Capillary ion chromatography

This review summarizes progress in capillary ion chromatography. Theoretical aspects and practical limitations of packed and open tubular capillary columns are considered. Applications of packed and open tubular capillary IC are described. Emerging technologies such as chip-scale IC and the use of monolithic columns are discussed.

Key Words: Capillary ion chromatography; Capillary separations; Inorganic ions

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1 Introduction

Ion chromatography (IC) is an analytical technique for the separation of ionic and ionizable compounds. IC exploits the differences in ion exchange affinities of different analytes. Lucy [1] has suggested that the documented use of ion exchange goes back to biblical times: when Moses arrived in Marah after crossing the Red Sea he used a tree to “sweeten” brackish water [2]. While any biological material undoubtedly contains capillaries, we shall confine ourselves here to a less ambitious time frame. The clock in this review begins with the publication of the seminal paper by Small, Stevens, and Baumann on IC in 1975 [3]; this paper has since been designated one of the milestone papers in Analytical Chemistry [4]. For the first time, they used a chromatographic system with two columns, a separation column filled with a low capacity ion exchanger, a second, high capacity ion exchange column for eluent counterion exchange to reduce the conductivity of the background, and an electrical conductivity detector. The key new concept was the use of the second column that “neutralized” the eluent ions resulting in “conductivity suppression” so that a flow-through conductometric detector could monitor the second column effluent for eluting analyte ions with high sensitivity. A detailed discussion of all significant developments in IC in the last three decades is beyond the scope of this review. Some of the more important milestones include the introduction of membrane suppressors [5–7], the electrodialytic generation of eluents [8–10], single column or “non-suppressed” IC [11–13], in which only one low capacity column is utilized in conjunction with an eluent of high elution strength but low conductivity, ion exclusion chromatography [14–16], in which weak acids or bases are separated in their uncharged form, and more recently the use of electrically polarized ion exchange resin beds that can be used for generation, suppression, and even purification of eluents [17–19]. These and other innovations, notably in the development of novel ion exchanger phases, have contributed to the popularity of IC such that anion analysis today is unthinkable without IC. The present worldwide market for IC equipment and related consumables is estimated to be ca. US $ 220 million.

At the present time, capillary IC equipment or columns are not commercially available. Researchers have explored capillary scale IC, however, since the early years. The introduction of capillary LC in late seventies, generally attributed to Horvath et al. [20, 21], and related key publications in the area [22–25] had an early impact on carrying out IC on a miniature scale. As early as 1983, suppressed capillary IC was reported by Rokushika et al. [26]. Due both to theoretical and to practical limitations, including the lack of commercially available instrumentation, capillary IC has not yet emerged beyond research laboratories. In this review some theoretical aspects of capillary format chromatography are discussed from the perspective of performing IC, and then practical achievements and applications thus far are discussed.

2 Capillary (ion) chromatography

Herein we consider a column to be a capillary column if the inner diameter is less than 1 mm. We consider here (i) packed, (ii) open tubular (OT), and (iii) monolithic capillary columns.

Packed capillary columns are analogous to conventional large bore columns and are typically made by the same slurry packing methods as their large bore counterparts. A frit is made at one end of the capillary that retains the packing material. The capillary columns are typically packed under pressure with particles <15 μm in diameter. Drawn packed capillaries [27] (drawing a large bore glass capillary packed with particles), explored in the early days of capillary LC have never been used in capillary IC; most
ion exchange packings are unlikely to survive such thermal conditions.

Open tubular capillary columns are usually fused silica capillaries of inner diameter in the range of <100 μm. A few practitioners of capillary LC have ventured to columns of <10 μm ID: the multiple problems of reliably pumping very small flow rates, reproducibly injecting minute quantities of samples, and sensitively detecting the very small amounts of analyte are formidable. The stationary phase is attached to the capillary walls by electrostatic interactions or by covalent bonding. The wall can also be coated with a polymer – the coating thickness can be optimized to achieve a compromise between acceptably high mass transfer rates and high loading capacity. Extraordinary efficiencies (over a million theoretical plates) have been demonstrated in capillary LC, such efficiencies have not been reported in IC. While many ionic analytes are small and thus have relatively high diffusion coefficients, ion exchange kinetics can sometimes be rate-limiting relative to nonion adsorption-desorption equilibria. As the horizons of capillary IC broaden, it will therefore be interesting to observe how the attainable efficiencies compare in LC vs. IC in the capillary domain. Nevertheless, achieving appropriately small injection and detector volumes while maintaining detection sensitivity is likely to remain a challenging task. In this context, the mainstay of IC detection, conductometry, enjoys an advantage. Unlike optical detection methods, conductometric detection can be downscaled without penalties.

Monolithic columns consist of a rigid polymeric structure inside the capillary produced by thermally initiated or photo-initiated polymerization of monomers with desired functional groups, giving the polymer matrix a variety of tailored properties. Sol-gel processes are typically used for preparation of silica-gel monoliths. Relative to packed columns, they provide comparable separation efficiency, relatively simple fabrication, and lower flow resistance.

While there are a number of publications on packed capillary IC and fewer (but still several) reports on OT IC, monolithic column capillary IC still appears to be in its infancy.

3 Theory of chromatography: Consequences for capillary IC

All processes contributing to the final zone width are important in capillary IC. These include resistance to mass transfer, eddy diffusion (in packed columns and monoliths), longitudinal diffusion, and extra-column effects that include broadening in the suppressor and the detector. The overall peak variance is the sum of the individual variances:

\[ \sigma_{\text{tot}}^2 = \sigma_{\text{res}}^2 + \sigma_{\text{eddy}}^2 + \sigma_{\text{long}}^2 + \sigma_{\text{sup}}^2 + \sigma_{\text{det}}^2 \]  

The final zone width, \( \sigma_{\text{tot}}^2 \), is the measure of column efficiency and for ideal (Gaussian) analyte peaks, the width of the peak being directly related to the variance, \( \sigma^2 \).

Some important questions have to be answered: What are the advantages and disadvantages of the capillary format vs. the conventional one? Under what conditions will capillary IC (in any column format) be competitive with its macroscale counterpart? Capillary separation systems can generally produce greater column efficiencies per unit length but what other limitations are posed by the capillary format?

Knox and Gilbert [28] theoretically studied the efficiency of capillary columns in LC. They derived the optimum particle diameter \( d_{\text{opt}} \) for packed capillary columns and the optimum inner diameter of an open tube capillary column \( d_{\text{opt}} \), for a given number of total theoretical plates \( N \), mobile phase viscosity \( \eta \), analyte diffusion coefficient \( D_m \), and the total pressure drop \( \Delta \rho \):

\[ d_{\text{opt}} = (4000 N \eta D_m / \Delta \rho)^{1/2} \]

\[ d_{\text{opt}} = (128 N \eta D_m / \Delta \rho)^{1/2} \]

For typical conditions, such as \( N = 10^4 \), \( \eta = 10^{-3} \) N m/s², \( D_m = 10^{-9} \) m²/s, \( \Delta \rho = 200 \) bar \( (2 \times 10^7 \) N/m²), Knox and Gilbert compute \( d_{\text{opt}} \) to be 1.4 μm. Although the use of very small particles in the 1–1.5 μm particle size range has been in vogue for some time in reverse phase liquid chromatography, this is not the case for IC. The smallest available particle size for commercially available columns is 3 μm [29]. However, comparison of chromatograms from columns from another manufacturer that uses a larger particle size packing clearly indicates that particle size alone is not the only factor in determining column efficiency. Moreover, it is not clear that the same theoretical consideration for optimum packing diameter strictly applies to IC columns which more often than not consist of surface-agglomerated packing. While pumping systems capable of pumping at very high pressures have become commercially available, the maximum pressure tolerance of polymeric particles typically used in IC must also be considered.

The optimum inner diameter for open tubular capillaries \( (d_{\text{opt}}) \) is similarly calculated from Eq. (3) to be 0.26 μm. Capillaries with this diameter are not at this time commercially available and even if they were, injection (the smallest commercially available injector at the time of this writing has a loop volume of 10 nL), detection, and column plugging problems may be nearly insurmountable.

However, open tubular columns can and often are operated at conditions far from theoretical optima and can still generate enough plates for the desired separation. With injection and detection volumes of 1 nL and a pressure difference of 100 bar (1500 psi), a performance comparable...
with conventional packed columns can be theoretically achieved at \( N = 30,000 \), using a 5 m long column of 10 \( \mu m \) ID at a flow rate of 30 nL/min and diffusion coefficient \( D_n = 10^{-9} m^2 s^{-1} \). With a 100-fold larger injector/detector a comparable performance at 10-fold greater plate count (\( N = 30,000 \)) can be achieved.

Often the column performance is measured in terms of number of theoretical plates per unit time based on Golay’s performance index \([30]\) that measures the efficiency with which a given column achieves a certain resolution with a given pressure differential at a given time. This may not be satisfactory because efficiency can be generally increased by increasing the pressure or by decreasing the eluent viscosity. For general comparison of various columns types, Knox \([31]\) introduced a term separation impedance, \( E \):

\[
E = \frac{b \Delta p}{N \eta} = h^2 \phi
\]

(4)

where \( b \) is the void retention time, \( \Delta p \) is the pressure difference across the column, \( \eta \) is the eluent viscosity, \( h \) is the reduced plate height, and \( \phi \) is the column flow resistance factor.

\( E \) is dimensionless; the lower the value of \( E \), the better is the column performance. If two columns are to be compared, they can be compared based on the value of \( E \).

Based on the calculation of \( E \) under optimum conditions, Knox and Gilbert concluded that (i) very small detection/injection volumes are essential for packed capillary columns to be competitive with large bore packed columns in performance, (ii) OT capillaries will be particularly attractive at very high values of \( N \) and will necessitate low injection/detection volumes and very small diameters: such conditions may be feasible in the future in on-chip chromatographic systems.

At room temperature, the low diffusion coefficient of the analyte in the mobile phase results in a low mass transport rate in OTLC, resulting in poor plate heights. Some have believed that hydrodynamic means to improve mass transfer to the wall will be useful, i.e., mass transfer to the wall should be changed from a strictly diffusive regime (as in laminar flow) to systems where there is active convective transport to the wall. Coiling the separation capillary (as in Helical Open Tubular columns) enhances the mass transfer by radial convection due to the secondary flow. Tijssen \([32, 33]\) (who has called such capillaries HOT columns) and Tsuda and Novotny \([27]\) have demonstrated that radial convection indeed improves the plate height in relatively large bore (typically 200–300 \( \mu m \) ID) tubes. However, noticeable improvement is observed primarily at high flow rates, and the separation column must then be sufficiently long to allow sufficient time for the interaction of the analyte with the stationary phase. Thus far, the experimental data have been observed to agree well with theory for unretained analytes but actual chromatographic performance falls far short for retained analytes. Because diffusion coefficients in the stationary and mobile phases in LC/IC are not as different as in GC, there are, comparatively speaking, less limitations on the permissible thickness of the stationary phase. For reasonably permeable coatings, relatively little loss of efficiency should occur for wall coating thicknesses up to 25% of the tube diameter. Thus, a thick layer of porous or otherwise permeable ion exchanger phase will improve column capacities in OTIC. The astute reader will observe that the boundary between this and highly porous monolithic columns in a capillary scale will become blurry.

4 Applications of packed capillaries in IC

While packed capillary chromatography is completely analogous to conventional column chromatography, preparation of capillary columns requires very small amounts of packing material. As such, stationary phases that are expensive to prepare, are more easily used in the capillary format. Kryptand-based stationary phases that have been introduced for variable capacity gradients \([34, 35]\) can, for example, profit from a capillary column adaptation. Further, the consumption of eluent and sample solutions is decreased and this becomes invaluable when samples are of the order of \( \mu L \) or sub-\( \mu L \) and cannot easily be analyzed by conventional scale LC or IC.

4.1 The introduction of capillary IC

The evolution of capillary IC has closely followed that of capillary LC. The first publication on suppressed capillary IC appeared in 1983 \([26]\). The development of IC with conductivity suppression on the macroscale inspired Rokushika et al. to use capillary IC columns of 0.19 mm diameter and couple these to a 0.2 \( \times \) 10 mm Nafion\textsuperscript{®} perfluorosulfonate hollow fiber suppressor immersed in a large reservoir of a low-bleed, large counterion acid regenerant, dodecylbenzenesulfonic acid. Using a surface agglomerated anion exchanger of 10 \( \mu m \) base particle size, sodium carbonate-bicarbonate as eluent, they not only demonstrated the separation and detection of several inorganic anions and organic acids at low ppm levels, they showed the applicability of the method to real samples ranging from river water to several types of fruit juice. Figure 1 shows the separation of a standard mixture. The plate heights ranged from 0.15–0.28 mm for \( \text{NO}_2^- \) and \( \text{NO}_3^- \), respectively, at a linear velocity of 0.67 mm/s and increased to 0.25–0.45 mm as the velocity was increased to 2.4 mm/s. Later in the same year, using the same column and carbonate or hydroxide based eluents with or without added methanol, this group illustrated the use of UV detection in capillary IC for the sep-
aration and analysis of UV-absorbing anions, many organic acids including aromatic acids such as aminobenzoic acids, nucleotides and nucleobases [36]. Detection wavelengths ranged from 195 nm upwards and subsequently the applicability of UV detection was also demonstrated for polyallylamino-coated silica gel columns (3 and 10 μm particle size) to separate and quantitate hydroxybenzoic acids and nitrophenols with carbonate-bicarbonate eluents [37].

4.2 Contributions from the Takeuchi Laboratory

The use of capillary chromatography techniques for the analysis of ionic species has been extensively investigated by Takeuchi et al. beginning in 1988 and continuing to date. They have explored a variety of separation and detection methods in packed fused silica capillary columns. In 1988, they demonstrated the analysis of inorganic anions by indirect UV detection on a 0.32 mm particle size column. In 1988, they demonstrated the analysis of inorganic anions by indirect UV detection on a 0.32 mm particle size column. In 1988, they demonstrated the analysis of inorganic anions by indirect UV detection on a 0.32 mm particle size column. In 1988, they demonstrated the analysis of inorganic anions by indirect UV detection on a 0.32 mm particle size column. In 1988, they demonstrated the analysis of inorganic anions by indirect UV detection on a 0.32 mm particle size column.

4.2.1 Stationary phases coated with immobilized bovine serum albumin

In 1996, Takeuchi et al. published three separate accounts of ionic determination on columns containing bovine serum albumin (BSA) immobilized on 5 μm ODS particles. As the eluent pH decreases, more of the BSA is protonated and anionic analyte retention generally increases. Using 0.35 × 75–150 mm columns and an eluent containing NaI and tartaric acid, the retention of chloride, nitrite, and bromide was studied by indirect photometric detection. The method was also applied to tap water [46]. Direct UV detection was subsequently used with these columns with various acid eluents in the pH range 3–4 for the separation and detection of iodate, bromide, nitrate, iodide, and thiocyanate. The latter three ions were determined in the saliva of smokers vs. non-smokers [47]. In a final publication of this series [48], they compared NaI, KHP, Na-salicylate, and 2,6-AQDS as eluents for indirect photometric detection on a BSA-based capillary column under weakly acidic to neutral conditions; the latter eluent ion had the highest absorptivity and provided the best detection limits. In principle, BSA-coated columns can be used as cation exchangers at high pH. In practice, the column packing degrades under these conditions and cation exchange chromatography cannot be
carried out on BSA-based columns. The authors earlier carried out cation exchange chromatography on 0.35 × 50 mm silica gel columns for the separation of alkaline metal cations using a solution of benzyltrimethylammonium chloride (BTMAC, typically 2 mM in 30% acetonitrile) as the eluent [49]. Applicability of the method to real samples such as whisky and ketchup was shown. Subsequently, the effort towards cation determination by indirect photometric detection was extended to include alkaline earth metals Mg$^{2+}$ and Ca$^{2+}$ on a column of similar dimensions using a more strongly absorbing cation 1,1′-dimethyl-4,4′-bipyridinium as the eluent (in the dichloride form) [50]; the applicability of the method was demonstrated for beverage samples, including red wine.

### 4.2.2 Ionically coated ion exchangers

In 1997, taking a cue from the success of Dionex Corporation in modifying surface-sulfonated resins with ion exchange particles of opposite charge, Takeuchi et al. began experimenting with modifying ion exchangers with polysaccharides that contain ionizable groups [51]. A coating of chondroitin sulfate (the sugar skeleton contains a −COOH group, a −CH$_2$OSO$_3$H group, and a −NHCO-CH$_3$ group, potentially providing both anion and cation exchange sites) was tested on two different stationary phases, TSKgel IC-Anion-SW and TSKgel IC-Anion-SW, each in a 0.32 × 100 mm column format. These resins are respectively silica- and polymer based, differ greatly in their ion exchange capacities (the former having an order of magnitude greater ion exchange capacity than the latter) and their pore size distribution such that the chondroitin sulfate (estimated average MW 4.1–6.5 × 10$^4$) was completely excluded from the pores of the first resin but not of the second. Coating led to quite different effects on retention. For the silica based high capacity low pore-size exchanger, absolute retention (using nitrate as a probe analyte) decreased substantially upon coating. With 1–100 mM Na$_2$SO$_4$ as eluent, the retention of nitrate with increasing concentration of the eluent before coating the column, as may be expected. However, after coating the column, the retention increased with increasing eluent concentration. This most unusual behavior clearly indicated that the mode of anion retention was not simple ion exchange. In contrast to the silica based exchanger, coating the polymer based stationary phase led to increased retention compared to the uncoated column and predictable retention behavior as a function of eluent concentration. Further work was conducted only with the silica column. Separation and detection of common UV absorbing anions such as NO$_3^-$, I$^-$, and SCN$^-$ was readily possible with low concentrations (10 mM) of Na$_2$SO$_4$ as eluent and direct UV detection. The same conditions could be readily used in detecting these ions in saliva. That the mechanism was not ion exchange was indeed apparent in that pure water could be used as the eluent and these three ions could be separated as their sodium salts. This ability of pure water-based elution is known for other zwitterionic stationary phases [52]. Finally, the authors showed the conductometric detectability of a number of anions, using very dilute tartaric acid as eluent.

They followed up this work with heparin-coated columns [53]. Like chondroitin, heparin contains an amidosulfonic acid group aside from carboxylate and sulfonate groups. Retention behavior of both anions and cations was investigated. Retention of anions on the anion exchanger was remarkably reduced after heparin-modification. Eluent cations were found to affect analyte anion retention behavior. With Na$_2$SO$_4$ and MgSO$_4$ eluents, retention factor of anions increased with increasing eluent concentration but opposite (more normal ion exchange like) behavior was observed with eluents containing Al$_2$(SO$_4$)$_3$, CuSO$_4$, and H$_2$SO$_4$. With CuSO$_4$ as the eluent, the retention and elution of both cations and anions on the modified stationary phase could be indirectly visualized by monitoring the absorption due to copper ions. With just water as eluent, the heparin-modified stationary phase showed no anion retention. The general area of chromatography on such mucopolysaccharide modified anion exchanger stationary phases in microcolumn format was then summarized by the authors [54].

In 1999, Takeuchi et al. began studying dextran sulfate (DS). This anionic polysaccharide is available in pure form in different molecular weight ranges [55]. Using DS of different molecular weight fraction and anion exchange resins of different pore size, they conclusively proved that when the DS is large compared with the pore size of the resin, the negatively charged DS merely attaches to the exposed cationic surface sites of the resin, resulting in decreased anion exchange capacity and normal elution behavior (log $k^′$ of an analyte anion linearly decreasing with increasing log eluent concentration) is maintained. When the DS is smaller than the pore size of the resin, the DS can get into the pores of the resin and can attach as well to the cationic sites inside the resin. The greater the pore size relative to the size of the DS, the more DS is adsorbed. Under these conditions, the retention of the analyte anion does not show ion exchange like behavior: log $k^′$ increases linearly with increasing log eluent concentration. The authors suggest in a qualitative fashion that repulsion from the cation exchange sites (i.e., excess unbound sulfate groups in DS) may be increasing with decreasing eluent concentration and overrides the normal ion exchange retention that is affected by the eluent anion. Experiments involving a series of eluent cations and anions of different ion exchange affinities at the same ionic strength will be necessary to better understand the
behavior of such systems in light of the Stern-Guoy-Chapman double layer theory [56, 57].

Using low MW DS-coated columns, separation of alkali and alkaline earth cations was demonstrated using both CuSO₄ and the much more strongly absorbing ruthenium tris(bipyridyl)³⁺ as the eluent; anion separation performance was not particularly attractive. Using the same column dimensions (0.32 × 100 mm), the technique was subsequently demonstrated for analysis of blood serum samples [58]. The general behavior of positively charged anion exchange columns coated with substances that are negatively or zwitterionically charged are similar to nonpolar columns coated with zwitterionic substances; Takeuchi et al. have also contributed to this area; this is discussed later.

4.2.3 Recent efforts

The fluorimetric detection of Mg and Al as their oxine complex was studied in the microcolumn format (0.32 × 100 mm, ODS packing), with (a) oxine present in the eluent (comprising a buffered micellar sodium dodecylsulfate medium to effect the solubilization of oxine) or (b) oxine added postcolumn [59]. The incorporation of oxine in the eluent was found to produce substantially superior performance relative to postcolumn addition. The LOD for Mg was 18 µM. The method was significantly more sensitive for Al but blanks could not be reduced below 0.80 µM. There is great potential of these type of approaches for detecting a variety of metal ions. The sulfonated derivative of oxine is water soluble and has been extensively studied for the numerous fluorescent metal complexes it forms [60]. Attractive detection of many metal ions has been demonstrated in macroscale systems [60–62]. High intensity light emitting diodes that are ideally suitable to excite metal-sulfoxine complexes have become inexpensively commercially available [63]. In combination with capillary scale liquid core waveguides based on Teflon AF-coated fused silica tubes can form the basis of dedicated, monochromatorless, highly sensitive fluorescence detectors [64].

The environmental nitrogen budget, in particular the supply of nitrogen to bodies of water, is often a topic of considerable interest. The dominant forms of soluble nitrogen consist of nitrate-N and ammonium-N, with small amounts of nitrite-N being occasionally present. The Takeuchi group developed a method for determining NO₂⁻ and NO₃⁻ by direct UV detection at 206 nm using a 0.32 × 100 mm column packed with a commercial anion exchanger and a 5 mM Na₂SO₄ eluent. Ammonium is not significantly retained by the column and comes out first. The UV detector effluent is mixed with o-phthalaldehyde-mercaptoethanol reagent in a tee and heated to temperatures up to 65°C in a 0.5–2 ml long 50 µM ID reaction coil to form an isoindole derivative that is detected by its fluorescence. The UV detector itself responds to eluted NO₂⁻ and NO₃⁻. Application to analysis of river water was demonstrated [65]. The Takeuchi group has also studied large volume injections in a microcolumn format; this is discussed in a later section.

Takeuchi et al. have obviously made impressive contributions to capillary chromatography in general and capillary IC in particular, especially in reference to exploring novel stationary phases and retention mechanisms. The average IC practitioner still needs to be sold on performance, however, especially in comparison with conventional systems.

4.3 Capillary IC on bile salt and other zwitterionic micelle coated columns

The work of Takeuchi et al. described above provides an appropriate backdrop to next consider zwitterionic micellar bile-salt coated reverse phase columns that have been used for IC, both on the capillary scale and otherwise. The first reported application of such a stationary phase for IC was by Hu, Takeuchi, and Haraguchi in 1992 in which the separation of alkali metal ions was achieved by indirect photometric detection using CuSO₄ [66]; the detection limits were modest. The same technique was used by the authors more sensitively and effectively with a CeCl₃ eluent and it was possible to separate both alkali and alkaline earth metals [67] and applied extensively to different types of beverages. Hu and Haraguchi then applied the method for the determination of UV-absorbing ions, notably NO₂⁻, NO₃⁻, I⁻, and SCN⁻, in saliva of subjects of different age, sex, and smoking habits [68]. The LODs ranged from 0.5 µM for I⁻ to 2.3 µM for SCN⁻ with a phosphate buffer as eluent and UV detection at 230 nm. In smokers [SCN⁻] was significantly elevated and also depended on the age and sex, while the other anion concentrations did not show any particular pattern.

The best known publication in this area, by Hu, Takeuchi, and Haraguchi, appeared in 1993; “Electrostatic Ion Chromatography” was performed in this report on both the conventional and capillary scales [69]. An illustrative separation using pure water as eluent is shown in Figure 2. The lure of using pure water as eluent is considerable. However, the intervening time has proven that this quest may be elusive. On such a system, the actual separation is considerably dependent on the ionic strength of the sample itself (as would be predicted from the Stern-Guoy-Chapman theory of the double layer) and the possibility that the exact elution time of an anion may be dependent on the counterion present. The exact mechanism of separation that was proposed in this paper has also since been revisited. Hu and Haraguchi also revisited determination of analytes in saliva [70] and proceeded to show that simultaneous determination of cations and anions is possible on such a column using CuSO₄ as the eluent to...
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4.4 Unique applicability of capillary IC

There have been few occasions where the scale of the separation/detection system has been vital to the success of the mission. Bächmann et al. [73] wanted to analyze size-classified raindrops for both their cation and anion composition. According to their relative sensitivity analysis, the optimum sample volumes for conventional scale IC, capillary IC (0.5–0.75 mm ID), and capillary zone electrophoresis (CZE, 75 μm ID) are: 50, 0.5, and 0.01 μL (these translate to spherical drop diameters of 4.6, 1.0, and 0.25 mm), respectively. Depending on windspeed, raindrops can span these size ranges. Accordingly, the researchers used all three techniques. (Interestingly, while chromatograms for actual raindrops were presented for both scales, only standard electropherograms were shown. Perhaps it is not surprising that CZE is no longer considered a viable competitor to IC for ion analysis!)

Cation chromatography involved 110–250 mm long 0.75 mm ID capillary columns with a Ce(NO3)3 eluent and indirect photometric detection via fluorescence. Injection volumes ranged from 0.5 to 2.5 μL. Anion chromatography was conducted with a 0.5 × 150 mm reversed phase column coated with dodecylamine with indirect absorbance detection at 205 nm using K3[Fe(CN)6] as the eluent. For all of the common anions and cations measured (Na+, NH4+, Ca2+, Mg2+, Cl−, NO3−, SO42−), the concentration in the raindrops increased dramatically with decreasing size.

The second application involves for the same reason that capillary LC is finally beginning to enjoy commercial success – it is the optimum scale for coupling with a mass spectrometer. Buchberger and Haider first demonstrated the advantages of capillary scale IC-MS for the analysis of inorganic anions and aminopolycarboxylic acids [74]. The S/N = 3 LOD for a variety of anions were in the 20–80 ng range.

4.5 Large volume injections in capillary IC: On-column enrichment

In liquid chromatography, direct large volume injections are often impractical because the sample matrix cannot often be made weak enough to prevent premature elution of sample components. In ion exchange chromatography, water as an eluent has near-zero eluent strength. As such, on-column enrichment by large volume injections of low ionic strength samples are straightforward. Kourilova et al. [75] used sample volumes ranging from 200 nL to 1 mL on 0.7–0.9 × 150 mm columns packed with 7.5 μm cation exchange particles. Using eluents containing ethylenediamine and tartaric acid and conductometric detection, they were able to obtain 5–50 nM detection limits for 1 mL injection volumes for a variety of metals. This is particularly impressive in view of the equipment used and the relatively poor column efficiencies attained. Although at

Figure 2. Chromatogram of inorganic anions. Column, Develosil ODS-5 coated with CHAPSO micelles, mobile phase: pure water, flow rate: 2.8 μL/min, UV detection at 230 nm, analytes concentration: 0.1 mM each. Reprinted from Ref. [69] by permission from the American Chemical Society.

Figure 3. Simultaneous separation of inorganic cations and anions: column, 0.35 mm ID × 150 mm packed with Develosil ODS-5 coated with NaTDC micelles; mobile phase: 5 mM copper(II) sulfate aqueous solution, UV detection at 210 nm, analytes concentration: 0.5 mM each. Reprinted from Ref. [71] by permission from the American Chemical Society.

permit optical detection [71]. An illustrative separation is shown in Figure 3. Both CuSO4 and CeCl3 were used as eluents for the separation and determination of Na, K, Mg, and Ca in human saliva on 0.35 × 150 mm columns; the results agreed very well with values determined by plasma-atomic emission methods [72].
1 mm column ID, it is on the border of what can be called capillary scale. Ito’s work [76] on the direct determination of iodide in seawater by using large volume (2 mL) injection and UV detection at 226 nm is worthy of mention because it solves a practical need using a very short column (1 × 35 mm). With a high capacity ion exchange packing, an eluent containing 30 mM NaClO₄, 500 mM NaCl, and 5 mM Na-Phosphate buffer (pH 6.0), the author was able to achieve an LOD of 200 ng/L, a 2 nM, practical for iodide determination in both oceanic and estuarine waters. Lim et al. have also recently reported on the separation and detection of ppb levels of different anions using a 10 µL injection on a 0.32 × 100 mm column (5 µm high capacity ion exchanger) using an NaCl eluent and UV detection at 210 nm [77]; the separation efficiency is, however, not competitive with commercial macroscale counterparts.

### 4.6 Suppressed IC in capillary format

Since the original work of Rokushika et al., few researchers have ventured into membrane suppressed capillary IC, attempting to miniaturize precisely what in macroscale has proved to be a considerable success. Sjögren et al. [10] constructed the first capillary IC complete with an electrodialytic generator (EDG) for the eluent. Indeed, the capillary scale greatly facilitated fabrication of an EDG on the high pressure side which had not previously been achieved in macroscale systems. The system diagram is shown in Figure 4. A commercially available low cost stepper motor driven syringe pump containing a 500 µL glass syringe is used with a 3-port header, where a mildly pressurized water reservoir is connected through an inlet check valve. The syringe itself and the downstream components are connected through the other two ports. To begin operation, the syringe begins to aspirate, which opens the inlet check valve and allows water to come in to the syringe. During the dispensing process, a rapid initial movement builds up pressure and seals the inlet check valve and water flows out via the pressure sensor tee to the high pressure EDG. The EDG consists of a lower flow path cut into a stainless steel (SS) block through which the syringe output flows. This is separated by a thick cation exchange membrane from an upper flow path cut in a polymer insert. A donor NaOH solution is made to flow at a low flow rate by modest pneumatic pressure through the upper flow channel to waste. A platinum wire is also present in the upper flow path. Application of voltage between the SS block (−) and the Pt wire (+) causes Na⁺ to migrate across the membrane and form NaOH and H₂ gas on the cathode side, the amount being faradaically proportional to the microampere level current flowing through the system. The NaOH output from the SS channel is connected by a PTFE tube (under the operating pressure, the hydrogen gas present in the stream is removed by permeation through the polymer tube wall) to a low-volume miniature in-line conductivity cell to measure the generated NaOH concentration. The eluent concentration can be programmed to change by changing the EDG current. The eluent flows through a 100 nL volume loop injector, a 0.18 × 560 mm capillary column packed with AS–11 packing material from Dionex, a 80–100 µm ID Nafion tube based suppressor (≈11 mm active length), and a capillary conductivity cell. The small column diameter led to high permeability and allowed the use of a long, high plate number column at a flow rate of 2 µL/min (1.3 mm/s, equivalent to the flow rate of 1 mL/min in a 4 mm ID column) at a pressure drop of <800 psi. In isocratic operation, the column efficiencies per unit length for all the tested ions were significantly better than a 2 mm ID column packed with the same packing. The difference was as large as 2.8 times for an early eluting ion like fluoride to a point of no difference for late eluting phosphate (suggesting that suppressor/detector contributions to loss of plates in conventional bore systems could have been significant). A gradient separation on this system is shown in Figure 5. Experience with these systems suggests that...
the high permeability of capillary systems may allow longer columns operated at higher than optimum flow rates to achieve same separations faster than a conventional bore system.

In the following year, Boring et al. reported a field-portable version of this system that fits into a custom briefcase (28 × 43 × 15 cm, 10 kg) [78]. Several novel features were introduced: a trap column consisting of a chelating resin and a mixed bed resin was used ahead of the EDG to remove impurities, a polystyrene capillary (80 μm ID, 250 μm OD, 10 cm long) was used downstream of the EDG for more efficient H₂ gas removal with a lower residence volume, a 0.05 × 19 mm radiation grafted sulfonated PTFE ion exchange fiber provided suppression and a novel conductivity cell where the electrode spacing was readily changeable was used for detection along with bipolar pulse electronics, a 10 cm long 180 μm ID preconcentration column was used for preconcentration experiments. The total power requirement of the system, not including that of the laptop computer used for instrument control and data acquisition was 17.5 W.

Boring et al. [79] then coupled a preconcentration column equipped capillary IC system described above to a miniature parallel plate denuder used for collecting soluble gases. They showed that low parts per trillion level detectability of trace ionogenic gases is readily attainable (Figure 6).

IC equipment manufacturers may finally have realized the merit of the capillary scale as evidenced from a recent presentation from Dionex Corporation [80]. Although many details are still proprietary, attractive performance has already been demonstrated. The impressive repeatability of an isocratic chromatogram is shown in Figure 7.
portional to \(d/t\), characteristic time

characteristic time

analytes, especially at elevated temperatures, are typically used in capillary gas chromatography with good separation efficiencies, the diffusion coefficients of the analytes, especially at elevated temperatures, are typically \(10^4\) times larger than those encountered in LC. The characteristic time \(t\) for diffusive travel of distance \(d\) is proportional to \(d^2/D\) where \(D\) is the diffusion coefficient. It follows that ideally capillary internal diameters for use in LC should be about \(10^2\) times smaller than in GC. However, the Knox and Gilbert [28] optimum ID of \(<0.3\ \mu m\) is unlikely to be practical in the near future. Going down in column diameter also places corresponding burdens on injection and detection techniques. The injector demands can be bypassed by splitting the flow after the injector but there is no way to bypass the small detection volume requirement. As a result, progress with attractive performance has not been plentiful.

As early as in 1981, Ishii and Takeuchi demonstrated open tubular cation-exchange chromatography [81]. In their work, 30–60 \(\mu m\) soda lime glass capillaries were coated with phenyltriethoxysilane or 2-mercaptoethyltriethoxysilane, and subsequently ion exchange groups were introduced by treatment with concentrated \(H_2SO_4\) at 90 °C for 4 hours or by oxidation with permanganate. Separation of four nucleosides was demonstrated. Elevated temperature operation was shown to be beneficial due to the increase of the diffusion coefficients of the analytes. Further, surface treatment of the soda lime capillary with \(1N\ NaOH\) over a prolonged period prior to coating resulted in 9–18 times greater surface area and correspondingly higher column capacity.

In 1983, Manz and Simon [82] illustrated the use of a potentiometric microelectrode with a demonstrated detection volume \(<500\ pL\), with the best estimate of the effective volume being 10 \(\mu L\) with a 25 \(\mu m\) ID column. A 1 \(\mu m\) tip diameter ion selective electrode was inserted into the open end of a 25–30 \(\mu m\) diameter column. The detector performed admirably but the separation system showed poor efficiency for significantly retained analytes. By 1989 this problem was solved and the group was able to coat the interior of columns 4.6 to 9.5 \(\mu m\) in ID with polybutadienesulfonic and polybutadiene-maleic acid (PBMA) pre-polymers and polymerize them in situ. For the 4.6 \(\mu m\) ID PBMA column (estimated coating thickness of 0.1 \(\mu m\)), 100% and 89% of the theoretically expected efficiency was achieved for a 90 cm long column, with 680,000 and 246,000 plates for analytes with \(k'\) values of 0 and 0.16, respectively. The authors were able to separate alkali and alkaline earth metals in about 4 min and alkali metals as a group in under 90 s, reaching LODs of 0.2–20 fmol [83]. They also showed separations of various amino acids. Using coatings of sulfopropylhydroxysilane and aminoalkylalkoxysilane in 4.6 \(\mu m\) ID columns, 0.33–0.37 m in respective length, the group was then able to show separations of alkali metal cations (10 \(\mu L\) injection, 20 mM HCOOH, 10 \(nL/min\) in under 25 s and common anions (10 \(\mu L\) injection, 10 mM HCOOH, 26 \(nL/min\) in under 35 s [84]. These are shown in Figure 8. They also showed that, on operation at an alkaline pH (\(NH_3/NH_4Cl\) buffer), it is possible to separate alkali cations in a 2.3 \(\mu m\) ID bare silica column, by virtue of ionization of the silanol groups.

Rather than going to smaller capillaries to solve the problem of slow mass transfer to the wall, Pyo et al. [85] attempted a different and in some sense a more direct approach: increasing the diffusion coefficient of the analyte. They accomplished this by raising the column temperature as high as 150 °C. Relative to analytes in LC, IC is already better off in that most analytes of interest are small high mobility ions. According to the Stokes-Einstein

5 Open tubular IC

Open tubular capillary IC provides the advantage over packed columns of low flow resistance, permitting the use of low pressure pumping systems. This may lead to the natural progression on IC separations on a chip, where the limited number of attempts thus far have used either electroosmotically generated flow or off-chip HPLC pumps and split flow. The possibilities of using low pressure, pneumatic or even gravity-induced flow for chromatographic separations makes the OT approach attractive not only with respect to microfabricated systems but with field-portable chromatographs in general. The open tubular approach however has also some inherent disadvantages that stem from the relatively small diffusion coefficients of analytes in the liquid phase at room temperature. While open tubular capillaries up to 530 \(\mu m\) in ID are routinely used in capillary gas chromatography with good separation efficiencies, the diffusion coefficients of the analytes, especially at elevated temperatures, are typically \(10^4\) times larger than those encountered in LC. The characteristic time \(t\) for diffusive travel of distance \(d\) is proportional to \(d^2/D\) where \(D\) is the diffusion coefficient. It follows that ideally capillary internal diameters for use in LC should be about \(10^2\) times smaller than in GC. However, the Knox and Gilbert [28] optimum ID of \(<0.3\ \mu m\) is unlikely to be practical in the near future. Going down in column diameter also places corresponding burdens on injection and detection techniques. The injector demands can be bypassed by splitting the flow after the injector but there is no way to bypass the small detection volume requirement. As a result, progress with attractive performance has not been plentiful.

Figure 7. Prototype 380 \(\mu m \times 250\ mm\) column, capillary electrogenerated KOH eluent, flow rate: 12 \(\mu L/min\), temperature: 35°C. Tubular Capillary Suppressor, suppressed conductivity detection. 20 separate runs overlaid on one another. Courtesy Yan Liu, Dionex Corporation.

Figure 8. 250 mm column, capillary electrogenerated KOH eluent, flow rate: 12 \(\mu L/min\), temperature: 35°C. Tubular Capillary Suppressor, suppressed conductivity detection. 20 separate runs overlaid on one another. Courtesy Yan Liu, Dionex Corporation.
equation, the analyte diffusion coefficient $D$ changes with temperature according to:

$$D = \frac{kT}{4\pi \eta r} \quad (5)$$

where $k$ is Boltzmann’s constant, $T$ is the absolute temperature, $\eta$ is the mobile phase viscosity, and $r$ is the spherical equivalent radius of the analyte. As it happens, for a largely aqueous eluent, the $T/\eta$ ratio increases by a factor of four from 25 to 100 °C. They demonstrated that up to a 6-fold increase in the number of theoretical plates is observed in anion separations in 50 µm capillaries from room temperature to 120–150 °C. However, at least in some cases, this increase in efficiency is not due to the increase in the diffusion coefficient of the analyte alone; the retention itself also decreases significantly at higher temperatures, particularly when adsorbed ion exchangers are used (vide infra). One way in which these experiments were conducted was through the use of positively charged (anion exchanger) latex particles electrostatically bound on the negatively charged walls of the fused silica column. This approach provides relatively good retention but quaternary ammonium functional groups degrade rapidly at increasing temperature due to Hofmann degradation. They experimented therefore with dynamically adsorbed ion exchanger coatings (e.g., with eluents such as hexadecyltrimethylammonium chloride). This was also not without problems: the adsorption of the surfactant on the column walls decreased markedly at temperatures above 100 °C. (The eluent was maintained in the liquid state at temperatures over its normal boiling point by using an exit restriction capillary.) Pyo et al. also studied the relation between the optimum flow rate (the Van Deemter minimum) as a function of the column temperature. They showed that the optimum velocity moves to higher flow velocities at increased temperature [86, 87]. Further, the relative rate of loss of plates with increasing flow velocity past the optimum is much less pronounced at higher temperatures than at lower temperatures. Fast separations therefore should be more easily attained at higher temperatures.

Functionalizing the interior walls of a small capillary is a nontrivial task. Especially when used in IC, the siloxane bonds obtained with alkoxysilane chemistries [82, 83] are not acceptable for practical use because of their hydrolytic instability. Recently, Pohl et al. have pioneered an approach where diepoxide-amine chemistry can be used to build up a unique hydroxide selective anion exchanger layer by layer [88]. In unpublished work, Kuban et al. [89] have fabricated open tubular capillaries using a 75 µm ID column and 25 alternating layers of 1,4-butanediol diglycidyl ether and methylamine. Electron microscopy shows that the interior coating is only 0.7 µm thick (Figure 9). Using such a column, suppressed open tubular IC was carried out for the first time (Figure 10) using a 11 mm long 80 µm diam. membrane suppressor. At the present time, such approaches are not practical because the separation still takes too long and the column exchange capacity is still rather inadequate. However, it definitely provides a path forward for future experiments with smaller diameter columns.

6 Monolithic columns in IC

Monolithic columns occupy an intermediate position between packed and open tubular columns albeit they are more closely related to the former. Flow resistance in

Figure 8. Fast separation of alkali metal cations. Reprinted from Ref. [84] by permission from Wiley InterScience.
monolithic columns is lower than that in packed columns. In properly designed monoliths, column efficiencies are maintained at relatively high flow velocities, enabling high velocity rapid separations. The use of commercially available porous silica-based monolithic rods in a conventional size (i.e., 4.6 mm x 10 mm) [90] for high speed IC separations using ion interaction chromatography and indirect conductometric detection was recently demonstrated by Hatsis and Lucy [91, 92]. They were able to accomplish the separation of 8 common inorganic anions in an amazing 15 s at a flow rate of 16 mL/min. The eluent consumption in this mode is obviously very high; however, in a capillary format this will not be an issue. It should be noted, however, that unlike the case of transition between conventionally sized packed columns to packed capillaries where column permeability generally increases markedly, an increase in permeability in going from conventional to capillary sized monoliths may not be realized. The pressure drop on monolithic columns will still be high enough to require high pressure pumping systems.

Monolithic columns have been created inside a microbore capillary by polymerization or by a sol-gel process. In what is thus far the only report on use of monolithic silica columns in capillary format IC, Motokawa et al. [93] prepared porous silica monoliths in 50 to 200 μm fused silica capillaries with various skeleton and through-pore sizes and used it for high speed ion chromatographic determination of acidity [94].

7 Ion chromatography on a chip

Liquid chromatography on a chip was first demonstrated by Manz et al. [95] in 1990. Although capillary electrophoresis has been widely implemented in a chip format, reports on chip scale LC/IC are scarce, due to the obvious difficulties of integrating pumping, injection, and detection systems. If all else is located off-chip, it is still necessary to fabricate efficient chip-scale microcolumns. He et al. [96] have used a chromatographic structure fabricated on...
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a quartz wafer for LC on a chip with flow being electroosmotically generated. The fabrication of a monolithic column inside the microfluidic structure on a chip was reported recently by Ericson et al. [97]. They have used both electroosmotic flow and pressure driven flow for on-chip LC. The integration of a chromatographic pumping system on a chip, capable of providing high pressure eluent flow, has not yet been achieved.

Thus far, there are only two reports on chip-scale IC. Kang et al. [98] described a microfabricated device with multiple parallel channels for conducting IC. The stationary phase was prepared in situ and each channel contained its own dedicated conductivity detection electrodes. The limit of detection (LOD) of injected analytes was stated to be about 50 mM, which may have been well above the concentration (unspecified) of the biphthalate or carbonate eluent used. This performance level underscores the importance of the need to develop some means of carrying out continuous ion exchange based suppression. Murrihy et al. [99] described an IC system in which separation takes place in a latex-coated channel microfabricated on silicon (as well as in non-negligible lengths of connecting tubing) with off-chip optical detection. In both reports, the pumping was achieved by an off-chip conventional HPLC pump.

8 Evolution of suppressors

At least in the conventional format, most find ion chromatography synonymous with suppressed conductometric detection. Suppressed conductometry is both uniquely applicable to ions and provides sensitive detection that is not degraded by scaling down. Naturally, efforts are made to continue to perform both on the capillary scale; however, this is a challenging task. The issue of miniaturization of conductometric detectors is discussed in the next section. Presently we discuss the issue of suppression. Although packed column based suppression has been used in the capillary scale [40], this is a dead end; it has even more disadvantages on the capillary scale than in macroscale IC. Early on Rokushika et al. [26] used a short length of a thermally stretched Nafion membrane as a suppressor and small diameter (75–80 μm ID) Nafion membranes have since been extruded. However, this may be the limit of extrusion technology. Such membranes and radiation grafted tubular membranes have been used both in suppressed capillary IC [10, 78–80] and suppressed conductometric capillary electrophoresis [100–104]. Low dispersion interconnections between the membrane and the suppressor and the suppressor and the detector on this scale are not a trivial task. Tubular membranes of even smaller diameter can be custom-fabricated. In unpublished work, Kuban and Dasgupta have demonstrated that very small diameter membrane tubes can be cast with Nafion solutions, using a platinum wire inserted in a capillary as a mandrel. A photograph of such a membrane is shown in Figure 11. In principle, this technology can obviate the need for interconnects. When miniaturizations proceed to the chip-scale, the construction of suppressors may need to be rethought. PolymericIC of appropriate construction may lend themselves to conversion of a portion of the chip into a membrane. With glass/quartz/silicon wafers, a liquid or suspension based ion exchange suppression will be practical and can be scaled to almost any dimension. Microscale continuous ion exchange has recently been demonstrated by Kuban et al. in both chip scale flows occurring in horizontal side-by-side streams [105] and vertical, layered streams [106], using both an ion exchanger dissolved in an immiscible solvent or a colloidal size ion exchange resin dispersed in water.

9 Miniaturizing conductivity detection

Early attempts at miniaturizing conductivity detectors largely involved putting together stainless steel tubular electrodes face to face in close proximity. Although not coupled to capillary systems, a variety of electrode/cell designs using small diameter metallic capillaries/needles were described in the literature early on [107]. Thin-walled stainless steel tubes are readily available down to 50 μm in diameter and readily permit making such detectors or detectors of the wall-jet geometry (such as that deployed by Boring et al. [78]). Conductivity detectors have been extensively used in capillary electrophoresis as well. The first commercial conductivity-based capillary electrophoresis detector is also essentially of a shielded wall-jet design [108]. Huang et al. [109] described an on-column conductivity detector which involved laser-drilling 40-mm holes on opposite sides of the capillary walls, followed by
the insertion of two 25-mm diameter Pt wires inside the holes to act as electrodes. Subsequently, a simpler grounded end-column arrangement was reported for both conductometric and amperometric detection. The sensing electrode (Pt wire, 50-mm in diameter) in this set up was placed at the outlet of the separation capillary [110]. Some further refinements of this end column detection arrangement were later reported [111]. The suppressed conductometric detectors used by the Dasgupta group [100, 102–104] were constructed by inserting two 100-μm-diameter Pt wires through the wall of a 190-μm-ID poly(vinyl chloride) (PVC) capillary in parallel and as close to each other as possible. Basically the same geometry was later modified to be a reproducible detection system by using a bifilar wire [112]. The group at Dionex [101] used disk-shaped metallic electrodes with 75-μm holes in them separated by a similar but insulating spacer disc as the conductivity detection cell for the same purpose. Given current microfabrication technology, conductivity detectors are perhaps best fabricated on-chip [98]; there may be merits to such detectors even when separations are carried out in conventional capillaries. Very low dispersion chip-capillary connectors have been designed [113]. Meanwhile, nongalvanic contact conductivity detection by using high frequency ac excitation voltages has been developed. Such techniques have some obvious advantages in that detection can be conducted directly on the separation capillary, without having to resort to a separate cell. Such detectors are described in the next section. However, unlike galvanic contact conductivity detectors, signal/noise in a nongalvanic contact conductometric detector (hereinafter called capacitively coupled contactless conductometric detector, C 4D) may be somewhat scale-dependent. This has not yet been systematically studied but it is believed that conductivity of the double layer near the capillary wall dominantly governs the observed signal [114].

9.1 C 4D and its potential for capillary IC

Contactless conductometric detection based on oscillography [115] was used as early as 1983 by Pungor et al. [116], who constructed a flow-through conductivity detector without galvanic contact. The detector cell was connected to a commercial oscillogrator and consisted of two concentric electrodes coated with either methylsilicone or Teflon. It was used for flow injection based measurements of conductivity and permittivity. In 1988 they used this detector in an end-column format for IC [117]. The electronic circuitry was modified from the previous version and a high frequency (42 MHz) crystal oscillator was used for excitation. Detector performance was evaluated in both suppressed (carbonate/bicarbonate) and non-suppressed (salicylate/salicylic acid or benzoate/benzoic acid) eluant systems: the performance of the detector was shown to be comparable with state-of-art commercial detectors using galvanic contact.

Almost a decade later, C 4D was revived for capillary format separation techniques, notably capillary electrophoresis (CE). Independently, Zemann et al. [118] and da Silva and do Lago [114] refined C 4D. In the on-column design, the C 4D detector cell consists of two cylindrical electrodes that are positioned directly on the outer wall of the separation capillary (typically made of an insulating material, commonly silica) and are not in galvanic contact with the solution inside the capillary. They are separated by a small gap, typically 1–2 mm, that defines the length of the solution inside the separation capillary, the conductivity of which is measured. See Figure 12. The electrodes are painted directly on the capillary outer wall using a silver conductive epoxy or alternatively, the separation capillary may be inserted inside two independent syringe cannulae. The latter design is more flexible and is thus preferred. High frequency alternating voltage (typically 20–600 kHz sine wave) is applied to the first electrode. The alternating current output at the second electrode is rectified and amplified using relatively simple circuitry. Excitation amplitude was typically 10–20 V; the scheme has been widely used in CE [114, 118–123]. Tanyanyiwa et al. [124] have subsequently demonstrated that if the excitation voltage amplitude is increased (in the range of 25 to 250 V), the sensitivity increases nearly linearly with the voltage applied while the noise remains almost the same. In their detector configuration, the noise was largely generated by the detector electronics and not the detection cell.

Since its resurgence in 1998, the number of C 4D publications has crossed the half century mark at the time of this writing. A recent review by Zemann [125] indicates that C 4D has been used primarily for electrophoresis, both in capillary and chip formats. The only exceptions are the work of Hilder et al. [119] and Kuban et al. [126] where the detector was used in IC. Kuban et al. used a high voltage
C⁺D on a 150 μm ID fused silica capillary in series with a commercial galvanic contact based conductometric detector while monitoring the effluent from a conventional scale ion chromatograph. The performance of the two detectors in terms of S/N, etc. was essentially the same, confirming the conclusion of Pal et al. [117] from two decades ago.

The on-column C⁺D design offers several advantages. The detector cell can be positioned at any location along the separation capillary (unlike the detectors with galvanic contact or the original oscillometric detector used by Pun-gor et al.). While there may be some dependence of the detection sensitivity and/or the S/N ratio on the capillary ID, this does not appear to be nearly as great as in optical detection. Mayrhofer et al. [127] have shown that C⁺D detection of inorganic cations, separated by electrophoresis in capillaries of 10 μm ID is feasible. Hilder et al. [119] used C⁺D in anion-exchange capillary electrochromatography; detection was performed directly across a 75 μm ID packed bed. Obviously, the concept should be of utility in both packed and open tubular capillary IC systems. It is inexpensive to build C⁺D electronics (∼US $200 in components) and it is compact so that it is particularly attractive in portable instrumentation [128]. Multiple C⁺D systems can be used on a single separation capillary, and in combination with other detection techniques, such as UV detection [129].

Capillary IC is an evolving field that is still very much in the making. Whether it will finally emerge as a viable entity in the form of open, packed, or monolithic columns, in a capillary form or on a chip, will require a sharp enough vision to see inside a capillary!

References