Review

What are the unanswered (and unasked) questions in ion analysis?

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Abstract

This article “free-associates” through a few of the questions that remain unanswered and unasked in ion analysis. Some of the questions nosed into include: What limits precision and accuracy in ion chromatography and capillary electrophoresis?; What is the cause of nonlinear calibrations curves in suppressed ion chromatography?; Why do we use ion chromatography?; What is meant by speciation in ion chromatography?; What self-imposed limits are restricting our application of ion chromatography?; and What is the relationship between ion chromatography and capillary electrophoresis for ion analysis?. © 1998 Elsevier Science B.V.

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1. Introduction

When one thinks about the “unanswered questions”, one first thinks about what further research must be done in the field. However if one looks up the definition of “question” one finds “an interrogative question used to test knowledge”. Such is not so daunting a task. But how do you take up the task?

Previously when asked to review recent advances in ion chromatography (IC) [1], I found it useful to use as a conceptual framework “The Seven Ages of an Analytical Method” proposed in a classical editorial by Herb Laitinen [2]. This provided a systematic basis from which to see where IC was, and where it was going. Such a muse would be useful herein. However, the present discussion requires a more random framework—one that allows “brainstorming” on what are the unanswered and unasked questions in ion analysis.

After much thought, and increasing trepidation, I finally turned to the story of Cyrano de Bergerac. Not the classical story by Edmond Rostands, but rather the modernized version presented in the movie “Roxanne” starring Steve Martin. In this movie Steve Martin plays C.D. Bales, the fire chief who
happens to have an enormous nose. At one point in the movie, C.D. Bales must defend his honor by coming up with twenty jokes about his nose better than “Hey, big nose.”. C.D. Bales’ method for doing this was to categorize each joke. For instance, “Fashionable: You know, you could de-emphasize your nose if you wore something larger, like Wyoming”. or “Religious: The Lord giveth, and he just kept on giving, didn’t he.” These categories allowed C.D. Bales to free-associate, and thus come up with the jokes needed to defend his honor. This then is the methodology I will use in this article. I will use C.D. Bales’ jokes as the seed for my own free-associations about the unanswered and unasked questions in ion analysis.

2. The questions

2.1. “Obscure: Hoo, I’d hate to see the grindstone”

The concept of “putting your nose to the grindstone” reminds me of the fanaticism of classical analytical chemists regarding precision. Nonetheless most IC and capillary electrophoresis (CE) papers quote a precision of 2–5% without comment. So what are the causes of imprecision in ion analysis?

Grushka and Zamir have made a detailed analysis of factors affecting the precision of retention times and peak area measurements in high-performance liquid chromatography (HPLC) [3]. Errors in flow-rate result in proportional errors in the retention times and peak areas. That is, a 0.2% error in the flow-rate causes a 0.2% error in the peak area. This is significantly smaller than a number of the other sources of imprecision discussed below, and so can generally be ignored. Grushka and Zamir demonstrate that temperature can also have a significant effect on precision in HPLC [3]. In IC, temperature is of even greater importance as conductivity changes by approximately 2% for each degree of temperature change. Thus, thermal isolation of the conductivity detector is essential to produce maximum sensitivity and precision [4].

One often overlooked source of poor precision is injection. Most chromatographs introduce sample into the eluent stream using a valve possessing either an internal or external loop. Sample is flushed through this loop, and then the valve is switched to inject the sample onto the column. But how much sample must be flushed through the loop to ensure that the solution within the loop is representative of the sample? Fig. 1 shows the relationship between observed peak area and the number of loop volumes flushed through the injector [5,6]. The observed response does not reach a plateau (indicating a fully filled loop) until a minimum of three loop volumes have passed through the injector. The nonlinear response below three loop volumes results because of the parabolic flow profile of laminar flow. In laminar flow, fluid at the walls experiences viscous drag, and flows much more slowly than the bulk fluid. Since some of this fluid flows slower than the rest, more than a single loop volume is needed to fully flush the loop. Thus, for maximum precision the injector should be flushed with at least three loop volumes. However such laminar flow also exists in tubing connecting the sample port to the injector. For instance, our Dionex DX-100 ion chromatographs are fitted with 25 μl injection loops. However the tubing connecting the injection port to the injector is ~100 μl in volume, for a total volume of ~125 μl. Therefore it takes at least 0.4 ml to fully flush the loop of this injector.

Fig. 1. Effect of number of loop volumes flushed through an injector on the peak area observed. Adapted from Figure 10.7 of Ref. [6].
However, one should not assume from Fig. 1 that any volume greater than three loop volumes is appropriate. There have been numerous reports of precision problems arising from sample adsorbing onto components of the injector [7,8]. These problems arise when the sample matrix is a weak eluent. As sample passes through the injector, analyte may adsorb onto components of the injector. The amount of analyte adsorbed increases as volume of sample flushed through the injector increases. Subsequent switching of the injection value results in injection of the sample and desorption of the adsorbed analyte by the eluent. Diagnostic characteristics of sample adsorption onto injectors include [7]: peak size increases with the volume of sample flushed through the injector; peak size varies with speed that the sample is flushed through the injector; and peak size decreases with increased sample residence time in injector.

An alternative problem that occasionally arises is that there is not enough sample solution to properly flush the injection loop. Placing a small air bubble at each end of the sample prevents mixing between the sample and the eluent [8,9]. This allows full flushing of the injector loop in as little as one loop volume, rather than the three loop volumes normally required.

To date there have been no reports of adsorption onto injectors in the IC literature. Nonetheless, the matrices of many of the samples analyzed in IC are weaker eluents than the mobile phase. Thus, it is prudent to take precautions to avoid precision problems due to adsorption. The primary means of minimizing this source of poor precision is to always flush the same volume of sample through the injector. Use of the same flush rate and minimizing the sample residence time would also help improve precision if injector adsorption is occurring.

Injection precision in CE would seem even more problematic. Injection precision can be as poor as 10% relative standard deviation (R.S.D.) with manually operated systems, or as good as 2–3% with automated systems. Use of an internal standard in CE improves the precision to less than 1% R.S.D. [10–12]. Dose and Guiochon [12] found that use of one internal standard was effective for hydrodynamic injection but that two internal standards of differing electrophoretic mobility are needed for electrokinetic injection.

2.2. “Polite: Ah, would you mind not bobbing your head? The, ah, orchestra keeps changing tempo”

The concept of tempo brings up a second influence on precision, the data acquisition rate. Alarmingly, not a single paper from the last “International Ion Chromatography Symposium” quoted the data acquisition rate used [13]. Many manufacturers state that a minimum of ten data points are required to represent the peak to within 0.5% accuracy. However, this statement provides no guidance as to what the effect will be if more or less data points are collected, or if there is noise present.

The most exhaustive analysis of the effect of data acquisition on the precision of peak area measurements is that of Hayashi and Matsuda [14]. In their analysis the R.S.D. of peak area measurements for fully resolved peaks is given by [14]:

$$\text{R.S.D.} = \frac{2\pi^{1/2}\sigma_j\Delta T\bar{W}^2}{A_j^2}$$

(1)

where $\sigma_j$ is the standard deviation related to the peak width, $\Delta T$ is the sampling interval of the analog-to-digital converter, $\bar{W}$ is the standard deviation of white noise in the output, and $A_j$ is the area of peak $j$. This equation can be rewritten in terms of signal-to-noise ($S/N$) and number of data points collected over a peak ($n_{data}$):

$$\%\text{R.S.D.} = \frac{184}{S/N} \sqrt{n_{data}}$$

(2)

This rearrangement is based on the following assumptions. Firstly, $n_{data}$ refers to the number of data points over a span of $6\sigma$ (i.e., essentially baseline to baseline). Secondly, the peak shape is assumed to be Gaussian, such that:

$$A = \sqrt{2\pi\sigma h}$$

(3)

where $h$ is the height of the peak. The signal, $S$, is equal to the peak height, $h$, and the noise, $N$, is equal to $\bar{W}$, the standard deviation of the white noise.

Fig. 2 shows the effect of $S/N$ on the precision observed for peak area measurements. Below a $S/N$ of 50, the error in integrating the peak area increases...
In analogy to the question above regarding what limits precision in ion analysis, we should ask what limits the accuracy. IC results have been validated versus a number of other techniques [20]. However, the accuracy of the IC results is critically dependent upon the calibration procedure used [20–22]. Single point calibrations usually give the poorest results [20]. Linear regression using multiple standards over two orders of magnitude in concentration yield correlation coefficients better than 0.99, with calibration curves based on peak area generally being more linear than those based on peak height. Over about one order of magnitude of analyte concentration, the errors from using linear regression are small [22]. However over wider concentration ranges linear calibrations overestimate the lowest and highest anion concentrations, and underestimate the midrange concentrations of anions [21]. The error is typically less than 10%, but can be as much as 300% at low concentrations. Numerous studies have demonstrated that use of quadratic regression greatly lowers the analysis error [20–22]. Use of higher order polynomials such as cubics rarely result in significant improvement [21]. For alkali metal and alkaline earth determinations, the calibration curves are more linear than for the anions [21]. Ammonium, like the anions, required quadratic regression, al-
though in the case of ammonium the curvature of calibration is opposite to that of the anions [21]. The distinctive calibration behavior for ammonium results from its weak acid character.

2.4. “Scientific: Say, does that thing there influence the tides?”

So, why are calibrations in IC nonlinear? A number of fundamental studies have investigated this question [22–25]. In suppressed IC, the eluent is protonated in the suppressor. Typically the protonated form of the eluent (e.g., carbonate–hydrogencarbonate) is a weak acid \( \text{pK}_a(\text{H}_2\text{CO}_3) = 6.35 \):

\[
\text{H}_2\text{CO}_3 \rightleftharpoons \text{H}^+ + \text{HCO}_3^-
\]  

(4)

Thus, a portion of the carbonic acid dissociates to \( \text{H}^+ \) and hydrogencarbonate. It is this dissociation that causes the \( \sim 15 \, \mu \text{S} \) background observed with carbonate–hydrogencarbonate eluents. Conversely, the analyte (e.g., chloride) is a strong acid:

\[
\text{HCl} \rightarrow \text{H}^+ + \text{Cl}^-
\]  

(5)

Therefore, after suppression, the analyte ion is accompanied by an equivalent amount of \( \text{H}^+ \). This extra acid causes the equilibrium governing the background conductance (Eq. (4)) to shift to the left, causing a decrease in the background conductance.

Fig. 3 illustrates this effect. Plot A shows the idealized (i.e., no background conductivity) response for a strong acid analyte, such as depicted by Eq. (5). Plot B shows the baseline. In the absence of analyte (first portion of plot), the baseline corresponds to the dissociation of the weak acid eluent (\( \sim 15 \, \mu \text{S} \) for carbonate–hydrogencarbonate). In the presence of the strong acid sample, the presence of excess \( \text{H}^+ \) reduces the dissociation of carbonic acid (Eq. (4)). This decreases the background conductivity at the same retention time as the eluting peak. The higher the analyte concentration, the more severe the reduction in the background conductivity. The solid line in plot C shows the observed response. The peak height, and also the area, of this observed response is reduced from that expected for an idealized system (dotted line). As a consequence of this baseline shift, calibration plots in IC are nonlinear.

The degree of nonlinearity of a calibration is reduced by decreasing the magnitude of the background conductivity. This is achieved either with more dilute eluents or with weaker acid eluents. Eluents such as borate [\( \text{pK}_a(\text{boric acid}) = 9.24 \)] and hydroxide [\( \text{pK}_a(\text{water}) = 14 \)] yield essentially linear calibration curves [22,26]. Similarly, strong acid eluents used for cation separations should give linear calibrations, as has been observed [21].

2.5. “Humorous: Laugh and the world laughs with you; sneeze and it’s goodbye Seattle”

So, what is it that can destroy an ion analysis? A
common source of problems in IC results from interferences due to the eluent or from the sample matrix. An often overlooked source of interference is the water used in the preparation of standards and eluents [27]. For routine determinations this water should be freshly distilled and deionized (1 MΩ-cm). However, 1 MΩ-cm water contains up to 200 ppb ionic impurity. Therefore for trace anion analyses 18 MΩ-cm water is recommended. Such water may still contain 20 ppt chloride, 100 ppt sodium and 50–60 ppt ammonium. With eluents, an obvious source of interference is impurities within the salts used to prepare the eluent. As such, analytical grade salts should be used to prepare eluents.

But what is the effect of impurities in the eluent? In isocratic elution, the impurities are continuously pumped onto the column. If these impurities are strongly retained the column performance will deteriorate. Thus, it is always advisable to use a guard column to protect the expensive separation column. Alternatively, the impurities may be only weakly retained by the column, and so elute continuously from the column. This causes an increase in the longterm noise and/or a higher baseline [28]. In nonsuppressed IC and ion-pair chromatography complex “ghost” and “vacancy” peaks may also be observed if impurities are present in the eluent [29,30]. If such extra peaks are observed, the best solution is to eliminate the impurity from the eluent by using high quality reagents. If this is not possible, these artifact peaks can be minimized by using the eluent as the sample solvent and by minimizing the injection volume [30].

With gradient elution, eluent impurities may concentrate on the column under the initial weak eluent conditions and elute later as the eluent concentration is increased [31]. Eluent impurities thus cause positive peaks in gradient methods. These peaks appear at the retention time of the impurity ion. Their magnitude depends on the impurity concentration and how long the weak eluent was pumped through the column before initiation of the gradient.

The sample matrix can interfere with determinations by coeluting with ions of interest, shifting retention times and disrupting the baseline. A recent paper discussing the analysis of mineral waters illustrates these effects [32]. Mineral waters contain high concentrations of dissolved CO₂. Initially, it might be expected that CO₂, being a gas, would not affect the analysis since it would simply pass through the column unretained and elute at the dead volume. However, in aqueous solution CO₂ is in equilibrium with carbonic acid (H₂CO₃). When injected into a HCO₃⁻/CO₃²⁻ eluent, the CO₂ thus makes the eluent more acidic which alters the retention times. Further, the high CO₂ concentration results in microbubble formation in the suppressor, which causes peak deformation and lower signals for chloride which coelutes with the matrix.

Such problems can be eliminated by removing the matrix using a technique such as solid-phase extraction (SPE) [33]. Sample acidity or basicity can be eliminated using SPE cartridges prepared from cation-exchange resin in the H⁺ and anion-exchange resin in the OH⁻ form, respectively. SPE cartridges are also available for elimination of chloride and sulfate matrices [33]. Alternatively, a suppressor can be used to neutralize alkaline samples. This procedure was recently approved for the IC determination of trace anionic impurities in concentrated NaOH and KOH [34]. A similar procedure was used for the mineral water analysis discussed above [32].

2.6. “Obvious: Excuse me is that your nose, or did a bus park on your face?”

The most obvious question is: “Why do we use IC and CE for ion analysis?” For anions such a question is relatively easy to address. Colorimetric methods are available for a wide array of anions [35]. These methods can be adapted to flow injection analysis to provide rapid determinations [36]. However such systems are limited to the determination of one to two analytes. Ion-selective electrodes can also be used for anions. Currently commercial electrodes are available for Br⁻, Cl⁻, CN⁻, SCN⁻, S²⁻, NO₃⁻, and ClO₄⁻ [37]. However the detection limits for such electrodes typically are in the 20–400 ppb range. This is well above the ~10 ppb detection limits typical of IC and comparable to the 200 ppb detection limits achievable with CE [38]. Furthermore both IC and CE can achieve <1 ppb detection limits using preconcentration, a technique that is not easily performed with ion-selective electrodes. Further, ion-selective electrodes are unsuitable for many applications because of their slow response (several minutes) at low analyte concentration, interferences, and inability to provide information about more than
a single analyte. Thus, IC and CE are the only means of analyzing samples for multiple anionic components.

But what about cation analysis? Again one can consider colorimetric analysis [39] and ion-selective electrodes [37]. These can be extremely useful in some applications, but suffer from the limitations discussed above. The obvious alternative techniques are the atomic spectroscopic methods. Chief among the advantages of these techniques are the wide dynamic range and lack of interferences in inductively coupled plasma atomic emission spectrometry (ICP-AES). However, often overlooked is the relatively poor sensitivity of ICP-AES for alkali metals (from 1–40 000 ppb!). Thus, the low ppb detection limits of IC for these metals are highly competitive. Furthermore, in an interlaboratory study analyzing simulated rainwater, IC provided comparable precision to spectroscopic methods, and at low concentrations IC performed better than atomic emission spectroscopy for Na and K and atomic absorption spectrometry for Ca [20]. IC also offers some advantages over ICP-AES for lanthanide determinations. While detection limits for ICP-AES determination of lanthanides are reasonable (1–10 ppb), the spectra are exceedingly complex [40–42]. In contrast, dynamic ion-exchange chromatography of lanthanides achieves nanogram detection limits [43] with no cross lanthanide interferences. Coupled column chromatography yields detection limits of 0.02 µg/g for lanthanides in uranium [44]. Thus, IC and CE possess some niche applications within metal ion analyses.

However atomic spectroscopy is limited to metallic species. In many applications it is necessary to monitor both metal ions and other cationic species, such as amines. Fig. 4 is an illustrative example of the simultaneous determination of metal ions and amines by IC [45]. Thus IC and CE’s true place within cation analysis is in their ability to determine both inorganic and organic cations.

2.7. “Philosophical: You know, it’s not the size of a nose that’s important, it’s what’s in it that matters”

Another rationale often stated for using IC is speciation. Given the commonality of this argument, closer examination of what we mean by “speciation” is required. Speciation refers to differentiating the various forms of a species. However in order for a “species” to be analyzed by chromatography or electrophoresis it must be stable (i.e., must not reequilibrate) on the time scale of the separation. Table 1 presents some examples of speciation using IC. A similar table could be constructed for CE separations, but the examples given in Table 1 will serve the current discussion. Table 1 illustrates that speciation in IC normally refers to “oxidation state speciation”. There were, however, fewer examples of metal oxidation state separations than I had initially anticipated. For instance, Sn⁡²⁺ /Sn⁡⁴⁺ is often quoted as an example of metal oxidation state speciation. Separations of organotin [46] and organotin chlorides [47,48] have been reported. However I could not find a practical application of IC for measuring Sn⁡²⁺ and Sn⁡⁴⁺. This is probably because the facile equilibria causes this redox equilibria to shift during the chromatographic separation. Similarly, many of the other metal analytes (As, Se, Te) listed in Table 1 required atomic spectroscopy.
detectors to achieve analytically useful detection limits. Thus they are not, in the strictest sense, IC methods.

Surprisingly one area of metal speciation not yet explored by the IC community is the determination of “free metal” concentrations. Free metal concentration refers to that portion of the total metal concentration that is not complexed in solution. In many physiological and environmental situations it is the free metal concentration and not the total metal concentration that is important. Thus there is a growing interest in means of measuring free metal concentrations. Typically such free metal concentrations are measured using ion-selective electrodes. However, in many applications there either is no suitable ion-selective electrode (e.g., \( \text{Mg}^{2+} \)) or ion-selective electrodes cannot achieve the required detection limits (e.g., \( \text{Cu}^{2+} \)). An emerging technique for free metal determination is the column equilibration method [49,50]. In this technique a small column containing ion-exchange or chelating resin is equilibrated with the sample. That is, sample is loaded onto the column until the solution eluting from the column is of the same composition as that being loaded onto the column. In effect, the sample matrix is acting as the eluent of this small column. Under these conditions the amount of metal adsorbed onto the column is related to the free metal concentration. In current applications of the column equilibration method, the retained metal is eluted and analyzed by atomic absorbance spectrometry [49,50]. Given the extensive expertise in chelating preconcentration columns in the IC community [51,52], it is surprising that this “new” area of speciation has not been explored.

2.8. “Paranoid: Keep that guy away from my cocaine”

The discussion of “speciation” above shows that we often do not attempt something simply because we think it is “wrong” or “impossible”. However technology and knowledge follow Lamarckian evolution, and so are constantly moving forward. Thus, practices that were impossible or forbidden just a few years ago may now deserve reexamination.

For instance, typically IC columns operate at back pressures of 2000 to 3000 p.s.i. (1 p.s.i. = 6894.76 Pa). Recently MacNair et al. demonstrated that solid silica particles could withstand pressures as high as 60 000 p.s.i. [53]! As many as 300 000 theoretical plates were generated for reversed-phase separations performed on a 66 cm \( \times \) 30 \( \mu \text{m} \) column packed with 1.5 \( \mu \text{m} \) nonporous particles. Much of IC is performed on nonporous particles. It is intriguing to consider what separations might be achieved in IC with such plate counts.

Alternatively, consider the manner in which separations of metal ions such as the lanthanides are performed. The sample is often acidic due to dissolution or extraction, or simply for sample preservation. However, the sample is injected into an eluent buffered to around pH 4. Care must be taken to ensure that the acidity of the sample does not exceed the buffering capacity of the eluent, otherwise retention behavior may be altered and the column

---

Table 1
Examples of speciation by ion chromatography

<table>
<thead>
<tr>
<th>Species</th>
<th>Example Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \text{NO}_2^- / \text{NO}_3^- )</td>
<td>[70,71]</td>
</tr>
<tr>
<td>( \text{Cl}^- / \text{ClO}_2^- / \text{ClO}_3^- )</td>
<td>[70]</td>
</tr>
<tr>
<td>( \text{Br}^- / \text{BrO}^- )</td>
<td>[70]</td>
</tr>
<tr>
<td>Sulfur oxyanions</td>
<td>[72]</td>
</tr>
<tr>
<td>( \text{Fe(II)}/\text{Fe(III)} )</td>
<td>[73]</td>
</tr>
<tr>
<td>( \text{Al}^{3+} ) inner sphere complexes</td>
<td>[74–76]</td>
</tr>
<tr>
<td>( \text{As(III)}/\text{dimethylarsenic acid/monomethylarsonic acid and}/\text{As(V)} )</td>
<td>[77,78]*</td>
</tr>
<tr>
<td>( \text{Se(IV)}/\text{Se(VI)} )</td>
<td>[79]</td>
</tr>
<tr>
<td>( \text{HTeO}_4^- / \text{TeO}_4^{2-} )</td>
<td>[80]</td>
</tr>
<tr>
<td>( \text{Cr(III)}/\text{Cr(VI)} )</td>
<td>[80]</td>
</tr>
</tbody>
</table>

* As(III) and As(V) only.
potentially damaged. Thus, many metal ion samples must be diluted prior to injection to reduce the sample acidity. Upon elution from the column, the eluate mixes with acidic Arsenazo III to yield a colorimetric response. This going from acidic to around neutral to acidic is a very awkward manner in which to perform a determination. It would be preferable to keep the sample under acidic conditions throughout the analysis process. The historic rationale for not working with strongly acidic eluents was that the stainless steel components of the pump, injector and connecting tubing would corrode, and the silica based column would degrade. Currently, IC systems are constructed of polyether ether ketone (PEEK). PEEK is extremely chemically resistant, being attacked only by concentrated acids and swelled by methylene chloride, dimethyl sulfoxide and tetrahydrofuran [54]. Thus the historic instrumental limitation for not working with strongly acidic eluents is gone. With respect to the column, an alternative stationary phase for metal ion analysis might be based on zirconia. Zirconia possesses much greater pH stability than silica. Recent work by Carr and coworkers has yielded high efficiency zirconia based chromatographic media [55–57]. It would be interesting to investigate the potential of such materials for IC separation of transition metals and lanthanides.

2.9. “Enquiry: When you stop to smell the flowers, are they afraid?”

The question that most practitioners of ion analysis dread is: “What is the relationship between IC and CE?” Recently, Haddad compared the two techniques on the basis of: stage of development; separation efficiency; analytical performance parameters; method development procedures; applications; strengths; weaknesses; and future directions [38]. This detailed and thoughtful review is strongly recommended for practitioners in the field. Rather than restate what has already been stated so well, I will focus on one of Haddad’s conclusions. He stated that on the basis of separation selectivity (amongst other factors), CE and IC are complementary [38]. For the sake of clarity, Haddad limited his comparison to the determination of inorganic anion by suppressed IC and by coelectroosmotic flow (coeof) CE. The selectivities observed using these two procedures are:

\[
\text{IC: } F^- < Ac^- < Cl^- < NO_2^- < Br^- < NO_3^- < PO_4^{3-} < SO_4^{2-} < I^-
\]

(6)

\[
\text{CE(coeof): } Br^- < Cl^- < SO_4^{2-} < NO_2^- < I^- < NO_3^- < F^- < PO_4^{3-} < Ac^- 
\]

(7)

These two techniques clearly offer different selectivities. However as Haddad noted “ion chromatography should not be considered as a single chromatographic technique” [38]. Thus, let us consider the selectivities provided by other IC techniques. For instance, if a moderate capacity anion-exchange column (e.g., Dionex AS10, 170 µequiv) is used rather than the low capacity column (Dionex AS4A, 20 µequiv) quoted in Eq. (6), the selectivities are [58]:

\[
\text{IC(moderate capacity): } F^- < Ac^- < Cl^- < SO_4^{2-} < PO_4^{3-} < NO_3^- < NO_2^- < I^-
\]

(8)

The higher capacity column results in much lower retention of the higher charged anions than was evident in Eq. (6) (see reference [59] for an explanation of this phenomenon). The hydrophobicity of the anion-exchange column will also alter the selectivity.

Alternatively, if ion-exclusion chromatography (IEC) is used, the selectivities are once again radically altered [60]:

\[
\text{IEC: } Br^- , Cl^- , SO_4^{2-} , I^- , NO_2^- \ll NO_3^- \approx F^- \ll Ac^-. 
\]

(9)

Most significantly, fluoride and acetate are well separated with ion exclusion. This is significant since the CE’s ability to separate fluoride and acetate is often the primary example used to illustrate that IC and CE are “complementary”.

The key point of this discussion is that significant variations in selectivity for the ions in Eqs. (6)–(9) can be achieved by alteration of the chromatographic conditions. Further, the range of selectivities achievable using chromatographic techniques include that observed in CE.

So, what do I conclude the relationship between IC and CE is? Why, they are “complementary”!!
Ion-exchange selectivities are strongly dependent upon the ionic charge. This is best illustrated by the uptake of cations by a strong acid cation-exchange resin. The selectivities are approximately [61]:

\[
\begin{align*}
\text{Pu}^{4+} & \gg \text{La}^{3+} > \text{Ce}^{3+} > \text{Pr}^{3+} > \text{Eu}^{3+} > \text{Y}^{3+} \\
> \text{Sc}^{5+} & > \text{Al}^{3+} \gg \text{Ba}^{2+} > \text{Pb}^{2+} \\
> \text{Sr}^{2+} & > \text{Ca}^{2+} > \text{Ni}^{2+} > \text{Cd}^{2+} \\
> \text{Cu}^{2+} & > \text{Co}^{2+} > \text{Zn}^{2+} > \text{Mg}^{2+} \\
> \text{UO}_{2}^{2+} & \gg \text{Ti}^{4+} > \text{Ag}^{+} > \text{Cs}^{+} > \text{Rb}^{+} \\
> \text{K}^{+} & > \text{NH}_{4}^{+} > \text{Na}^{+} > \text{H}^{+} \\
> \text{Li}^{+} &
\end{align*}
\]

The effect of charge on selectivity is so pronounced that until recently rapid separations of both alkali metals and alkaline earth metals required column switching. This strong dependence of ion-exchange selectivities has two pronounced effects on IC. Firstly, IC most commonly deals with lower charged ions, typically ±1 or ±2 as shown in Figs. 4 and 5. Separations of higher charged solutes, such as lanthanides, generally require complexion to lower the charge and/or are performed using ion interaction
chromatography where the column capacity can be easily manipulated and/or gradient elution [62]. This is not to say that separations of highly charged solutes are not possible by chromatography. Indeed, inositol phosphates of charge up to $-7$ [63] and metal complexes with charges as high as $-10$ have been separated [64]. However such separations are far from trivial. Secondly, IC separations generally do not encompass ions of significantly different charge. That is, the separations shown in Figs. 4 and 5 only involve $\pm 1$ and $\pm 2$ ions.

Contrast the low solute charge and low solute charge range characteristics of IC with the separation shown in Fig. 6 [65]. This CE separation encompasses ions from $-1$ to $-4$, and achieves its greatest efficiency with the most highly charged ions. Similar separations can be achieved using ion interaction chromatography [66]. However the optimization is much more complex in ion interaction chromatography, and the efficiencies and run times are inferior to those shown in Fig. 6.

Thus my conclusion is that IC and CE are complementary. For samples possessing low charge ions and a small range in ion charge, IC is preferred. This more mature technique offers greater reliability and confidence for such separations than does CE [1]. However for samples possessing ions of high charge and more particularly large ranges in ion charge, CE is preferred.

2.10. “Meteorological: Everyone take cover, she’s going to blow”

So, CE is the technique of choice for high charge analytes. However, not all of the questions associated with analysis of high charge analytes have been solved. For instance, Fig. 7 illustrates the effect of trace Fe$^{3+}$ and Ca$^{2+}$ impurities on the CE separation of multiply charged benzoates [67]. Fig. 7a shows the separation in the absence of any secondary effects. Fig. 7b shows the severe impact of even low levels of metal impurities in the buffer. Iron(III) present in the buffer caused adsorption of the benzoates onto both coated and uncoated capillary walls. The effect was most severe for the benzoates forming the strongest complexes with Fe$^{3+}$ (peaks 4, 5 and 6 in Fig. 7).

Similarly, multiply charged cations strongly adsorb onto bare fused-silica capillaries. This results in severe tailing and even total peak loss [68]. Thus further understanding of buffer interactions and means of preventing wall interactions is required before CE will be able to fully achieve its promise for determinations of highly charged solutes. In essence, this is a similar state of development as liquid chromatography was in the mid-1980s when secondary adsorption plagued the analysis of amines.

Finally, there is the problem of electrodispersion. Typically CE analysis of small ions is performed using indirect detection. In the dilute buffers necessary for indirect UV detection, electrodispersion
broadening can be severe unless the mobility of the ion and the buffer co-ion are well matched [69]. Thus, not all of the questions associated with the determination of analytes of widely differing charge have been answered either. However, the greater understanding of transfer ratios provided in the recent literature [69] provides the insight necessary to begin to address this problem.

Thus more study is required on a number of questions before CE can truly take its place as a complementary technique to IC.

3. Concluding remarks

3.1. “Personal: Well, here we are, just the three of us”

Up to this point I have dealt with questions that arose in my own mind in response to the C.D. Bales’ nose jokes. However, these are not the only questions that are important. It is the role of symposia such as this one to allow discussion of the latest developments in the field. It is, however, equally important that the symposium fosters discussion amongst the participants. For it is in such discussions that one’s mundane and stupid questions are answered. Except that, of course, there is no such thing as a mundane or stupid question. Rather, as in the words of James Thurber:

“It is better to know some of the questions than all of the answers”

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