (Fig. S25), a nanopump-based system is simpler (Fig. 1). The former also cannot generate eluent gradients without a suitable eluent generator or a two-reservoir configuration. Fig. 6 shows a standard chromatogram generated by the nanopump system. Although the injection volume (7 nL) was somewhat higher than optimum, baseline separation of six common anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, and SO_4^{2-}) is easily achieved. Some post-injector dispersion takes place in the transition channel between the injector and the column. Exact alignment of the PEEK column bore, and the transfer channel may also be an issue. Both can be minimized in a future design. A thorough cleaning of the nanopump for an extended period with acetonitrile followed by an alkaline eluent is recommended before any use, as traces of organic acids, likely from machining lubricants, that elute near or before fluoride are otherwise detectable for a long time.

While the isocratic chromatogram in Fig. 6 provides performance comparable to larger macroscale systems, the capability for gradient elution is vital in many applications and is readily implemented by the

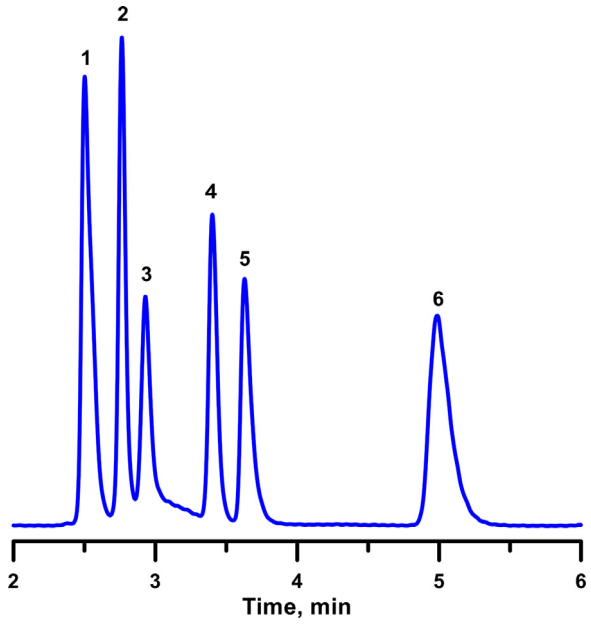

Fig. 6. Isocratic suppressed ion chromatogram using a Nanopump. Eluent: 10.0 mM KOH. Eluent, flow rate 170 nL/min. AS18 latex coated sulfonated PEEK column: 25 μm i.d., 360 μm o.d., 755 mm in length. Exit capillary: silica, 25 μm i.d., 360 mm o.d., 100 mm in length; TraceDec admittance detection: voltage, 0 dB; gain, 200%; frequency, 38 kHz; offset, 0. Detection probe located 45 mm from suppressor center. Electrodialytic suppressor active length ~1 mm, current 20 μA. Peak identification: 1, F^-; 2, Cl^-; 3, NO_2^-; 4, Br^-; 5, NO_3^-; 6, SO_4^{2-}, 0.10 mM each, 7 nL.

nanopump system. Fig. 7 demonstrates a gradient separation of seven common anions (F^-, Cl^-, NO_2^-, Br^-, NO_3^-, SO_4^{2-}, and PO_4^{3-}), representing monovalent, divalent, and trivalent anions, in 9 min.

4. Conclusions

We have successfully developed an AEX latex coated sulfonated PEEK OT column. The sulfonation of PEEK capillaries was achieved without significant dissolution of the polymer under static sulfonation conditions using sulfuric acid of intermediate concentrations at elevated temperatures. Considerable variations in overall column capacities are seen, depending on the exact method of preparation of the column (both initial sulfonation and subsequent steps). The retention factor for any ion is seen to be directly proportional to the measured column capacity. Contamination of a column from residual impurities in the pumping system or other sources can be efficiently remedied by washing with 80% aqueous CH_3CN containing ~200 mM HCl. By using an AEX latex coated PEEK OT column and an electrodialytic capillary suppressor coupled to commercially available components, namely a nanopump system and an on-column admittance detector, both isocratic and gradient SOTIC were demonstrated with attractive performance.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

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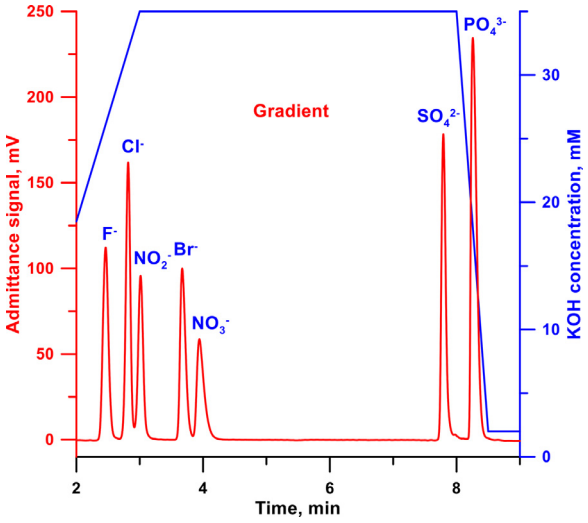

Fig. 7. Gradient chromatogram on AS18 coated sulfonated PEEK column (25 μm i.d., 360 μm o.d., 721 mm in length) using Nanopump. Exit capillary: silica, 25 mm i.d., 360 mm o.d., 100 mm in length; TraceDec admittance detection: voltage, 0 dB; gain, 200%; frequency, 38 kHz; offset, 0; with detection probe located 35 mm from suppressor center. Suppressor active length 0.85 mm, current 20 μA. Eluent: Pump A, 50 mM KOH; pump B, DI H_2O. Each analyte has a concentration of 200 μM, except for sulfate (100 μM). Injection volume: 7 nL. Linear gradient expressed as *t* (min) and KOH concentration (mM): 0–1, 2.0; 3, 35.0; 8, 35.0; 8.5, 2.0; 16, 2.0. Flow rate = 170 nL/min.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.talo.2020.100029.

References

[1] H. Small, T.S. Stevens, W.C. Bauman, Novel ion exchange chromatographic method using conductimetric detection, Anal. Chem. 47 (11) (1975) 1801–1809, doi:10.1021/ac60361a017.
[2] P.K. Dasgupta, O.G. Raabe, T.R. Duvall, B.K. Tarkington, Generatation and characterization of sodium sulfite aerosols for applications in inhalation toxicologic research, Am. Ind. Hyg. Assoc. J. 41 (9) (1980) 660–665, doi:10.1080/15298668091425464.
[3] D.J. Pyo, P.K. Dasgupta, L.S. Yengoyan, High temperature open tubular capillary column ion chromatography, Anal. Sci. 13 (1997) 185–190 http://www.journalarchive.jst.go.jp/jnlpdf.php?cdjournal=analsci1985&cdvol=13&noissue=Supplement&startpage=185&lang=en&from=jnltoc.
[4] A. Sjogren, C.B. Boring, P.K. Dasgupta, J.N. Alexander, Capillary ion chromatography with on-line high pressure electrodialytic NaOH eluent production and gradient generation, Anal. Chem. 69 (7) (1997) 1385–1391, doi:10.1021/ac961215n.
[5] B.C. Yang, M. Zhang, T. Kanyanee, B.N. Stamos, P.K. Dasgupta, An open tubular ion chromatograph, Anal. Chem. 86 (23) (2014) 11554–11561, doi:10.1021/ac503249t.
[6] W. Huang, S. Seetasang, P.K. Dasgupta, Characterization of ion exchange functionalized cyclic olefin polymer open tubular columns, Anal. Chim. Acta 1036 (2018) 187–194, doi:10.1016/j.aca.2018.06.063.
[7] www.globalfia.com. Milligat Pump Manual. https://www.globalfia.com/images/Documents/milliGAT_3.07.pdf, Last accessed December 31, 2020.
[8] P.L. Xiang, Y. Yang, Z.T. Zhao, M.L. Chen, S.R. Liu, Ultrafast gradient separation with narrow open tubular liquid chromatography, Anal. Chem. 91 (16) (2019) 10738–10743, doi:10.1021/acs.analchem.9b02190.
[9] P.L. Xiang, Y. Yang, Z.T. Zhao, A.P. Chen, S.R. Liu, Experimentally validating open tubular liquid chromatography for a peak capacity of 2000 in 3 h, Anal. Chem. 91 (16) (2019) 10518–10523, doi:10.1021/acs.analchem.9b01465.
[10] S. Sharma, A. Plistil, H.E. Barnett, H.D. Tolley, P.B. Farnsworth, S.D. Stearns, M.L. Lee, Hand-portable gradient capillary liquid chromatography pumping system, Anal. Chem. 87 (20) (2015) 10457–10461, doi:10.1021/acs.analchem.5b02583.
[11] X.F. Zhao, X.F. Xie, S. Sharma, L.T. Tolley, A. Plisti, H.E. Barnett, M.P. Brisbin, A.C. Swensen, J.C. Price, P.B. Farnsworth, H.D. Tolley, S.D. Stearns, M.L. Lee, Compact ultrahigh-pressure nanoflow capillary liquid chromatograph, Anal. Chem. 89 (1) (2017) 807–812, doi:10.1021/acs.analchem.6b03575.
[12] M.L. Lee, Brigham Young University. Personal communication, 2020.
[13] S. Sharma, H.D. Tolley, P.B. Farnsworth, M.L. Lee, LED-based UV absorption detector with low detection limits for capillary liquid chromatography, Anal. Chem. 87 (2) (2015) 1381–1386, doi:10.1021/ac504275m.

[14] X.F. Xie, L.T. Tolley, T.X. Truong, H.D. Tolley, P.B. Farnsworth, M.L. Lee, Dual-wavelength light-emitting diode-based ultraviolet absorption detector for nano-flow capillary liquid chromatography, J. Chromatogr. A 1523 (2017) 242–247, doi:10.1016/j.chroma.2017.07.097.

[15] Axcend Focus LC. www.axcendcorp.com, Last accessed December 31, 2020.

[16] D.L. Strong, P.K. Dasgupta, K. Friedman, J.R. Stillian, Electrodialytic eluent production and gradient generation in ion chromatography, Anal. Chem. 63 (5) (1991) 480–486, doi:10.1021/ac00005a019.

[17] Y.F. Lu, L.T. Zhou, B.C. Yang, S.J. Huang, F.F. Zhang, Online gas-free electrodialytic KOH eluent generator for ion chromatography, Anal. Chem. 90 (21) (2018) 12840–12845, doi:10.1021/acs.analchem.8b03365.

[18] B. Chouhan, C.P. Shelor, W.X. Huang, Y.J. Chen, P.K. Dasgupta, Nanovolume gas-free hydroxide eluent generator for open tubular ion chromatography, Anal. Chem. 92 (7) (2020) 5561–5568, doi:10.1021/acs.analchem.0c00505.

[19] J.P. Hutchinson, E.F. Hilder, M. Macka, N. Avdalovic, P.R. Haddad, Preparation and characterisation of anion-exchange latex-coated silica monoliths for capillary electrochromatography, J. Chromatogr. A 1109 (1) (2006) 10–18, doi:10.1016/j.chroma.2005.08.076.

[20] M. Zhang, B.C. Yang, P.K. Dasgupta, Polymethylmethacrylate open tubular ion exchange columns: nondestructive measurement of very small ion exchange capacities, Anal. Chem. 85 (16) (2013) 7994–8000, doi:10.1021/ac4018583.

[21] W. Huang, S. Seetasong, M. Azizi, P.K. Dasgupta, Functionalized cycloolefin polymer capillaries for open tubular ion chromatography, Anal. Chem. 88 (2016) 12013–12020, doi:10.1021/acs.analchem.6b03669.

[22] P.K. Dasgupta, Linear and helical flow in a perfluorosulfonate membrane of annular geometry as a continuous cation exchanger, Anal. Chem. 56 (1) (1984) 96–103, doi:10.1021/ac00265a027.

[23] R. Prakash, A. Wong, K. Pabbaraju, R. Tellier, K.V.I.S. Kaler, Detection of arboviruses in blood and mosquito slurry samples using polymer microchip, in: Proceedings of the 2017 IEEE Healthcare Innovations and Point of Care Technologies (HI-POCT), Bethesda, MD, 2017, pp. 168–171, doi:10.1109/HIC.2017.8227611.

[24] Topas Polyplastics. UV transmission. https://topas.com/tech-center/performance-data/uv-transmission. Last accessed August 13, 2020.

[25] A.F. Kadjo, P.K. Dasgupta, C.P. Shelor, Optimum cell pathlength or volume for absorbance detection in liquid chromatography: transforming longer cell results to virtual shorter cells, Anal. Chem. 92 (9) (2020) 6391–6400, doi:10.1021/acs.analchem.9b05464.

[26] L. Paturzo, A. Basile, A. Iulianelli, J.C. Jansen, I. Gatto, E. Passalacqua, High temperature proton exchange membrane fuel cell using a sulfonated membrane obtained via H_2SO_4 treatment of PEEK-WC, Catal. Today 104 (2-4) (2005) 213–218, doi:10.1016/j.cattod.2005.03.050.

[27] T.S. Stevens, J.C. Davis, H. Small, Hollow fiber ion-exchange suppressor for ion chromatography, Anal. Chem. 53 (9) (1981) 1488–1492, doi:10.1021/ac00232a044.

[28] D.T. Gjerde, J.S. Fritz, G. Schmuckler, Anion chromatography with low-conductivity eluents, J. Chromatogr. 186 (1979) 509–519 Dec, doi:10.1016/S0021-9673(00)95271-3.

[29] D. Ishii, T. Takeuchi, Study of the performance of cation-exchange columns in open-tubular microcapillary liquid-chromatography, J. Chromatogr. 218 (1–3) (1981) 189–197, doi:10.1016/S0021-9673(00)82055-5.

[30] A. Manz, W. Simon, Picoliter cell-volume potentiometric detector for open-tubular column LC, J. Chromatogr. Sci. 21 (7) (1983) 326–330, doi:10.1093/chromsci/21.7.326.

[31] S.R. Muller, W. Simon, H.M. Widmer, K. Grolimund, G. Schomburg, P. Kolla, Separation of cations by open-tubular column liquid-chromatography, Anal. Chem. 61 (24) (1989) 2747–2750, doi:10.1021/ac00199a013.

[32] S. Muller, D. Scheidegger, C. Haber, W. Simon, Fast separations with open tubular ion-exchange capillary columns, HRC-J. High Resolut. Chromatogr. 14 (3) (1991) 174–177, doi:10.1002/jhrc.1240140307.

[33] P. Kuban, P.K. Dasgupta, Capillary ion chromatography, J. Sep. Sci. 27 (17-18) (2004) 1441–1457, doi:10.1002/jssc.200401824.

[34] P. Kuban, P.K. Dasgupta, C.A. Pohl, Open tubular anion exchange chromatography. Controlled layered architecture of stationary phase by successive condensation polymerization, Anal. Chem. 79 (14) (2007) 5462–5467, doi:10.1021/ac070690q.

[35] X.J. Huang, P.K. Dasgupta, Controlled porosity monolithic material as permselective ion exchange membranes, Anal. Chim. Acta 689 (2) (2011) 155–159, doi:10.1016/j.aca.2011.01.020.

[36] X.J. Huang, F.W. Foss, P.K. Dasgupta, Multilayer chitosan-based open tubular capillary anion exchange column with integrated monolithic capillary suppressor, Anal. Chim. Acta 707 (1) (2011) 210–217, doi:10.1016/j.aca.2011.09.028.

[37] W. Huang, P.K. Dasgupta, Electrodialytic capillary suppressor for open tubular ion chromatography, Anal. Chem. 88 (2016) 12021–12027.

[38] W.X. Huang, C.A. Pohl, P.K. Dasgupta, Ion exchange column capacities. Predicting retention behavior of open tubular columns coated with the same phase, J. Chromatogr. A 1550 (2018) 75–79, doi:10.1021/acs.analchem.6b03667.

[39] W. Huang, Open tubular ion chromatography: a state-of-the-art review, Anal. Chim. Acta 1143 (2021) 210–224, doi:10.1016/j.aca.2020.08.012.

[40] F. Maleki, P.K. Dasgupta, Moldable strong cation exchange polymer and microchannel fabrication, Anal. Chem. 92 (19) (2020) 13378–13386, doi:10.1021/acs.analchem.0c02754.

[41] P. Kuban, P.C. Hauser, Contactless conductivity detection for analytical techniques: developments from 2016 to 2018, Electrophoresis 40 (1) (2019) 124–139, doi:10.1002/elps.201800248.

[42] E. Pungor, Oscillometry and Conductometry, Pergamon Press, London, 1965.

[43] M. Zhang, B.N. Stamos, N. Amornthammarong, P.K. Dasgupta, Capillary scale admittance detection, Anal. Chem. 86 (23) (2014) 11538–11546, doi:10.1021/ac503245a.

[44] B. Gaš, M. Demjaněnko, J. Vacik, High-frequency contactless conductivity detection in isotachophoresis, J. Chromatogr. 192 (2) (1980) 253–257, doi:10.1016/S0021-9673(80)80001-X.

[45] A.J. Zemann, E. Schnell, D. Volgger, G.K. Bonn, Contactless conductivity detection for capillary electrophoresis, Anal. Chem. 70 (3) (1998) 563–567, doi:10.1021/ac9707592.

[46] J.A.F. da Silva, C.L. do Lago, An oscillometric detector for capillary electrophoresis, Anal. Chem. 70 (20) (1998) 4339–4343, doi:10.1021/ac980185g.

[47] M. Zhang, P.K. Dasgupta, Conductance or admittance? Q&More (2015). http://q-more.chemeurope.com/q-more-articles/215/conductance-or-admittance.html.

[48] do Lago, C. Open C4D project. https://github.com/claudimir-lago/openC4D Last accessed August 17, 2020.

[49] W.X. Huang, B. Chouhan, P.K. Dasgupta, Capillary scale admittance and conductance detection, Anal. Chem. 90 (24) (2018) 14561–14568, doi:10.1021/acs.analchem.8b04561.

[50] B. Chouhan, P.K. Dasgupta, Suppressor and contact conductivity detector for open tubular ion chromatography, in: Proceedings of the Pittsburgh Conference, Chicago, IL, 2020.

[51] D.Y. Qi, T. Okada, P.K. Dasgupta, Direct-current conductivity detection in ion chromatography, Anal. Chem. 61 (13) (1989) 1383–1387, doi:10.1021/ac00188a015.

[52] L.A. Knecht, E.J. Guthrie, J.W. Jorgenson, On-column electrochemical detector with a single graphite fiber electrode for open-tubular liquid-chromatography, Anal. Chem. 56 (3) (1984) 479–482, doi:10.1021/ac00267a042.

[53] K. Pihel, S.C. Hsieh, J.W. Jorgenson, R.M. Wightman, Quantal corelease of histamine and 5-hydroxytryptamine from mast cells and the effects of prior incubation, Biochemistry 37 (4) (1998) 1046–1052, doi:10.1021/bi9714868.

[54] S.N. Gamat, L. Fotouhi, Z. Talebpour, The application of electrochemical detection in capillary electrophoresis, J. Iran. Chem. Soc. 14 (3) (2017) 717–725, doi:10.1007/s13738-016-1023-7.

[55] M.A. Islam, P. Mahbub, P.N. Nesterenko, B. Paull, M. Macka, Prospects of pulsed amperometric detection in flow-based analytical systems – a review, Anal. Chim. Acta 1052 (2019) 10–26, doi:10.1016/j.aca.2018.10.066.

[56] E. Murray, P. Roche, M. Briet, B. Moore, A. Morrin, D. Diamond, B. Paull, Fully automated, low-cost ion chromatography system for in-situ analysis of nitrite and nitrate in natural waters, Talanta 216 (2020) art. 120955, doi:10.1016/j.talanta.2020.120955.

[57] S.K. Mishra, P.K. Dasgupta, Electrodialytic reagent introduction in flow systems, Anal. Chem. 82 (2010) 3981–3984, doi:10.1021/ac100882a.

[58] S.K. Mishra, P.K. Dasgupta, Capillary scale light emitting diode based multi-reflection absorbance detector, Anal. Chim. Acta 605 (2007) 166–174, doi:10.1016/j.aca.2007.10.030.

[59] J. Sestak, J. Planeta, V. Kahle, Compact optical detector utilizing light emitting diodes, 50 nL L-shaped silica capillary cell and CCD spectrometer for simultaneous multi-wavelength monitoring of absorbance and fluorescence in microcolumn liquid chromatography, Anal. Chim. Acta 1112 (2020) 80–91, doi:10.1016/j.aca.2020.03.020.

[60] N. Shibuya, R.S. Porter, Kinetics of PEEK sulfonation in concentrated sulfuric acid, Macromolecules 25 (24) (1992) 6495–6499, doi:10.1021/ma00050a017.

[61] R.Y.M. Huang, P. Shao, C.M. Burns, X. Feng, Sulfonation of poly (ether ether ketone)(PEEK): kinetic study and characterization, J. Appl. Polym. Sci. 82 (11) (2001) 2651–2660, doi:10.1002/app.2118.

[62] L.J. Anderson, X. Yuan, G.B. Fahs, R.B. Moore, Blocky ionomers via sulfonation of poly (ether ether ketone) in the semicrystalline gel state, Macromolecules 51 (16) (2018) 6226–6237, doi:10.1021/acs.macromol.8b01152.

[63] R.J.W. Cremlyn, Chlorosulfonic Acid: A Versatile Reagent, Royal Society of Chemistry, Cambridge, U.K., 2002.

[64] J. Eom, D.S. Lee, Low-temperature conductivity detection for ultrasensitive ion chromatography, Bull. Kor. Chem. Soc. 37 (3) (2016) 321–329, doi:10.1002/bkcs.10673.

[65] M.C. Bruzzoniti, E. Mentasti, C. Sarzanini, Determination of lanthanides by ion chromatography. Separation and retention mechanism, Anal. Chim. Acta 353 (2–3) (1997) 239–244, doi:10.1016/S0003-2670(97)87782-3.

[66] Thermo Scientific Dionex IonPac AS18-4μm Column Product Manual. http://tools.thermofisher.com/content/sfs/manuals/Man-065499-IonPac-AS18-4um-Column-Man065499-EN.pdf, Last accessed December 31, 2020.

[67] J.S. Fritz, D.T. Gjerde, in: Ion Chromatography, 3rd ed., Wiley-VCH, Weinheim; New York, 2000, p. 88.

[68] V. Pucci, M.A. Raggi, F. Svec, J.M.J. Frechet, Monolithic columns with a gradient of functionalities prepared via photoinitiated grafting for separations using capillary electrochromatography, J. Sep. Sci. 27 (10–11) (2004) 779–788, doi:10.1002/jssc.200401828.

[69] S. Currivan, D. Connolly, E. Gillespie, B. Paull, Fabrication and characterisation of capillary polymeric monoliths incorporating continuous stationary phase gradients, J. Sep. Sci. 33 (4–5) (2010) 484–492, doi:10.1002/jssc.200900720.