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Review

Capillary and microchip electrophoresis: Challenging the common conceptions

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ABSTRACT

Capillary electrophoresis (CE) has long been regarded as a powerful analytical separation technique that is an alternative to more traditional methods such as gel electrophoresis (GE) and liquid chromatography (LC). It is often touted as having a number of advantages over both of these, such as speed, flexibility, portability, sample and reagent requirements and cost, but also a number of disadvantages such as reproducibility and sensitivity. Microchip electrophoresis (ME), the next evolutionary step, miniaturised CE further providing improvements in speed and sample requirements as well as the possibility to perform more complex and highly integrated analyses. CE and ME are seen as a viable alternative to GE, but are often considered to be inferior to LC. This review will consider the strengths and weaknesses of both CE and ME and will challenge the common conceptions held about these.

Contents

1. Introduction

As this review is submitted it is exactly 30 years ago to the month that the seminal paper entitled 'zone electrophoresis in open-tubular glass capillaries' was published in *Analytical Chemistry* by Jorgenson and Lukacs [1]. Using 75 μm internal diameter capillaries, a 30 kV power supply and a homemade fluorescence detector, separations of amino acids, dipeptides and amines with efficiencies of 400,000 plates/m were obtained and immediately generated excitement within the separation community. There was of course earlier work undertaken in Europe and this has been covered in an outstanding contribution by Righetti that simply must be read by every practising 'electrophoreticist' [2]. There is no intention to downplay the work of Hjertén, who showed separations in slightly larger diameter capillaries (300 μm) over a decade before [3], nor to the work of Everaerts and others in the 70s [4], by mentioning the work of Jorgenson and Lukacs first, but it is this work more than any other that history (and citations) indicates has defined capillary electrophoresis (CE) as it is most widely known today. Then, almost 20 years ago now, the next evolutionary step occurred with implementation of electrophoresis in flat planar microchips (to give microchip electrophoresis, ME) by Harrison, Manz and Widmer [5,6]. Miniaturisation of the entire electrophoretic process resulted in separations being performed in seconds as opposed to minutes, and has spawned a new generation of research to make a μTAS or lab-on-a-chip. It is therefore a fitting and appropriate time to critically reflect upon the technique and its current state.

CE and ME occupy a unique position within the separation science community and are in many ways a hybrid between traditional slab gel electrophoresis (GE) and liquid chromatography (LC) and are ultimately always compared with these two. When compared to GE, CE and ME have a number of advantages. First and

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foremost, the ability to use higher electric fields due to more efficient heat dissipation in a capillary/microchip than a gel results in separations taking tens of minutes rather than several hours, and this is reduced to 2–3 min in microchips. Second, they are much easier to automate than a gel and do not require staining or scanning steps. Third, CE and ME are much better for quantification than a gel. Because of these reasons CE was rapidly uptaken for its ability to separate nucleic acid fragments which was one of the key techniques used to initially sequence the human genome [7]. For these reasons CE is now routinely used for many applications that were previously done with GE, but there are several reasons why GE is still used. For many applications it is necessary to fraction-collect bands for subsequent analysis, and it is much easier to obtain a sufficient amount of material from GE than CE or ME. Additionally, gels form the basis of a number of multi-dimensional systems, with 2D systems comprising IEF and PAGE and the various blotting techniques (such as Western blots) being regularly used for proteins. These approaches are not easy to implement in CE and have only been demonstrated in research labs in microchip format in the past 3–5 years. Finally, gels are multi-lane devices, allowing the simultaneous analysis of many samples at once. CE and ME typically do not. While there are a number of multiplexed CE instruments available from a number of companies, these are considerably more expensive than single capillary instruments, which are in turn more expensive than equipment for GE.

When compared to LC, CE and ME are seen as complementary analytical techniques for the analysis of non-volatile analytes. The situation is therefore different than GE, in which CE and ME do exactly the same thing but 'better', but when compared to LC the same outcome might be achieved, but not in exactly the same way. There are many reports in which we are told that CE (and by extension, ME) is 'better' than LC for a number of reasons. First it can provide separations that are much more efficient and much faster. Second, the sample size of CE is smaller as only tens of nL are injected into the capillary, making it ideal for the analysis of scarce and precious samples. Third, it is very flexible, and with the same instrument it is possible to perform a number of different separations simply by changing the electrolyte within the capillary. Fourth, that it is more portable than LC, particularly when implemented in microchips. Fifth it is a cheap alternative to LC as capillaries cost a tenth of the price of a LC column and uses much less solvent. But we are also told that it has a number of limitations. First, the limits of detection are poor when compared to LC. Second, it suffers from poor repeatability and it is therefore unreliable and difficult to use in a routine environment. Despite these limitations, CE has thrived where LC has struggled [8] and CE has been particularly important for the separation of large molecules such as nucleic acids, proteins and oligosaccharides, the resolution of enantiomers, and has found widespread use in the biotechnology, pharmaceutical and food industries.

In this review, the intention is to take a closer and more recent look at some of the advantages and disadvantages of CE and to provide an objective evidence-based analysis of the current literature to rationalise these. There are many instances where CE has replaced GE, and also where it has not because of the genuine need to do those remaining tasks on gels. The debate on GE is therefore much less contentious than the comparison of CE (and ME) to LC, and thus this review will be focused on this comparison and on addressing the advantages and disadvantages mentioned above and trying to present this in light of the most recent developments within both of these fields. On some of these topics it has been easy to find suitable evidence. For others it has not. CE (and ME) and LC are multi-faceted and highly intricate in their own right and there will be examples of each that far eclipse the performance of the others, but wherever possible, literature using similar conditions and samples has been identified to try and provide an objective basis upon which to draw conclusions. This literature has been sourced from the more general and widespread use of CE, ME and LC, and is heavily focused on the use of commercial instrumentation, but there are examples of each from research laboratories to highlight some of the more recent developments within the field to provide an up to date comparison. It is necessary to declare up front that the writer is a practitioner of CE and ME, with limited practical experience with LC, so it must be realised that this is the perspective from which this review has been written.

2. Speed

The 'advantage' of CE that is nearly always mentioned first is speed. Many readers will have seen a large number of examples showing that CE can provide much faster separations than LC. The caveat here is that some of these are contrived to deliberately make CE look favourable when compared to LC. How well CE performs with real and complex samples is a more considered and appropriate comparison. To illustrate the differences, two examples have been selected: the first is the separation of a small number of structurally similar compounds, the second a more comprehensive analysis of one class of compounds to profile a sample.

For the first example, the separation of the drug, itraconaozle (ITC) and its active metabolite, hydroxyitraconzaole (HITC) has been selected [9]. CE is often a good choice for the separation of drugs and their metabolites because there are often only very minor differences in structure and it can be quite challenging to separate them without the benefit of the higher efficiency of CE. In this case, HITC differs from ITC by a single hydroxyl group (MW change from 705 to 721). There are a large number of examples similar to this in the literature and this specific example has been selected because CE and LC were performed on a large number of the same samples in the same laboratory at exactly the same time. Fig. 1 shows the separation of these analytes and an internal standard by LC (A) and CE (B). In LC a single peak is obtained for HITC while in CE two peaks are obtained due to the separation of diasteroisomers, clearly demonstrating the superior resolving power of CE. The LC method was based on a C18 column and native fluorescence detection (λ_{ex} = 260 nm, λ_{em} = 366 nm) and took 14 min, while the CE method was based on MEKC and used UV absorbance detection (214 nm) and took 8 min. The LC method was isocratic, meaning that samples could be injected every 14 min. The CE method had a 1 min conditioning step between injections, and when accounting for the time for movement of the vials, then a sample could be injected roughly every 10 min. Thus the CE method took approximately 60% of the time of the LC method, excluding sample preparation times, and potentially provided more information due to the ability to separate the HITC diastereoisomers. However, in this example there was a difference in sample preparation, with LC only requiring a protein precipitation (evaporated to dryness and redissolved) while CE employed liquid–liquid extraction (evaporated to dryness and redissolved). Thus the sample preparation was slightly shorter on the LC method because of the use of the more sensitive and selective fluorescence detector. The UV absorbance detector used for CE required more extensive sample cleanup to isolate the targets from the matrix and allow the sensitivity to be improved through stacking. When considering the entire process, there is little difference in the time required to analyse samples by CE and LC, and thus the speed benefit of CE is not readily apparent.

The second application in which to consider speed is the separation of serum N-linked glycans. This is not only a very difficult and challenging separation problem that is highly topical at this point in time but there are also a number of separations of these by traditional CE and LC, as well as more recent evolutions of both of these. As both techniques require the glycans to be derivatised,

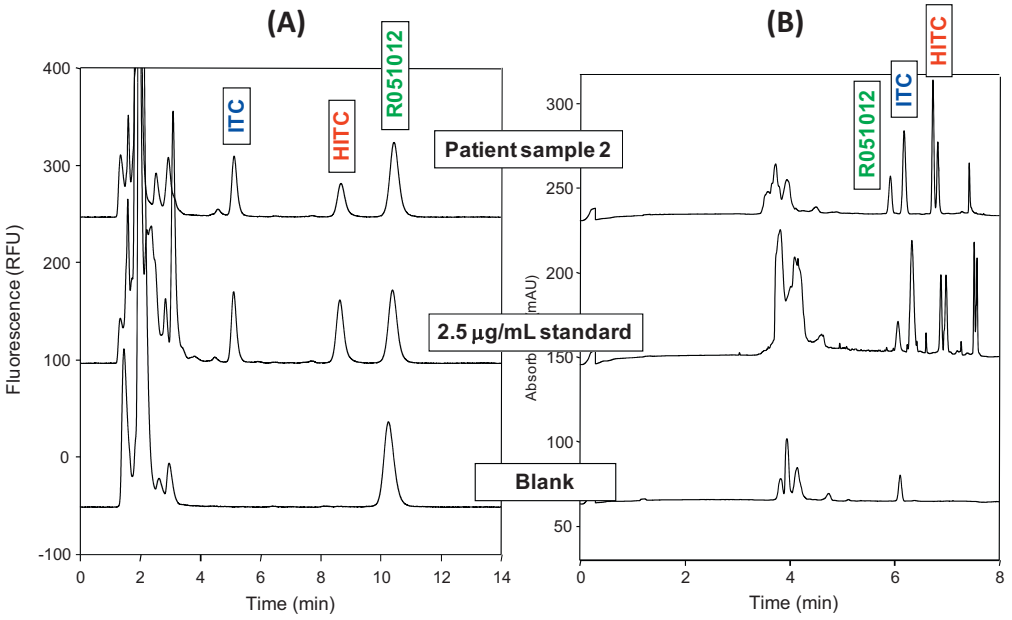

Fig. 1. Separation of the antifungal drug itraconazole (ITC) and hydroxyitraconazole (HITC) from human plasma by: (A) LC and (B) CE [9].

the sample preparation time has not been considered on the basis that it will be similar in both approaches. Fig. 2(A) shows the CE separation of 8-amino-1,3,6-trisulfonic acid (APTS) derivatised N-glycans. This separation is certainly not new, but in this recent paper the authors designed a complete workflow allowing routine analysis with a 48 capillary array instrument for high throughput glycomics [10]. The separation requires approximately 34 min and approximately 35 individual peaks can be identified. Fig. 2(B) shows a normal-phase LC separation of N-linked glycans after derivatisation with 2-aminobenzamide (AB) [11]. All sample preparation, including release of the glycans and derivatisation, is performed in 96-well plates and occurs over 2–3 days in a completely automated manner, with a subsequent 2 days required for complete analysis. Somewhere between 14 and 20 peaks were identified and the separation time was reduced significantly from 3 h to approximately 24 min on a newly introduced 15 mm column. These two examples are highly topical at the moment given the strong interest in glycomics for the development of diagnostics, especially since MS alone is incapable of distinguishing different positional isomers that occur within glycans. In this case, the CE method requires 35 min while the LC method needs about 25 min, and thus CE does readily demonstrate superior speed. However, what is illustrated is the superior resolution of CE as approximately 50% more peaks are observed in CE than LC. Similar resolution on LC would require a longer time, thus there is some credible evidence to support the notion that CE is faster than LC, although the difference may not be as significant as is commonly implied. However, a big difference in terms of speed can be gained from the multiplexing capability. The CE system can separate 48 samples simultaneously in 35 min, while the LC method cannot. While multiplexing can be applied

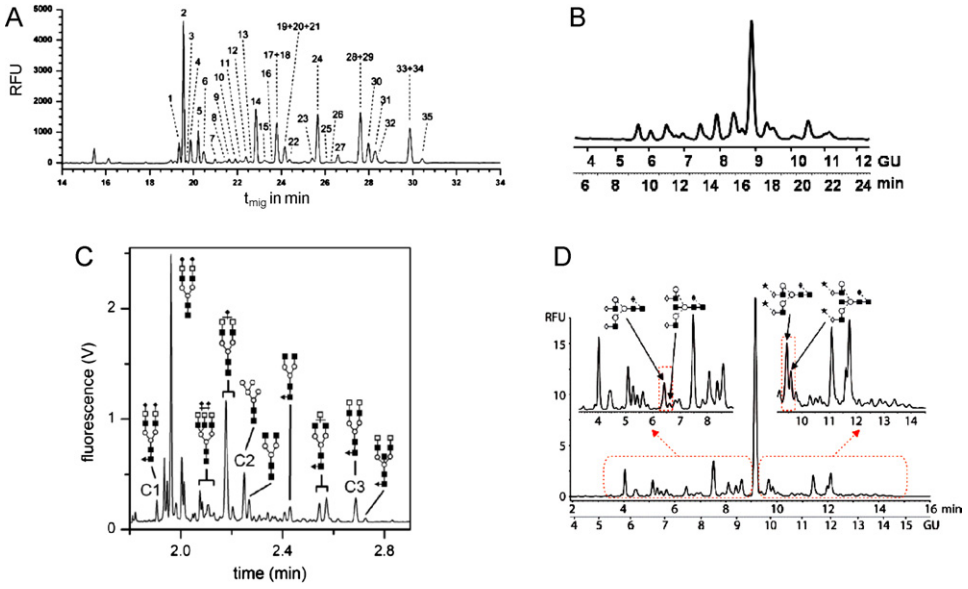

Fig. 2. Comparison of the separation of N-linked serum glycans by: (A) CE after derivatisation with APTS [10], (B) LC after derivatisation by AB [11], (C) ME after derivatisation with APTS [15] and (D) ultra high pressure LC after derivatisation with AB [16].

to LC and there are a few reports of 4- [12] and 9- [13,14] column systems in the literature, these are not as widespread as those for CE and do not yet show the same level of multiplexeing (8-, 12-, 48- and 96-capillary variants are all commercially available with CE). Thus, for situations when a large number of samples need to be analysed, multiplexing with CE will produce sample times that will be very difficult to match by LC.

The comparison above is made using current commercially available CE and LC equipment that is widely used around the world today. It is appropriate and timely to consider more recent developments within both CE and LC for a more up-to-date comparison. In CE, there has been considerable effort focused on performing separations in microchips and Fig. 2(C) shows the separation of APTS derivatised N-linked glycans from serum in a microchip containing a 22 cm spiral microchannel with a field strength of 750 V/cm [15]. The separation is achieved in under 3 min and when compared to Fig. 2(A) it can be seen that there is a very minimal change in resolution with in excess of 30 peaks being identified. This strongly indicates the ability of this platform to provide very rapid and highly efficient separations of complex samples. In LC, the development of ultra high pressure LC has been one of the main technological advances and Fig. 2(D) shows the separation of AB-derivatised glycans from serum using a 100 mm × 2.1 mm column packed with 1.7 μm particles [16]. The separation is complete within 16 min and has a 14 min retention time window allowing the detection of 45 features. What perhaps did not show up clearly from comparing current CE with LC becomes much more pronounced when comparing microchips to ultra high pressure LC, with the microchip separation being much more rapid while also providing a similar number of peaks. When ME separations are multiplexed to perform simultaneous separations which can be achieved on some of the Caliper Labchip® instruments (www.caliperls.com), throughput is enhanced even further to an extent that is very difficult to achieve by LC.

Based on these two examples, it is clear that CE can be significantly faster than LC when multiplexed or miniaturised, but on a comparison of CE with a single capillary with a high efficiency LC column, the speed advantage of CE is certainly disputable.

3. Flexibility and simplicity

CE (and to a lesser extent ME) is renowned for its simplicity and flexibility and this is one of the most frequently cited advantages of this technique. It has a number of different separation modes allowing analytes to be separated in a number of different ways and it is possible to change between these in a simple and cost-effective manner. Separations can be performed by zone electrophoresis, isotachophoresis (ITP), isoelectric focusing (IEF), gels/sieving matrices (GE/SE), electrkinetic chromatography (EKC) and electrochromatography (EC). Of course, chromatography has its different separation modes as well. Separations by normal phase (NPLC) or Hydrophilic interaction (HILIC), reverse phase (RPLC), size exclusion (SEC), ion exchange chromatography (IC) and ion exclusion chromatography (IEC), and it is even possible to perform pI based separations using chromatofocusing. The difference in terms of flexibility and simplicity comes in the ease with which you can change from one mode to another. In LC, you need to change the column and mobile phase. In CE and ME, in all instances, except EC, to change from one separation mode to the other is simply a matter of changing the composition of the separation electrolyte. But there is greater flexibility than that. In chromatography, if there is a need to increase the capacity of the column, then you need a new column. In EKC, the electrophoresis variant that allows LC selectivity to be achieved for neutral species, column capacity is simply a function of the concentration of the additive in the separation electrolyte. Thus it is readily feasible to optimise the amount of 'stationary phase' independent to the mobile phase conditions. The advantage of the flexibility of CE is illustrated in Fig. 3 which shows the separation of plasma proteins by both capillary isoelectric focusing (A, [17]) and a capillary size separation of SDS–protein complexes performed using a linear polyacrylamide polymer (B, [18]). The different selectivity of the separation is significant, but importantly these two complementary methods are useful because the isoelectric focusing separation allows determination of pI and evaluation of charge heterogeneity while the size based allows evaluation of the molecular weight of the protein, parameters which are important for characterisation of protein therapeutics.

While the flexibility and simplicity that can be achieved by electrophoresis is one of its greatest attributes, the increase in popularity of MS detectors has quickly countered this advantage. MS is an exceptionally powerful detector offering the ability to detect and quantitate overlapping components with a unique molecular mass and with the use of powerful accurate-mass MS instruments that are becoming frequently more common, it is possible to determine the identity of unknown peaks. As such it is one of the most intense areas of CE and ME currently being researched and is discussed in several excellent reviews [19–23]. Despite the power of this approach, interfacing to the MS in a way that preserves the resolving power of the separation without compromising the performance of the MS is challenging. This is most commonly achieved using an electrospray ionisation interface, and is much more complex than the interface required for LC due to the need to ensure a stable electrophoretic current and a stable electrospray is obtained. To achieve these, a make-up flow of solvent is used, typically 100 times the flow of the capillary, and this greatly improves the stability of the system, however at the expense of instrument simplicity, reliability and robustness and through dilution of the capillary effluent which compromises sensitivity. The other important issue is that it is preferable to use volatile electrolytes to ensure efficient ionisation in the MS, and is typically limited to combinations of ammonia and formate, acetate or (bi)carbonate [24]. The flexibility and simplicity of being able to rapidly change separation mechanisms is greatly diminished and EKC, one of the most powerful modes of electrophoresis, is problematic due to the high currents and non-volatile additives used. EKC–MS can be performed by only partially filling the capillary with additive, or through the use of alternative interface, such as atmospheric pressure photoionisation interface [25], but these approaches either result in a reduction in resolution or are not widespread. If it is a requirement that a MS detector be used, then many of the advantages of CE and ME are lost and LC is a more attractive option, but if this is not a requirement, then it the CE and ME may be considered to be more flexible and versatile than LC.

4. Portability

The instrumental simplicity purportedly gives CE and ME an inherent ability for miniaturisation. While recent effort has been focused on microchips, there are a number of commercial and research CE instruments that have been presented and these have been recently reviewed by Ryvolová et al. [26]. Focusing specifically on commercial instruments, the smallest commercial portable CE that is currently available is from CE Resources (www.ce-resources.com). It has a size of 32 cm × 23 cm × 15 cm and weighs 6 kg when using internal conductivity, electrochemical or LED-based absorbance detectors (as shown in Fig. 4(A)) and is capable of operating on an internal battery for up to 3 h. The instrument features a carousel and pressure system for liquid handling and automation and can apply up to 25 kV, thus the only compromise in performance maybe the need to use slightly lower

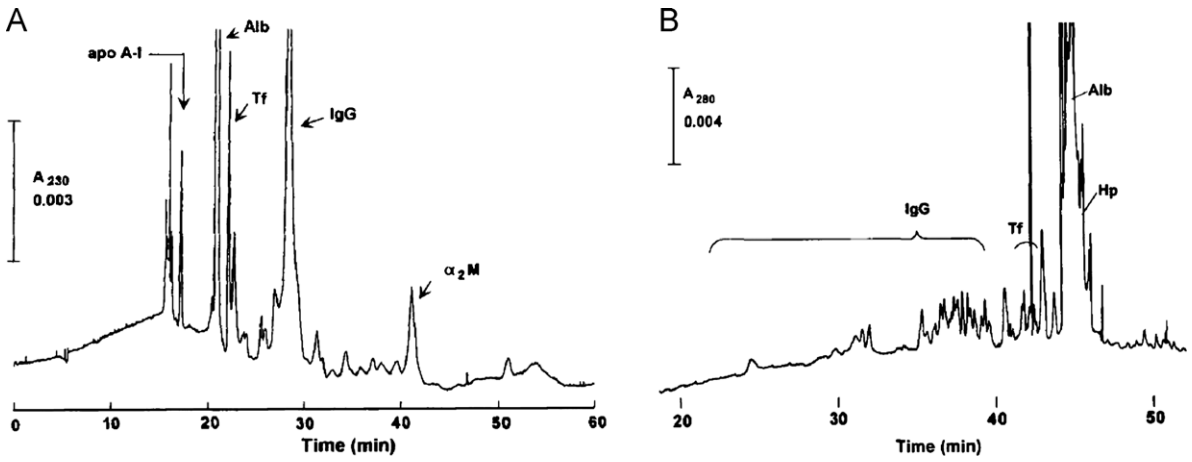

Fig. 3. Separations of plasma proteins by: (A) capillary isoelectric focusing [17] and (B) capillary SDS-PAGE [18].

voltages than is typically used in the laboratory. The comparison to portable LC instrumentation is surprisingly not as favourable as might be expected. There are currently two commercially available LC instruments. The first published commercially available instrument was the Minichrom® [27]. This was developed and commercialised in Russia in the late 90s and features two dual piston pumps allowing gradient capability, fixed wavelength absorbance detection, and has a size of 41 cm × 25 cm × 23 cm and weighs 9.5 kg without accessories. This was followed shortly afterwards by the Chance® Portable LC system introduced by Knauer in 1999 (www.knauer.net). This isocratic LC system (shown in Fig. 4(B)) has an approximate size of 11 cm × 19 cm × 40 cm and weighs only 3.5 kg. It contains a fixed wavelength UV detector, operates up to 400 bar (5800 psi) at flow rates from 1 μL to 10 mL/min and can be powered from a car battery. An additional pump to perform gradients can be added for an extra 1.5 kg. For a total weight of 5 kg it is possible to obtain a fully portable LC system for performing gradient separations with a weight and power requirement similar to that of a portable CE, although the LC system requires manual injection while the CE can sequentially analyse several samples through the use of its autosampler. Nevertheless, this comparison suggests that the 'portability' advantage that CE has over LC is not necessarily true. Of course, this does not consider the use and transport of reagents, but in reality, the weight and volume of these is much less than that of the equipment itself and is only likely to be of relevance in deciding between the two techniques for on-site analyses if chemicals difficult to transport are required.

When considering research grade instruments, as the review by Ryvolová et al. discusses portable CEs, the focus here will be on portable ME instrumentation. The space, weight and power savings made when making ME instrumentation are typically achieved with smaller high voltage power supplies and a reduction in

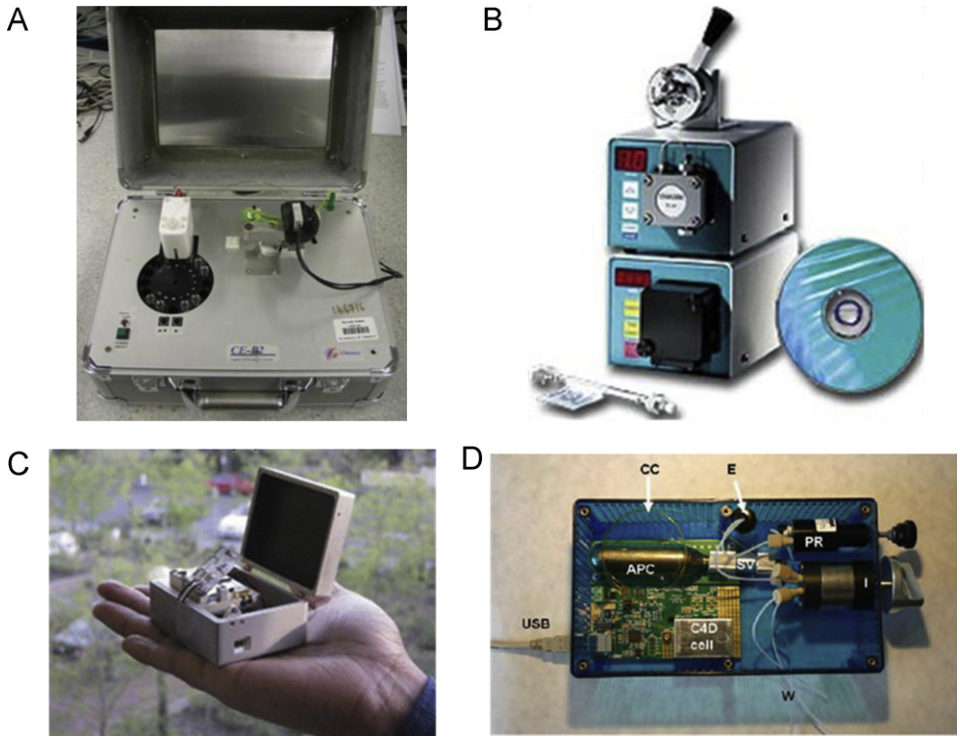

Fig. 4. Photographs of: (A) commercially available portable CE (www.ce-resources.net), (B) commercially available portable LC (www.knauer.net), (C) research grade portable ME [29], and (D) research grade portable IC.

capability. One exception is the Mars Organic Analyzer (MOA), a microfluidic amino acid analyser developed by the Mathies group [28]. This weighs 11 kg with a peak power utilisation of 15 W. The instrument includes a laser and optical detection system, production and control of electrophoresis potentials, actuation of the microfluidic valves and pumps through two rotary pumps, and control of the electrophoresis temperature. The compromise in performance is that a maximum voltage of −15 kV can be applied. This is sufficient to detect amino acids in soil at concentrations 1000 times lower than the GC–MS systems previously sent to Mars on the viking missions. On a weight basis, this not an extremely light and portable instrument, particularly when compared to the commercial portable CE and LC systems discussed above, but the MOA has been designed for complete remote operation and thus must be fully self sufficient, unlike the instruments above. One of the smallest ME instruments reported to date is shown in Fig. 4(C) [29]. The system has a size of 7.6 cm × 5.7 cm × 3.8 cm and weighs less than 240 g and includes a solid-state laser, a photodiode for detection and a high voltage power supply for separation, although it is limited to a maximum voltage of 154 V and 30 μA. Even with this low voltage, the authors were able to demonstrate the indirect detection of explosives and an endocrine disruptor in river water within 5 min. While small, it is important to realise that this has been achieved by limiting its instrumental flexibility, but its applicability has been maintained through using ITP which integrates sample pretreatment and separation. In the area of LC, there has been a very recent report of a light weight portable system for open tubular IC by Kiplagat et al. [30]. Their system is shown in Fig. 4(D) and was housed in a 25 cm × 15 cm × 8 cm plastic box with a weight less than 1 kg. The miniature air pressure cylinder provides a maximum pressure of 700 kPa (100 psi) through a pressure regulator to pressurise a 5 mL eluent chamber with detection by direct conductivity using a homemade capacitively coupled contactless conductivity detector (C^4D). This pressure is sufficient to use long narrow diameter open tubular capillary columns (100 cm × 30–75 μm ID). The whole system was powered through a USB port, through which data acquisition also occurred and the system was operational for up to 8 h. The performance of the system was demonstrated with the on-site analysis of 6 cations in river water, and while a powerful demonstration of the potential of miniaturisation, the compromises required to reduce the weight and power resulted in separation times of 40 min. This again shows that even when the performance of the ME and LC systems are slightly compromised to enhance portability that there is still a slight edge in terms of performance per weight/power that can be obtained through the use of electrophoresis.

5. Sample size

The next 'advantage' of CE (again by extension, ME) often quoted is the much smaller sample requirements, as 'only nL are injected onto the capillary'. This statement should however be interpreted in light of the actual requirements for analysis, and is nicely articulated in recent work by Büscher et al. [31]. They compared CE, LC and GC each coupled with TOF-MS for metabolomics to determine the best separation platform for quantification of central metabolic intermediates and key cofactors. Using a selection of 91 metabolites, which because of isomers reduced to 75 compounds with distinct molecular mass, comparison was made using specific parameters, such as coverage, matrix effects, isomer separation and a number of general considerations such as sample volume, time and reproducibility. Other points from this work will be considered later, but for now the focus will be on the sample volume. The authors found that the minimum sample amounts were 50 pmol (2.5 pmol on-column) for the GC/TOF-MS, 250 pmol (200 pmol on-column) for the LC/TOF-MS, and 400 pmol (0.2 pmol on-column) for the CE/TOF-MS. They noted that the on-column amount for CE was 100 times lower than that of LC but that a minimum volume of 10 μL was required in the sample vial when using an autosampler. Thus, they concluded that there was no real difference in sample requirements between these three separation platforms. This outcome is likely to be similar to the majority of cases in CE and that the smaller sample size requirement of CE is therefore debatable. This work did not consider microchips, but in practice, a volume of 5–10 μL of sample is typically employed in microchips, with smaller volumes having problems with evaporation.

It is reasonable to ask if there is any real advantage in the smaller volume of sample that can be analysed by CE (and ME). There is at least one area in which the answer to this question is a most resounding yes and that is the analysis of single cells. The internal contents of a single cell are on the order of the injection volumes typically employed in CE, making the technique ideally suited to this type of analysis. As such, there has been a considerable amount of interest in the ability to analyse single cells in capillaries and microchips [32–34]. Early work involved injection of a single intact cell into the capillary, lysis of the cell and separation of the intracellular contents. This is a very powerful approach, but it is destructive and given that there is considerable variation between cells it becomes necessary to analyse a statistically relevant number of individual cells in order to be able to draw any scientific conclusions from the results. This limitation can be overcome if the intracellular contents can be sampled without destruction of the cell, and this was demonstrated a few years ago by Woods et al. [35]. Using a 770 nm ID capillary the outer diameter was tapered from 150 μm to 2.5 μm at the tip by etching with HF. Analysis was performed by positioning the tip of the etched capillary against the cell and causing a slight depression in the surface. A small voltage (2–3 kV for 3–6 s) was then applied to form a small nanopore in the membrane, thus allowing the intracellular contents to be injected into the capillary. As little as 2% of the internal volume was injected and while this is a truly exciting development in single cell analysis, it is not without its issues. It is currently exceptionally manually tedious (but this drawback might be addressable in microchips) and there will always be the question of the effect of the analytical measurement on the behaviour of the cellular system. However, this does clearly indicate that there are some emerging applications in which the small volume of sample injected into CE and ME will be of value.

6. Cost

The final 'advantage' of CE to be discussed is that of cost. The purported cost advantage of CE is frequently stated with little or no substantive evidence, presumably because it is assumed to be obvious. In fact, there is very little evidence within the scientific (or general) literature of a full cost comparison between LC and CE. The only documentation where a full cost analysis has been reported was in relation to a multiplexed CE system (12 channel) for genetic analysis compared to conventional agarose gel electrophoresis [36]. While CE with GE have not been specifically compared at other sections of this review, it is useful here because of the general scarcity of information regarding cost. Considering the running costs (capital costs were approximately the same) and the analysis of 12 samples, the CE system came out at $6.40 per analysis while the agarose gel system was $38–68. Interestingly, 90% of the cost in the gel system was labour costs, whereas in CE this comprised 50% of the CE cost, and the CE labour cost was 10–20 times lower than that of the gel system, indicating the much higher level of automation that can be achieved with a CE when compared to a

Table 1
Estimated costs of GE, LC and CE.

	Technique	Instrument cost	Technical service cost	Reagent costs (100 tests)
Tricyclic antidepressant	LC	$46,000	$5000	$1150
	CE	$45,000	$5000	$700
Serum proteins	GE	$20,000	$2200	$200
	CE	$45,000	$5000	$20
Haemoglobin	LC	$46,000	$5000	$140
	CE	$45,000	$5000	$20

From LeLe et al. [38].

gel. An application note from Industrial Bio Development Laboratory (http://www.ibdl.ca) compared single channel CE to that of SDS-PAGE with a mini-gel, and found that the cost of CE was $6.82 for 30 samples, compared to $5.71 for SDS-PAGE [37]. No specific details were given on how these costs were calculated but it appears that the cited cost covers only the consumable costs and did not consider labour. Given the difference in labour costs discussed by Amirkhanian et al. it is likely that if these were included the CE method would be again be cheaper, but it is unclear by how much.

When CE is compared to LC, the best assessment of relative costs found was from 2001 in a book chapter by LeLe et al. who compared the cost of CE, GE and LC based on the authors' experiences in a clinical lab [38]. Their data have been reproduced (Table 1) and as can be seen the capital and service costs for LC and CE were similar, with the LC running costs ranging from 1.5 to 7 times those of the CE costs for 100 samples. The authors commented that labour costs were not included in this comparison because in their experience the times required for analysis by LC and CE were similar. They also noted that the main difference in cost between the two techniques was due to the fact that capillaries were cheaper than columns. Based on current prices, 10 m of capillary can be purchased for $150 (www.polymicro.com), while for LC a guard and analytical column will range from $500 to $1400 (www.sigmaaldrich.com) and in routine use there is no reason to believe that the lifetime of a capillary would be in any way less than that of a LC column. It was unclear how the capillary and column costs were included in the data in Table 1. It is important to note that from a clinical laboratory perspective it is likely that all solvents and solutions are outsourced (including water) and the cost for both CE and LC solutions will be similar, with the only major benefit being the reduced volume required for CE (3–5 mL per day) when compared to LC (typically a flow rate of 1–4 mL/min). Given that the time requirements per sample by CE and LC are similar, the cost for 1 sample will be similar, but each additional sample will cost more for LC but will not induce any additional costs for CE. When LC is downscaled through the use of a capillary column (typical flow rates of 0.2 mL/min), the LC consumable costs in Table 1 will be reduced by approximately 5–10×. Even with this reduction, the CE cost per sample is still likely to be cheaper but the case may be much more marginal with this comparison.

In a manner similar to the earlier discussion relating to the potential advantages offered by the speed of CE, it is clear from the above examples that while there may be a cost benefit of single capillary CE over LC, the real benefits only start to materialise when using larger batches of samples.

7. Sensitivity

An issue that has been of interest to the author for a number of years is that of the sensitivity of CE, and this is one of those often-stated disadvantages of CE (and by extension ME). The reason why the detection limits achieved are inferior to those achieved with LC is ultimately due to the use of narrow diameter capillaries. Small diameters are required to enable rapid dissipation of the heat generated by the application of the high voltage needed to achieve the highly efficient separations that are characteristic of CE. But this ultimately means that volume injected in CE (typically 10–50 nL) is much smaller than in LC (typically 10–50 μL). With physically less analyte to detect, it is therefore not surprising that the detection limits in CE are lower. Exactly how much lower depends upon the actual comparison made.

In order to see exactly how much less sensitive is CE than LC, it is useful to compare them on an equal footing in order to try and elucidate the differences in sensitivity. Starting in the mid 90s, Chervet et al. [39] published one of the first reports on nanoflow LC. They used a 30 cm × 75 μm ID capillary packed with 5 μm C18 particles, a 20 nL injector which was split 1:10 with a flow splitter, and an off-column UV detector featuring a 3.0 nL detection cell with an 8 mm optical path length. Detection limits for 5 standard proteins were 17–42 pg (8.5–21 μg/mL based on an injection volume of 2 nL), and for BSA was 17.5 μg/mL. This is an important place to begin the discussion because it allows almost a direct comparison with protein separations performed using CE in which the injection volume will be similar. Also, because this LC method uses an absorbance detector, which is arguably the most common form of detection in CE, it provides an opportunity for a robust comparison given that this form of detection is path-length dependent and thus suffers from losses in sensitivity when miniaturised (i.e. it is a concentration dependent detector). Work from the same period by Recio et al. on the separation of milk proteins using a Beckman CE with a 75 μm ID capillary and on-capillary detection (with a path length of 75 μm) gave a detection limit of 5.0 μg/mL for BSA [40]. This is actually lower than that of the nanoflow LC system, thus on an approximately equal footing, CE is not less sensitive than LC at all. If this is taken to its logical end and a similar detector optical path length had been used on the LC system, then CE would give greatly superior sensitivity.

Consider now a more recent example, involving two articles published within the last 5 years, both using MS detection which has become a highly favoured detection method over the past 10 years because it is a mass dependent detector and is not compromised in the same way as an absorbance detector when miniaturised, but in this case using small molecules (plant hormones) to negate the potential inherent advantage that CE may have over LC for the separation of proteins. The nanoflow LC–MS/MS method developed by Izumi et al. used as an ion-trap MS and a 15 cm × 75 μm ID capillary packed with 3 μm C18 paticles [41] and the their separation is shown in Fig. 5(A). They obtained detection limits from 0.2 to 18 fmol for a number of zeatin hormones (Table 2). Ge et al. reported a CE–MS/MS method for the same zeatin hormones also using an ion-trap MS [42] and their separation is shown in Fig. 5(B). Using a 50 μm ID capillary and non-stacking conditions in which the dried extract was prepared in background electrolyte (BGE), they obtained detection limits of 5.8–12 fmol. The LC–MS/MS method was 100 times more sensitive than the CE–MS/MS method for *trans*-Zeatin O-glucoside, but the CE–MS/MS method was 1.4 times more sensitive than the LC–MS/MS method for *trans*-zeatin. If instead of redissolving the extract in BGE, water was used in order to induce field amplified sample stacking, then the LODs of the CE–MS/MS approach were

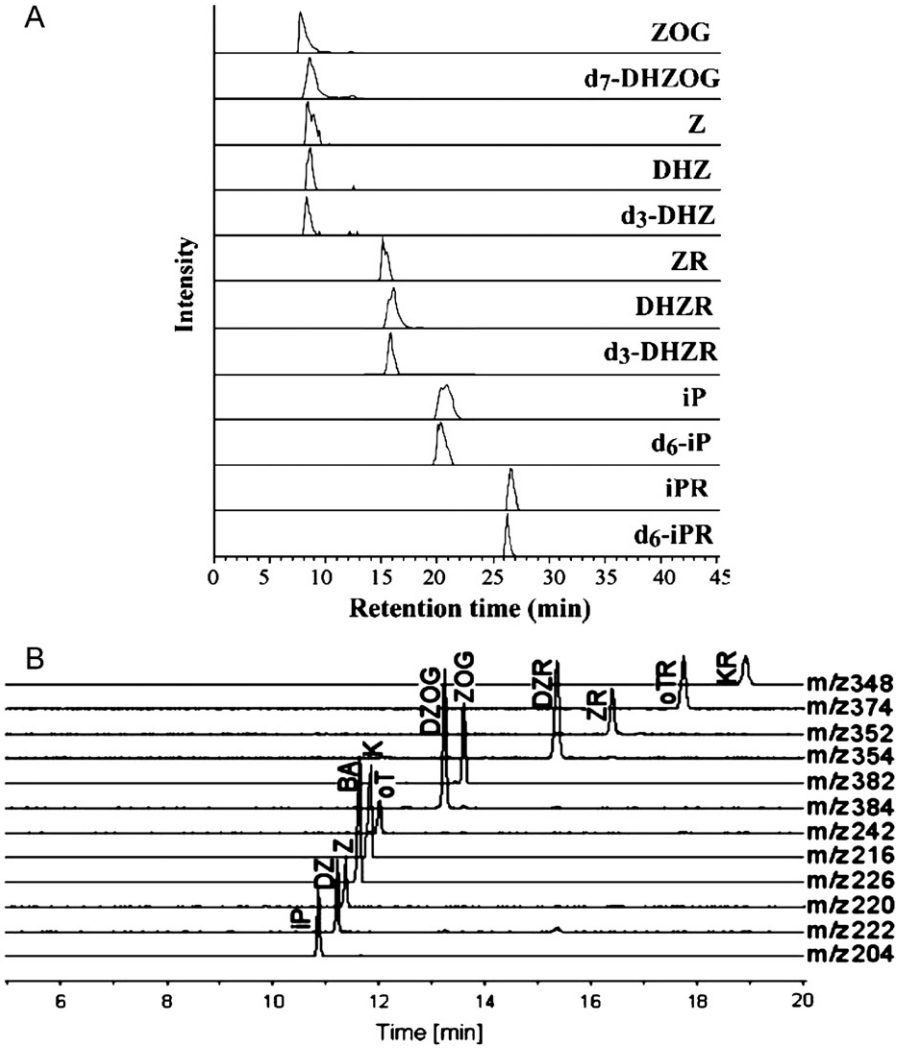

Fig. 5. Separation of plant hormones by: (A) LC [41] and (B) CE [42].

reduced by 10–20 times, and the CE–MS/MS gave detection limits up to 17 times lower than the LC–MS/MS method, and at worst was only 3 times higher. While this may initially appear to be an unfair comparison as the CE method is using sample enrichment through stacking, a closer examination of the details reveals that this is perhaps not such a significant issue. First, both methods had a similar sample preparation process: extraction, evaporation and redissolution. Thus to induce stacking in the CE method, it is simply necessary to redissolve the sample in water, not BGE, and thus there is no real difference or increase in the time required to induce stacking. Second, stacking allows the injection volume to be increased, but in this work there was no change in injection volume between the stacking and non-stacking methods. Finally, the sample for the LC method was dissolved in an aqueous buffer that did not contain acetonitrile (which was used in the mobile phase in that work) thus there will be a natural column-front focusing that will have a similar effect to the stacking process in the CE method. Thus, both methods employ a certain amount of on-line focusing, and in this respect can therefore be regarded as approximately equal. What is exceptionally interesting about this comparison is that there is a sheath flow in the CE–MS/MS method (4 μL/min) while the LC–MS/MS method used a nanospray emitter, yet even with dilution from the sheath flow the CE method was able to achieve very similar detection limits. This is actually a very important point to consider given the

Table 2
Comparison of nanoflow LC–MS/MS [41] and CE–MS/MS [42] detection limits for plant hormones using an ion trap MS.

	Nanoflow LC–MS/MS[a]	CE–MS/MS (non-stacking)[b]			CE–MS/MS (stacking)[c]		
	fmol	uM	fmol	CE/LC (LC/CE)	uM	fmol	CE/LC (LC/CE)
trans-Zeatin *O*-glucoside (ZOG)	0.17	1.9	11	65	0.09	0.53	3.1
trans-Zeatin (Z)	18	2.1	12	0.68 (1.4)	0.18	1.1	0.06 (17)
Dihydrozeatin (DZ/DHZ)	8.5	1	5.8	0.69 (1.4)	0.11	0.65	0.08 (13)
trans-Zeatin riboside (ZR)	0.2	1.9	11	56	0.07	0.41	2.0
Dihydrozeatin riboside (DZR/DHZR)	0.33	0.89	5.2	16	0.05	0.29	0.89 (1.1)

[a] Dried extracted dissolved in water/acetic acid (100/0.05, v/v).
[b] Dried extract dissolved in BGE.
[c] Dried extract dissolved in water.

trend towards miniaturisation in microchips and the current use of nanoflow LC–MS for proteomic and metabolomic studies. The question must be asked why capillary LC methods would be preferred over CE methods on this basis, particularly given the above comparison of these two separations. This work also makes a strong case for the development of robust and reliable sheath-less nanoflow interfaces for CE, because if these can be developed, then the sensitivity of CE will be superior to that of nanoflow LC.

These two examples show that when the dimensions of the LC system are reduced to a similar scale to that of CE, there is not really any difference in sensitivity. The problem with this comparison is that LC typically uses columns with 2–4 mm ID, and not the 75 μm ID mm sized columns used above, and thus returning to an earlier point raised above, the often-stated difference in sensitivity is due to the fact that less sample is physically injected onto the CE separation capillary than in LC. How much of an influence does this have? Ahrer et al. have developed both LC–MS and CE–MS methods for the detection of non-steroidal anti-inflammatory drugs (NSAIDs) in environmental waters using the same quadrupole MS [43]. LC–MS was performed using a 2 mm column with 100 μL of sample injected onto column, resulting in detection limits from 0.1 to 1 μg/L for 7 NSAIDs. CE–MS was performed using a 50 μm capillary with a sheath flow of 4 μL/min and approximately 22 nL of sample was injected, giving detection limits from 20 to 134 μg/L. The amount of sample injected into the capillary was 4544 times less in the CE case, but the detection limits were only 94 times higher than those achieved by LC–MS. This difference in sensitivity of about two orders of magnitude is what many people would expect and there are many similar reports that can be found in the literature demonstrating similar differences in sensitivity. But this is not always the case.

Recently, the author's research group has undertaken the development of complementary IC [44] and CE [45] methods for the separation of inorganic anions and cations for the detection of improvised explosives. This is useful work to examine for a number of reasons. First, considerable time was spent developing the optimised conditions on both platforms, for both anions and cations. Second, both platforms used conductivity detection, with the IC system using a contact conductivity detector while the CE had a capacitively coupled contactless conductivity detector (C4D). Third, in the IC system for anions, the background conductivity was lowered after separation by using a suppressor, while the cation system had to be performed in the non-suppressed mode, which is also used for CE. This therefore allows a direct comparison to be made between CE with conductivity detection and to then gauge the influence of suppression. As expected from the results (shown in Fig. 6), the CE method is faster for both anions and cations, requiring approximately half of the time needed for the IC separations. More interestingly, data on the detection limits are shown in Table 3. The detection limits for CE are on average 2.7 times higher than those for non-suppressed conductivity detection of the cations and 10 times higher than suppressed conductivity detection for the anions. The fact that CE is only 10 times worse than IC with suppressed conductivity detection will come as a surprise to many. It is also worth noting at this point that we have also developed CE methods using indirect absorbance detection for these ions using light-emitting diodes as a light source and these give LODs which are 10× higher than those obtained by conductivity detection [46], and approximately 5 times lower than using a conventional deuterium lamp. Thus, prior to the use of contactless conductivity detection, CE would have been around 100–500 times less sensitive than IC, and this is probably more in tune with the preconception of most scientists.

From the literature discussed above, CE is at worst 10–100 times less sensitive than LC. What may surprise people even further is that in the examples used above there has been no attempt to improve

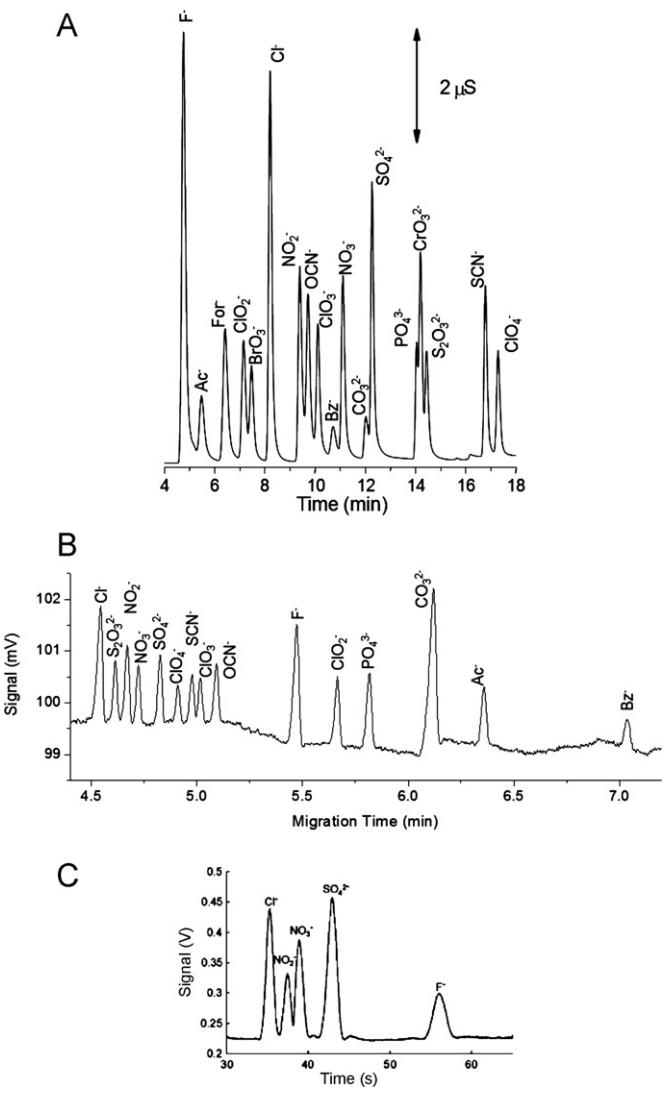

Fig. 6. Separation of inorganic anions by: (A) IC with suppressed conductivity detection [44], (B) CE with contactless conductivity detection [45], and (C) ME with top–bottom contactless conductivity detection [61].

the sensitivity of CE by using stacking to allow the injection of larger volumes of sample (and hence more analytes) to be injected. There are of course numerous approaches that can be employed to perform on-line concentration and these will not be discussed here as they have been covered in more detail in a number of specific reviews devoted to this topic [47–51]. These reviews show that it is relatively easy to achieve enhancements in sensitivity from 10 to 500 using approaches that exploit hydrodynamic injection, such as large volume sample stacking, isotachophoresis, dynamic pH junction and sweeping. Enhancements from 1000 to 100,000 can be easily achieved using electrokinetic injection by techniques such as field-amplified sample injection, electroaccumulation and electrokinetic supercharging. In relation to the specific examples discussed above to compare the sensitivity of CE with LC, there are numerous methods showing sensitivity enhancements of NSAIDs from 100 to 10,000 [52–56], with the lowest detection limits reported being 10 ng/L [57]. Enhancements for inorganic anions and cations as high as 100,000 have been reported, with detection limits for anions of 8 ng/L [58], and 1 ng/L for heavy metals [59,60]. When combined with on-line preconcentration, it is clear that CE can easily achieve detection limits considerably lower than those obtained with LC.

Table 3
Detection limit data for the separation of inorganic anions and cations by IC [44], CE [45] and ME [61].

	IC[a]	CE	ME[c]
Anions (μg/L)			
Fluoride	2	26	2.9
Acetate	12.6	110	
Chlorite	8.2	92	
Chloride	2.2	27	5.2
Nitrite	4.4	41	
Cyanate	4.8	53	
Chlorate	7.2	69	
Benzoate	27.4	240	
Nitrate	5.4	52	9.3
Sulfate	3.1	44	14
Phosphate	8	81	
Thiosulfate	7.6	52	
Thiocyanate	5.5	66	
Perchlorate	8.4	84	
	IC[b]	CE	ME[c]
Cations (μg/L)			
Sodium	14	52	6.9
Ammonium	12	31	5.4
Methylammonium	22	58	
Potassium	32	53	22
Ethylammonium	34	129	
Manganese(II)	30	40	
Magnesium	13	73	8.4
Calcium	25	48	12
Strontium	60	120	
Barium	115	240	

[a] Suppressed conductivity detection.
[b] Non-suppressed conductivity detection.
[c] Based on 2× signal-to-noise.

To conclude this section, it would be remiss not to discuss microchips and the perception that the sensitivity is even worse in ME than it is in capillaries. While there is as yet no absolutely perfect comparison between the performance of capillaries and microchips (using the same detector, BGE, analyte set, etc.), the work of Mahabadi et al. [61] using C4D electrodes both above and below the microchannel gives a good comparison with the inorganic ion separations above. The LODs in their system were 5–10 times lower than those discussed above for CE and are comparable to those obtained by IC (Table 3). While this is impressive, it is important to note that these separations were not performed with the same BGE or requirement for analyte resolution pertaining to the earlier example and the LODs were calculated at 2× the baseline noise, rather than the more commonly used 3× which was used in the CE and IC work. Mahabadi et al. also used a highly intricate microchip which had electrodes both above and below the microchannel and is therefore more akin to a capillary C4D detector, rather than the more common and more easily fabricated approach of using only electrodes on the top or bottom of the microchannel, which is about 10× less sensitive [62]. Even so, it is clear from this study that ME is equally as sensitive as CE and under the correct circumstances there may be no loss in sensitivity when moving to the microchip platform.

In summary, CE and ME can in general be considered to be less sensitive than LC. The exceptions are when stacking is used in CE and when the LC column dimensions approach those that are used in CE. In these cases, the sensitivity of CE can equal and even surpass that obtainable by LC.

8. Repeatability

In addition to sensitivity, the repeatability and reproducibility of migration times, peak areas and peak height and the general reliability of CE have often been stated as reasons why LC is preferred over CE, particularly for routine applications. There is some truth in this perception of CE, but it is important to recognise that there has been considerable research into addressing these issues over the past decade and some of these studies will be discussed in the following paragraphs.

It is an obvious requirement for analytical precision to be achieved that the same amount of sample should enter the capillary for each replicate injection. Differences in the volume injected will obviously impact upon the peak heights and areas, but in CE they will also impact upon the migration time. This is due to the way in which the electric field is distributed along the capillary in relation to the relative conductivities of the sample and electrolyte. Any variation in the length of the injected sample plug will obviously cause a change in this electric field distribution and this will impact upon the migration time. Commercial CE instruments have highly advanced pneumatic control in order to achieve precise sample injection, and significant developments occurred within these instruments in the 90s to improve the injection repeatability. However, pneumatic injectors require perfect sealing between the pressure source and the sample vial and constant viscosity and temperature in order to ensure the same volume of sample is introduced at each injection. The alternative is to introduce a defined volume of sample, and this has been achieved in CE through the use of a rotary injector similar to those used in LC and should provide repeatability similar to that exhibited in LC [63]. When five successive 10 nL injections were made using this approach, migration time and peak area repeatability of less than 1% was obtained. While promising, this approach clearly needs more validation to establish that it is a viable alternative to the more commonly used pneumatic injection approach employed in most instruments today. The ability to physically define the injection volume also has important application in microchip CE. This might be accomplished through appropriate design of the microchannel and should, when other issues are resolved, lead to improved analytical performance in ME.

Reproducible injection is not the only sample-related issue in CE. CE is also susceptible to variations in the composition of the sample, and this is a particular problem when using electrokinetic injection. This occurs because with electrokinetic injection any change in sample matrix causes a change in the electric field applied to the sample and, in turn, this impacts upon the number of ions that migrate into the capillary [64–66]. What is perhaps less obvious is that variation in the sample matrix is also important with hydrodynamic injection. For example, Van der Schans et al. showed that there is considerable variation in peak shape and migration time for the separation of dsDNA when the amount of salt in the sample changes [67]. What are the potential solutions? The ideal approach is to ensure that the electric field is distributed over the sample the same way for every injection. This may be achieved by removing the analytes from the sample matrix, through for example, off-line liquid–liquid or solid-phase extraction. However, this is not ideal as it requires additional time and expense. An alternative approach to minimise the significance of variations in sample composition is to ensure that, relative to the separation electrolyte, this variation is small. In other words, if the concentration of NaCl in a sample varies from 1 to 3 mM, which is a 2 mM variation, having a BGE with a conductivity equivalent to 200 mM NaCl would result in this variation being about 1% of that of the BGE, which is likely to be inconsequential. The alternative is to prepare the sample in a solution (often the BGE) such that there is no change in conductivity, however this approach is infrequently used as it does not promote field-amplified stacking (but may be adapted to induce isotachophoretic, dynamic pH junction or sweeping for on line concentration).

Repeatable migration times are also critical to obtaining reproducible peak heights and areas. Analytes move through the capillary due to the combination of electrophoresis and electroosmosis, and its minor variations in this latter component that are

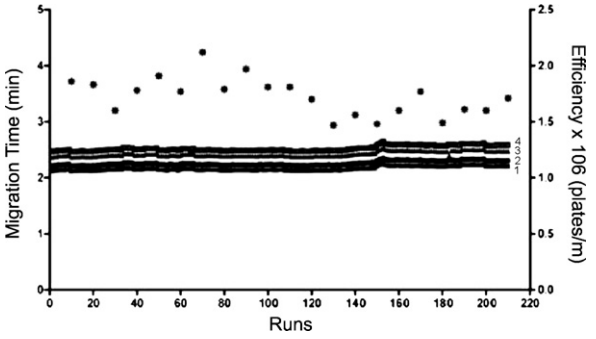

Fig. 7. Migration time and efficiency repeatability of protein separations of a 5 μm ID capillary coated with 0.5 mM DODAB.
Reproduced from [70].

responsible for many of the repeatability issues in CE. The majority of separations to date have been performed in fused-silica capillaries, and the problem with this is that silica is an amorphous material that does not have a highly defined surface and therefore the first requirement to obtaining a highly repeatable electroosmotic flow (EOF) is to generate a highly consistent and repeatable surface charge. Historically, this was addressed by conditioning capillaries with alkali and it is not uncommon to find that new fused-silica capillaries are immediately treated with 1 M NaOH to generate as many silanol groups on the surface as possible in an attempt to provide a fully charge-saturated and hence repeatable surface. This treatment may or may not be followed by flushing the capillary with water and/or acid prior to conditioning with the separation electrolyte. There may also be short combinations of these conditioning steps performed between each injection. Indeed, almost every CE practioner has their 'own' conditioning protocol that works for them. The second requirement to obtaining a repeatable and reproducible EOF is to ensure that nothing from the sample adsorbs irreversibly to the surface, which will change the surface charge and hence the EOF. This latter point has, for decades, been a specific issue for protein separations and considerable time and effort has been devoted to addressing this issue.

Perhaps the best currently available approach to provide a consistent and repeatable surface charge is to modify the inner surface of the capillary with a non-covalent semi-permanent coating, and the current state of this approach is covered in excellent reviews by Lucy et al. for protein separations [68] and by Huhn et al. for use with CE–MS [69]. These coatings are prepared by flushing the capillary with a solution of the coating agent in between separations, at the beginning of each day, or at some time interval as required to obtain sufficient repeatability. The coating agent may be neutral, cationic or anionic and a single coating agent may be used to generate a single layer on the capillary wall, or two coating agents can be used to generate multiple layers of alternating charge. Both small molecules, such as surfactants, and large molecules, such as a polymers, can be used. There are many advantages to this method, such as simplicity, low cost, and excellent repeatability. The reviews mentioned above give explicit examples of a number of approaches, but for illustrative purposes two examples will be discussed here. The first is very recent work by Gulcev et al. who showed impressive repeatability using dioctadecyldimethylammonium bromide (DODAB) as the coating agent for 5 μm capillaries [70]. Fig. 7 shows the migration time and average separation efficiency for the separation of 4 model proteins through 210 separations over a period of 17.5 h without regeneration of the capillary surface coating. The relative standard deviation (RSD) for migration time was less than 0.8% over the first 150 runs, and less than 1.9% over all 210 runs. Capillary-to-capillary repeatability was also excellent, with RSDs of 1.0–1.3% for 10 successive separations in 3 different capillaries. The second work is that of Puerta et al. who synthesised a new cationic polymer, poly-LA 313, for coating capillaries [71]. The intra-day RSD of the EOF for two capillaries was 0.4%, but much more impressively, the inter-day reproducibility of the EOF was 1.2% for 17 capillaries coated on 14 different days over a year, indicating that this coating agent created a highly repeatable surface. The intra-day repeatability was also good with migration time repeatability within 0.2%, but the inter-day repeatability was poor and regeneration of the coating with a 26 min procedure rectified this, producing a RSD for the EOF of less than 0.6%. This coating was also found to be very stable when organic solvents were present in the BGE, with RSDs for EOF over 30 separations of 0.4% and 1.5% when the BGE contained 20% and 50% of MeOH, respectively, and 0.4% and 1.2% when the BGE contained 20% and 50% MeCN, respectively. The authors also mention that this coating agent was also highly suited for use on polydimethylsiloxane, and thus could be useful to improve migration time repeatability in microchips, which is an important factor that has yet to receive any considerable attention.

Even taking into account all of these factors, there are still some residual issues that will cause changes in migration time between runs, leading to poor repeatability. One simple solution discussed by Schmitt-Kopplin et al. was to use a mobility-scaled axis for the electropherogram, rather than a time-scaled axis, by removing the variation in EOF [72,73]. Fig. 8 shows selected electropherograms from 76 separations of *p*-hydroxybenzoic acid and vanillic acid in both: (a) time-scaled and (b) mobility-scaled axes. The migration time of RSDs were 4.19% and 4.63% for *p*-hydroxybenzoic acid and vanillic acid, respectively, which is reduced to 1.46% and 1.48%, respectively, when translated to the mobility scale. Thus, simply by measuring and accounting for the EOF, in a manner similar to the way in which retention factors are calculated for LC, it is possible to significantly improve the repeatability of the position at which peaks occur, thereby improving the performance of CE both qualitatively and quantitatively.

Assuming the instrument is functioning properly and the method has been designed properly, can CE work reproducibly in a routine environment? The answer is yes, and there are a number of reports now showing that CE can match the performance of LC in a routine environment. Theurillat et al. presented data on the performance of a CE assay for the detection of lamotrigine in human plasma and serum over a 4 year period from 1998 to 2001 [74]. The authors discussed the rationale behind implementation of a CE method over that of LC, noting that the two platforms provided comparable performance with precision and sensitivity, but that sample preparation for CE was simpler (protein precipitation and direct injection of acidified supernatant, compared to liquid- or solid-phase extraction for HPLC) and the sample throughput was higher (9 min for CE, 13 min for HPLC). Evaluation of quality control data from 1998 to 2000 ($n = 71$) revealed RSD values of 9.15, 8.45 and 2.07% for the migration times of lamotrigine, the internal standard and the migration time ratio, respectively. All but 8 controls were within the target range of ±10% of the drug level, with those 8 being within ±20%. The authors also analysed 288 external inter-laboratory quality control samples, and were ranked 24/56 in 1998, 19/67 in 1999, 43/69 in 2000 and 35/72 in 2001, with an average ranking of 45.6%. This was the only CE method in the inter-laboratory study and the results clearly indicate that CE performed equally well to assays based on immunoassays, LC and GC. Weykamp et al. recently presented results on a 6 year study of a reference measurement system for haemoglobin [75]. Data were presented from 14 laboratories, 3 in Japan, 8 in the USA and 1 in Sweden, from 12 inter-laboratory comparison studies over the period 2001–2006. All laboratories used methods approved by the International Federation of Clinical Chemists, with 6 using LC–MS and 8 using CE. The data showed that there was no difference in haemoglobin outcome between laboratories that

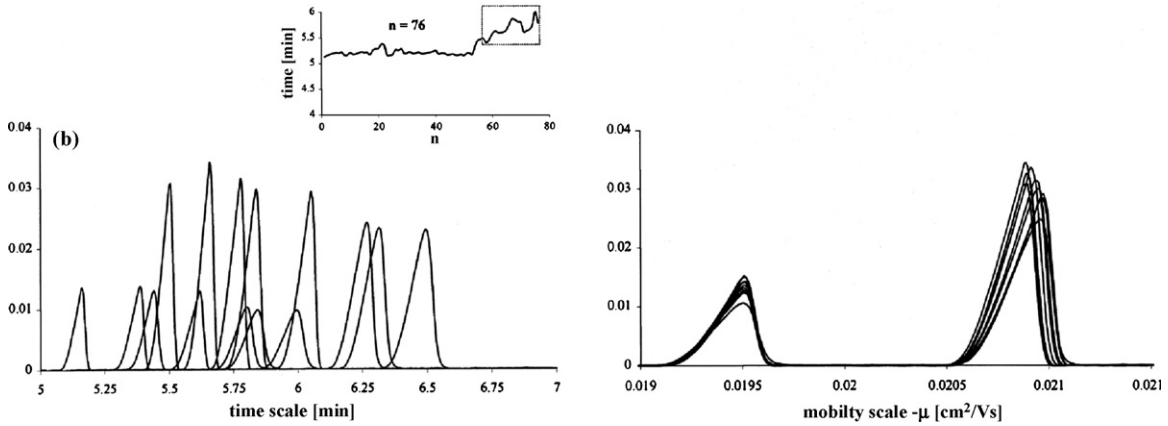

Fig. 8. Comparison of: (a) time scale and (b) mobility scale for the CE separation of *p-hydroxybenzoic* acid (left) and vanillic acid (right). The insert in (a) shows the variation in migration time of *p-hydroxybenzoic* acid.
Reproduced from [73].

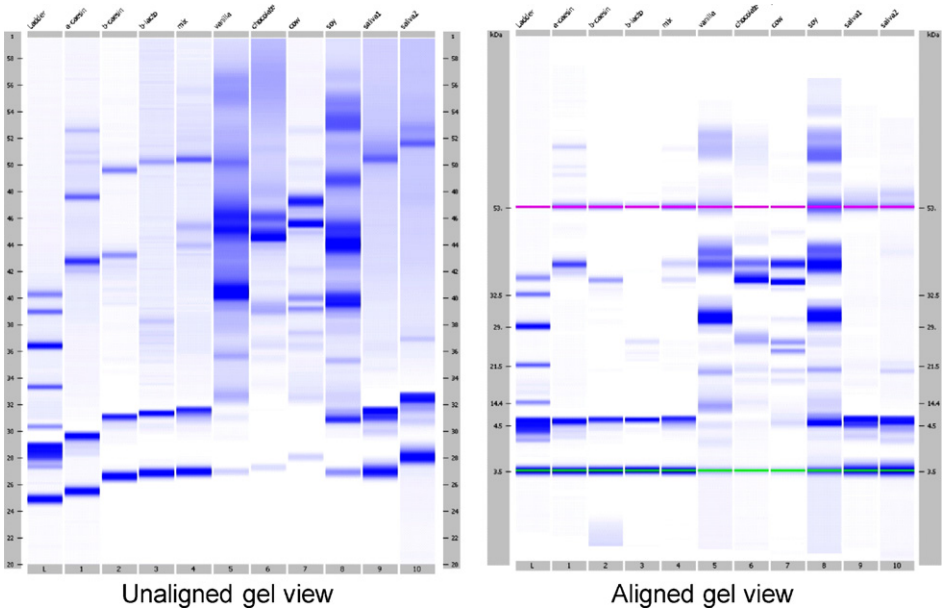

Fig. 9. Protein separations performed on the Agilent Bioanalyser 2100 in the analytical chemistry undergraduate unit at the University of Tasmania.

used LC–MS versus those that used CE methods (0.1 mmol/mol vs 0.0 mmol/mol) but that the MS-group had a significantly higher variation in haemoglobin (0.7 mmol/mol vs 0.4 mmol/mol). These two reports clearly show that if the method is developed properly and the instrument maintained, then CE is just as reliable and provides equivalent data to other analytical methods, particularly LC.

It is worth concluding this section by again considering microchips with discussion of some unpublished work taken from the undergraduate teaching program in the author's department. The final year analytical undergraduate course utilises the Agilent Bioanalyser and associated protein kit to introduce students to the microchip platform in an application-oriented experiment. The students run a number of standard proteins, and then a series of samples including protein shakes, cow and soy milk and some saliva samples. In addition to exposing students to microchips, this experiment also aims to introduce the use of internal standards to improve both qualitative and quantitative performance. Fig. 9 shows some results obtained during the development of this experiment, with the computer-generated gel image on the left representing the raw data. It can be seen from this image that there is a slight drift in migration time, as indicated by the time of the first alignment marker (the band at the bottom of each gel lane) changing from 25 to 28 s over the course of 11 separations. The average migration time of this marker is 26.6 ± 0.9 s, giving a 3.5% RSD (n = 11), indicating that the intra-chip repeatability is poorer than that which can be obtained in a capillary. This can be corrected for by using two alignment markers (right image in Fig. 9), and this also aids in improving inter-chip repeatability as well. This is the approach used commercially and can be used to accurately size and quantify both DNA [76] and proteins [77] with accuracy and precision similar to that obtainable by other methods. With the improved understanding of repeatability in CE and ME that we now have, it is likely that this will lead to improved absolute performance on the microchip platform as well.

9. Final comments

CE and to a lesser extent, ME, have been available for a considerable number of years, and it is clear from the discussion above that many of the conceptions held about CE and ME are not necessarily true. It can be quicker, use less sample and reagents, be simpler and more portable and, cheaper, but these generally require specific and specialised implementation. On the direct comparison of

a single capillary system with a single column LC, there is no significant difference in many of these attributes, but the situation may be different when a 'holistic' view is taken and all of these are required. It is also clear that the sensitivity of CE is inferior to that of LC when capillaries are compared with conventional LC columns, but it is less apparent when LC is performed with capillary columns and when on-line enrichment strategies are used. The repeatability of CE and ME has improved and can now match the general repeatability of LC. Even so, CE is still often considered to be inferior. Indeed in the comparison of GC, LC and CE with TOF-MS for metabolomics by Büscher et al. they stated that "In our experience, CE is the least suitable platform because its separation power and sensitivity are equivalent to both LC and GC, but it lacks the robustness required for analyzing biological samples" [31]. This view is most likely shared by other practitioners of CE, and from the authors' experience, is not just restricted to biological samples, but there is a general lack of robustness in general.

So what needs to be done? The instrumentation needs to be robust. There has been significant improvement in performance since the early commercial instruments became available, but perhaps it is time to consider different ways in which CE experiments can be performed. The current design used in all commercial instruments has been around for 20 years already. To paraphrase a friend and colleague 'if you turn a LC system upside down it will still function as a LC, but if you turn a CE upside you will only create a mess'. The growing opportunities in microchips to perform separations in 2 and 3 dimensions will allow creative solutions, but only if we are prepared to think beyond conventional approaches and start considering electrophoresis not chromatography. This has been used to great affect recently to create microfluidic multidimensional systems such as 2D gels and Western blots by using the novel ability to move in 2 dimensions, but many simple electrophoretic systems in chips are still performed in uni-dimensional manner similar to what is done in capillaries.

But even more importantly, the chemistry underlying CE separations needs to be robust as well. CE can be used successfully in an environment where the same method and the same chemistry are employed routinely, but in an environment where the chemistry is regularly changed, CE is a less attractive option. One of the most attractive options of CE is its flexibility and versatility, but this may come at the expense of repeatability and robustness. A great deal of progress has been made from thinking about CE from an electrophoresis perspective and not from a chromatography one, but we are still not there. In many instances method development begins by optimising the separation and perhaps the alternative of designing the separation to be compatible with the sample preparation rather than the other way around, might be one avenue towards improving robustness. The powerful array of on-line CE concentration mechanisms has primarily been used for sample enrichment, but there is also significant potential for integrated sample pre-treatment as well as enrichment, and it is likely that this will play a role in the development of functional and usable lab-on-a-chip assays that are simple, reliable and cheap.

As it has always been, the future of electrophoresis lies not in competing directly with chromatography, but rather in focusing on those areas where chromatography has limitations, and this is likely to be dominated with microchips over the next decade. Electrophoresis is inherently much more amenable to microchips than LC and the first commercially successful lab on a chip products have all been based on electrophoresis. This is unlikely to change anytime soon and the development of the recent microfluidic system for the detection of lithium in whole blood for point-of-care diagnostics [78] will pave the way towards next generation portable analytical technology. The challenge is ensuring that the knowledge developed and gained from capillaries is appropriately transferred into microchips to make functional, reliable and robust portable analytical systems.

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References

[1] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[2] P.G. Righetti, J. Chromatogr. A 1079 (2005) 24.
[3] S. Hjertén, Chromatogr. Rev. 9 (1967) 122.
[4] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[5] D.J. Harrison, K. Fluri, K. Seiler, Z. Fan, C.S. Effenhauser, A. Manz, Science 261 (1993) 895.
[6] D.J. Harrison, A. Manz, Z. Fan, H. Lüdi, H.M. Widmer, Anal. Chem. 64 (1992) 1926.
[7] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, K. Devon, K. Dewar, M. Doyle, W. Fitzhugh, R. Funke, D. Gage, K. Harris, A. Heaford, J. Howland, L. Kann, J. Lehoczky, R. Levine, P. McEwan, K. McKernan, J. Meldrim, J.P. Mesirov, C. Miranda, W. Morris, J. Naylor, C. Raymond, M. Rosetti, R. Santos, A. Sheridan, C. Sougnez, N. Stange-Thomann, N. Stojanovic, A. Subramanian, D. Wyman, J. Rogers, J. Sulston, R. Ainscough, S. Beck, D. Bentley, J. Burton, C. Clee, N. Carter, A. Coulson, R. Deadman, P. Deloukas, A. Dunham, I. Dunham, R. Durbin, L. French, D. Grafham, S. Gregory, T. Hubbard, S. Humphray, A. Hunt, M. Jones, C. Lloyd, A. McMurray, L. Matthews, S. Mercer, S. Milne, J.C. Mullikin, A. Mungall, R. Plumb, M. Ross, R. Shownkeen, S. Sims, R.H. Waterston, R.K. Wilson, L.W. Hillier, J.D. McPherson, M.A. Marra, E.R. Mardis, L.A. Fulton, A.T. Chinwalla, K.H. Pepin, W.R. Gish, S.L. Chissoe, M.C. Wendl, K.D. Delehaunty, T.L. Miner, A. Delehaunty, J.B. Kramer, L.L. Cook, R.S. Fulton, D.L. Johnson, P.J. Minx, S.W. Clifton, T. Hawkins, E. Branscomb, P. Predki, P. Richardson, S. Wenning, T. Slezak, N. Doggett, J.F. Cheng, A. Olsen, S. Lucas, C. Elkin, E. Uberbacher, M. Frazier, Nature 409 (2001) 860.
[8] L. DeFrancesco, Anal. Chem. 73 (2001) 497A.
[9] M.C. Breadmore, A. Procházková, R. Theurillat, W. Thormann, J. Chromatogr. A 1014 (2003) 57.
[10] L.R. Ruhaak, R. Hennig, C. Huhn, M. Borowiak, R.J.E.M. Dolhain, A.M. Deelder, E. Rapp, M. Wuhrer, J. Proteome Res. 9 (2010) 6655.
[11] L. Royle, M.P. Campbell, C.M. Radcliffe, D.M. White, D.J. Harvey, J.L. Abrahams, Y.G. Kim, G.W. Henry, N.A. Shadick, M.E. Weinblatt, D.M. Lee, P.M. Rudd, R.A. Dwek, Anal. Biochem. 376 (2008) 1.
[12] H. Lee, T.J. Griffin, S.P. Gygi, B. Rist, R. Aebersold, Anal. Chem. 74 (2002) 4353.
[13] L. Fang, J. Cournoyer, M. Demee, J. Zhao, D. Tokushige, B. Yan, Rapid Commun. Mass Spectrom. 16 (2002) 1440.
[14] L. Fang, M. Demee, J. Cournoyer, T. Sierra, C. Young, B. Yan, Rapid Commun. Mass Spectrom. 17 (2003) 1425.
[15] Z. Zhuang, J.A. Starkey, Y. Mechref, M.V. Novotny, S.C. Jacobson, Anal. Chem. 79 (2007) 7170.
[16] J. Bones, S. Mittermayr, N. O'Donoghue, A. Guttman, P.M. Rudd, Anal. Chem. 82 (2010) 10208.
[17] T. Manabe, A. Iwasaki, H. Miyamoto, Electrophoresis 18 (1997) 1159.
[18] T. Manabe, H. Oota, J. Mukai, Electrophoresis 19 (1998) 2308.
[19] M.R.N. Monton, T. Soga, J. Chromatogr. A 1168 (2007) 237.
[20] E.J. Maxwell, D.D.Y. Chen, Anal. Chim. Acta 627 (2008) 25.
[21] R. Haselberg, G.J. De Jong, G.W. Somsen, Electrophoresis 32 (2011) 66.
[22] C.W. Klampfl, Electrophoresis 30 (2009) S83.
[23] P. Schmitt-Kopplin, M. Englmann, Electrophoresis 26 (2005) 1209.
[24] P. Pantůčková, P. Gebauer, P. Boček, L. Kilvánková, Electrophoresis 30 (2009) 203.
[25] M. Himmelsbach, M. Haunschmidt, W. Buchberger, C.W. Klampfl, J. Chromatogr. A 1159 (2007) 58.
[26] M. Ryvolová, M. Macka, D. Brabazon, J. Preisler, Trends Anal. Chem. 29 (2010) 339.
[27] V.M. Tulchinsky, D.E.S. Angelo, Field Anal. Chem. Technol. 2 (1998) 281.
[28] A.M. Skelley, J.R. Scherer, A.D. Aubrey, W.H. Grover, R.H.C. Ivester, P. Ehrenfreund, F.J. Grunthaner, J.L. Bada, R.A. Mathies, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 1041.
[29] G.V. Kaigala, M. Bercovici, M. Behnam, D. Elliott, J.G. Santiago, C.J. Backhouse, Lab Chip 10 (2010) 2242.
[30] I.K. Kiplagat, P. Kubáň, P. Pelcová, V. Kubáň, J. Chromatogr. A 1217 (2010) 5116.
[31] J.M. Büscher, D. Czernik, J.C. Ewald, U. Sauer, N. Zamboni, Anal. Chem. 81 (2009) 2135.
[32] E. Rojas, M.C. Lopez, M. Valverde, J. Chromatogr. B 722 (1999) 225.
[33] L.A. Woods, T.P. Roddy, A.G. Ewing, Electrophoresis 25 (2004) 1181.
[34] W.H. Huang, F. Ai, Z.L. Wang, J.K. Cheng, J. Chromatogr. B 866 (2008) 104.
[35] L.A. Woods, P.U. Gandhi, A.G. Ewing, Anal. Chem. 77 (2005) 1819.
[36] V. Amirkhanian, M. Lui, A. Guttman, E. Szantai, Am. Lab. 38 (2006) 26.

[37] R. Anantharajah, D. Rubinger, J. Wang, J. Manual, A.C. Siegel, G.A. Levy, in Application Note – Industrial BioDevelopment Laboratory (www.ibdl.ca), 2011.
[38] M. LeLe, S.K. LeLe, J.R. Petersen, A. Mohammad, in: J.R. Petersen, A. Mohammad (Eds.), Clinical and Forensic Applications of Capillary Electrophoresis, Humana Press, New Jersey, 2001.
[39] J.P. Chervet, M. Ursem, J.P. Salzmann, Anal. Chem. 68 (1996) 1507.
[40] I. Recio, E. Molina, M. Ramos, M. De Frutos, Electrophoresis 16 (1995) 654.
[41] Y. Izumi, A. Okazawa, T. Bamba, A. Kobayashi, E. Fukusaki, Anal. Chim. Acta 648 (2009) 215.
[42] L. Ge, J.W.H. Yong, S.N. Tan, E.S. Ong, Electrophoresis 27 (2006) 2171.
[43] W. Ahrer, E. Scherwenk, W. Buchberger, J. Chromatogr. A 910 (2001) 69.
[44] C. Johns, R.A. Shellie, O.G. Potter, J.W. O'Reilly, J.P. Hutchinson, R.M. Guijt, M.C. Breadmore, E.F. Hilder, G.W. Dicinoski, P.R. Haddad, J. Chromatogr. A 1182 (2008) 205.
[45] J.P. Hutchinson, C. Johns, M.C. Breadmore, E.F. Hilder, R.M. Guijt, C. Lennard, G. Dicinoski, P.R. Haddad, Electrophoresis 29 (2008) 4593.
[46] J.P. Hutchinson, C.J. Evenhuis, C. Johns, A.A. Kazarian, M.C. Breadmore, M. Macka, E.F. Hilder, R.M. Guijt, G.W. Dicinoski, P.R. Haddad, Anal. Chem. 79 (2007) 7005.
[47] M.C. Breadmore, Electrophoresis 28 (2007) 254.
[48] M.C. Breadmore, M. Dawod, J.P. Quirino, Electrophoresis 32 (2011) 127.
[49] M.C. Breadmore, J.R.E. Thabano, M. Dawod, A.A. Kazarian, J.P. Quirino, R.M. Guijt, Electrophoresis 30 (2009) 230.
[50] A.T. Aranas, A.M. Guidote Jr., J.P. Quirino, Anal. Bioanal. Chem. 394 (2009) 175.
[51] S.L. Simpson Jr., J.P. Quirino, S. Terabe, J. Chromatogr. A 1184 (2008) 504.
[52] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, J. Chromatogr. A 1189 (2008) 278.
[53] A. Macià, F. Borrull, C. Aguilar, M. Calull, Electrophoresis 24 (2003) 2779.
[54] A. Macià, F. Borrull, M. Calull, C. Aguilar, J. Chromatogr. A 1117 (2006) 234.
[55] A. Macià, F. Borrull, M. Calull, F. Benavente, E. Hernández, V. Sanz-Nebot, J. Barbosa, C. Aguilar, J. Sep. Sci. 31 (2008) 872.
[56] I. Maijó, F. Borrull, C. Aguilar, M. Calull, Chromatographia 73 (2011) 83.
[57] M. Dawod, M.C. Breadmore, R.M. Guijt, P.R. Haddad, J. Chromatogr. A 1216 (2009) 3380.
[58] M.C. Breadmore, J.P. Quirino, Anal. Chem. 80 (2008) 6373.
[59] Z. Xu, K. Nakamura, A.R. Timerbaev, T. Hirokawa, Anal. Chem. 83 (2011) 398.
[60] Z. Xu, K. Kawahito, X. Ye, A.R. Timerbaev, T. Hirokawa, Electrophoresis 32 (2011) 1195.
[61] K.A. Mahabadi, I. Rodriguez, C.Y. Lim, D.K. Maurya, P.C. Hauser, N.F. De Rooij, Electrophoresis 31 (2010) 1063.
[62] P. Kubáň, P.C. Hauser, Electrophoresis 32 (2011) 30.
[63] C. Yao, R. Gao, C. Yan, J. Sep. Sci. 26 (2003) 37.
[64] X. Huang, M.J. Gordon, R.N. Zare, Anal. Chem. 60 (1988) 375.
[65] S. Qi, A. Huang, Y. Sun, Anal. Chem. 68 (1996) 1342.
[66] J.N. Van Der Moolen, H.F.M. Boelens, H. Poppe, H.C. Smit, J. Chromatogr. A 744 (1996) 103.
[67] M.J. Van Der Schans, J.K. Allen, B.J. Wanders, A. Guttman, J. Chromatogr. A 680 (1994) 511.
[68] C.A. Lucy, A.M. MacDonald, M.D. Gulcev, J. Chromatogr. A 1184 (2008) 81.
[69] C. Huhn, R. Ramautar, M. Wuhrer, G.W. Somsen, Anal. Bioanal. Chem. 396 (2010) 297.
[70] M.D. Gulcev, T.M. McGinitie, M.F. Bahnasy, C.A. Lucy, Analyst 135 (2010) 2688.
[71] A. Puerta, J. Axén, L. Söderberg, J. Bergquist, J. Chromatogr. B 838 (2006) 113.
[72] P. Schmitt-Kopplin, K. Fischer, D. Freitag, A. Kettrup, J. Chromatogr. A 807 (1998) 89.
[73] P. Schmitt-Kopplin, A.V. Garmash, A.V. Kudryavtsev, F. Menzinger, I.V. Perminova, N. Hertkorn, D. Freitag, V.S. Petrosyan, A. Kettrup, Electrophoresis 22 (2001) 77.
[74] R. Theurillat, M. Kuhn, W. Thormann, J. Chromatogr. A 979 (2002) 353.
[75] C. Weykamp, W.G. John, A. Mosca, T. Hoshino, R. Little, J.O. Jeppsson, I. Goodall, K. Miedema, G. Myers, H. Reinauer, D.B. Sacks, R. Slingerland, C. Siebelder, Clin. Chem. 54 (2008) 240.
[76] N.J. Panaro, P.K. Yuen, T. Sakazume, P. Fortina, L.J. Kricka, P. Wilding, Clin. Chem. 46 (2000) 1851.
[77] L. Bousse, S. Mouradian, A. Minalla, H. Yee, K. Williams, R. Dubrow, Anal. Chem. 73 (2001) 1207.
[78] A. Floris, S. Staal, S. Lenk, E. Staijen, D. Kohlheyer, J. Eijkel, A. Van Den Berg, Lab Chip 10 (2010).