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Review

Capillary electrophoresis–inductively coupled plasma-mass spectrometry: an attractive complementary technique for elemental speciation analysis

Sasi S. Kannamkumarath, Katarzyna Wrobel[1], Kazimierz Wrobel[1], Clayton B'Hymer, Joseph A. Caruso*

Department of Chemistry, Mail location 0172, University of Cincinnati, Cincinnati, OH 45221-0172, USA

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Abstract

Some basic and practical aspects of interfacing capillary electrophoresis to inductively coupled plasma-mass spectrometry (CE–ICP-MS) are reviewed in this article with emphasis on the use of this hyphenated technique for elemental speciation analysis. The principles behind the techniques of both CE and ICP-MS are introduced. The interfacing of CE to ICP-MS is discussed including several devices and nebulizers reported in literature. A brief account of their advantages and limitations is given. The various CE–ICP-MS applications for elemental speciation analysis are also reviewed. Some issues concerning the future of CE–ICP-MS for the elemental speciation analyses are discussed.
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Keywords: Reviews; Elemental speciation analysis; Capillary electrophoresis; Nebulizers; Interfaces; Inductively coupled plasma mass spectrometry

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*Corresponding author. Tel.: +1-513-556-5858; fax: +1-513-556-0142.
E-mail address: joseph.caruso@uc.edu (J.A. Caruso).

[1]At the University of Cincinnati while on the leave from Instituto de Investigaciones Cientificas, Universidad de Guanajuato, L. de Retana N° 5, 36000 Guanajuato, Mexico.

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1. Introduction

Environmental pollutants are a significant hazard to human health; among them trace elements have both beneficial and harmful effects depending on their quantities and chemical species in living organisms. In addition to the common elements (sodium, calcium, magnesium, etc.), a number of trace elements (selenium, zinc, molybdenum, manganese, etc.) are considered essential with specific biological functions at relatively low levels. However, when present in excess, these elements can be harmful. The elements present in biological and environmental materials are distributed among various chemical forms which include inorganic salts, organic and bio-metallic or bio-metalloid compounds. Determination of total elemental concentration is of limited value, as this information cannot be directly related to the chemical, biological and toxicological activity of a particular element. Oxidation state and/or chemical form of trace elements determine their toxicological effects or nutritional benefits.

Analytical elemental speciation has been defined as the separation, identification and quantification of the different chemical forms or species of a particular element in a given sample. Information on elemental speciation in environmental and clinical material is vital in studies on possible mechanisms of element transport and/or degradation in the environment, element bioavailability and on possible metabolic pathways within living organisms. The growing interest in elemental speciation is reflected in the number of recent publications in the literature, and it can be easily justified by a strong demand for analytical tools adequate for determination of elemental speciation in various fields, including environmental and life sciences. In the development of any analytical procedure for elemental speciation, it is necessary to consider: (a) the species/form to be detected; (b) the need to preserve the natural composition and distribution of species in the sample during the procedure; and finally, (c) the selectivity and detection capability of the analytical technique for quantification. Since usually more than one elemental species exists in a sample, a separation step is necessary. Separation is most often achieved by the use of modern chromatographic and electrophoretic techniques, because they offer efficient separation of the analytes.

Once separation is accomplished, the concentration of trace elemental species in the column effluent or in the fractions collected is often too low for response sensitivity of common chromatographic detectors. In the applications of liquid chromatography (LC) with UV–Vis or fluorimetric detection, this problem can be overcome by pre- or post-column derivatization of the analytes to form highly absorbing or fluorescent analytes. Other sensitive and selective detectors, such as ion selective electrodes or electrochemical detectors, are rarely used because of their lack of ruggedness and changes in sensitivity over time. The most recent additions for elemental speciation analysis are element specific detectors, specifically the inductively coupled plasma-mass spectrometer (ICP-MS) and the electrospray (ES) or the ion spray (IS) mass spectrometer. The use of atomic mass spectrometry with plasma ion source assures extremely low detection limits for the majority of the elements, has the advantage of linearity over a wide dynamic range, multi-elemental detection capability and the ability to perform isotopic analysis [1,2] with minimum matrix effects when compared to other detection systems. For the detection, identification and/or confirmation of species, molecular mass spectrometry techniques have been successfully applied. Many studies have been done coupling high-performance liquid chromatography (HPLC) [3–6], gas chromatography (GC) [7–9], supercritical fluid chromatography (SFC) [10–12] as well as capillary electrophoresis (CE) with ICP-MS detection. Recently, a series of review articles on hyphenated ICP-MS techniques has been published

[13–18]. Among the separation techniques previously mentioned, capillary electrophoresis (CE) offers a number of important attributes including: low sample-volume requirements, high plate number (peak efficiency), the ability to separate positive, neutral and negatively charged species in a single run, and, generally, short run times. This combination of the advantages of CE, coupled with the high sensitivity of ICP-MS detection, provides a valuable technique for elemental speciation. This review explains various aspects of coupling capillary electrophoretic systems to the ICP-MS detector. Emphasis in this review has been to give the reader a more practical view and points to focus on when interfacing CE to the MS detector.

2. Capillary electrophoresis

The advantage of conducting electrophoresis in capillaries was highlighted in the early 1980s by the work of Jorgenson and Lukacs [19–22]. Performing electrophoretic separations in capillaries allowed the possibility of automated analytical equipment, fast analysis times and on-line detection of the separated peaks. Typically, a capillary column has an internal diameter of 20–100 μm, an external diameter of 200–400 μm and a length of 20–100 cm. The high surface-to-volume ratios of capillaries with these dimensions allow for very efficient dissipation of joule heat generated by the applied field (typical voltage gradients range from 10 to 30 kV). Over the recent period, capillary electrophoresis has been extensively used for the separation and analysis of inorganic substances [23]. Furthermore, CE has the features of analyzing different sample types, small sample sizes, high resolution for cations, anions, small metal ions, metal–organic ligand complexes, organometallic molecules and biomacromolecules. Also, this makes CE particularly attractive for elemental speciation [24]. Because the CE separation is based on the mobility of analytes in an electric field, rather than a chemical interaction and partitioning between a stationary phase and a mobile phase, the advantage of this technique is that the natural distribution of the elemental species might be disturbed less severely when compared to the chromatographic methods [25]. It has to be stressed however, that in many CE applications, the natural elemental speciation in the sample can be altered due to the use of complexing electrolytes or pH conditions required for efficient separation [26,27]. The possible effect of sample composition (mainly the sample conductivity) is the second factor limiting CE capabilities for species separation [28].

The separation mechanism in CE is based on the charge as well as the size of the analytes, which move in electrical field applied across a capillary filled with a suitable electrolyte. The migration of ions in the capillary is controlled by two phenomena, the electrophoretic mobility and the electroosmotic flow (EOF) [29]. The electrophoretic mobility is due to the attraction between the ions and the oppositely charged electrode and is directly related to the charge to size ratio of the analyte. That is, highly charged smaller particles will migrate faster than less charged bulky particles. The electroosmotic flow arises from interactions between electrolyte ions and the negatively charged capillary wall rich in the ionizable silanol groups. To maintain electrical neutrality, an electrical double layer is formed on the surface of the capillary wall and, under the applied electric field, the hydrated cations in the Helmhotz layer move toward the more negative potential end of capillary. This produces a bulk solution flow, which depends on the voltage gradient applied across the capillary, type of electrolyte, pH, and the properties of capillary wall (density of silanol groups, coating, etc.). The important difference between the two migration forces is that the electroosmotic flow affects the migration of each analyte in the same way, so it does not cause any separation. However, by controlling the EOF and the voltage gradient applied, the combined effects of the two forces can improve the resolution of analytes and often makes possible the separation of the cationic, anionic and neutral species in a short time and in a single run. Moreover, the flat flow profile characteristic of electroosmotic flow gives better resolution of peaks and higher plate number (peak efficiency) unlike HPLC, which is affected by the parabolic flow profile characteristic of laminar flow.

Although the high plate number and separation versatility makes CE an attractive technique for elemental speciation purposes, the small capillary diameter seriously limits the possibility for the most

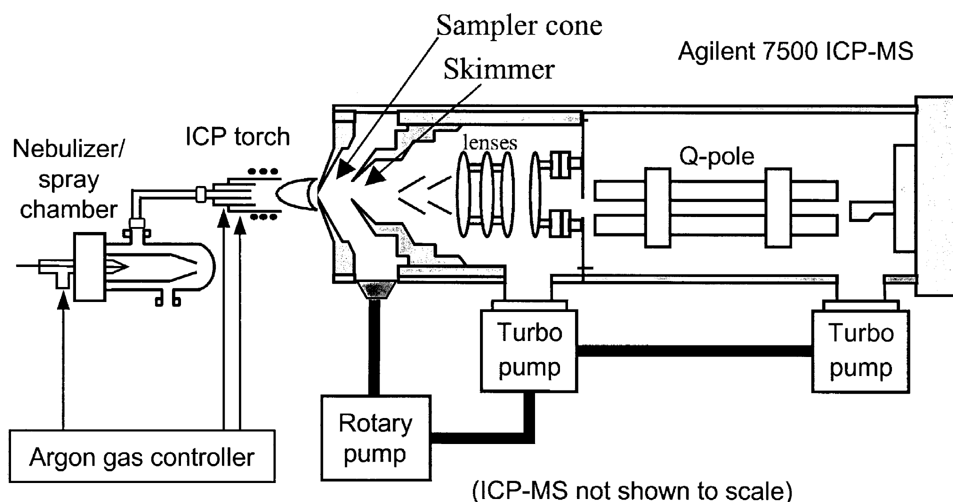

Fig. 1. Schematic of an ICP-MS.

sensitive detection when expressed as concentration. One approach for improving the detection limits is to increase loading of the CE system. This can be achieved by pre-separation clean-up or preconcentration procedures, by electrokinetic sample introduction, by applying electrostacking effect or isotachophoresis [23,25]. The other, complementary approach is the application of a sensitive and specific detection system [30]. The great majority of the commercial instruments are equipped with UV detectors. Because most of the metal ions and metallic or metalloid containing species do not absorb in UV region, pre- or on-column derivatization is commonly carried out to introduce a suitable chromophore. Another, widely used approach is indirect UV detection. In this case, a highly absorbing species is added to the electrolyte and the analytical signal is measured as the absorbance decay due to the elution of non-absorbing species (analytes) [31,32]. As reviewed recently, the two detection modes were used in variety of speciation studies at the concentration levels in the range 0.1–100 mg/l, but the limited applicability in ‘‘real world’’ elemental speciation has been highlighted [33]. The fluorescence and electrochemical detectors were also applied in metal speciation by CE, often offering higher detector sensitivity when compared to UV, but also presenting some interference problems [30,34]. The Barnes [35]and Olesik [36] research groups first described the technique of coupling CE to ICP-MS for rapid elemental speciation and several other research groups have since followed developing different interface devices. Detection limits have been lowered to the low μg/l range. The hyphenated CE-ICP-MS technique, despite being considered in an early stage of development, is an interesting alternative for elemental speciation studies in real samples at trace and ultra trace levels.

3. Inductively coupled plasma-mass spectrometry

In this technique, the high efficiency of atomization and ion formation of the inductively coupled plasma is complemented by the specific and sensitive multi-element detection capability offered by atomic mass spectrometry. The scheme of a commercial ICP-MS instrument is shown in Fig. 1. The ICP is an ion source, which operates at high temperatures (5000–10000 K) and at atmospheric pressure. The argon plasma is generated in a quartz torch [37] under the conditions of a radio frequency electromagnetic field (27–40 MHz, a power of 600–1800 W) [38]. The argon gas stream, which flows through the inner tube of the torch, is initially seeded with free electrons from a discharge coil. An eddy current of cations and electrons is formed as the charged

particles are forced to flow in a closed annular path. These fast moving ions and electrons collide with other argon atoms to produce further ionization, which leads to high thermal energy as they meet resistance to their flow. The second stream of argon gas, which passes through the outer tubes of the torch, helps not only in cooling the torch and preventing it from melting, but also provides a tangential flow of gas, which serves to center and stabilize the plasma [39].

A liquid sample is introduced into the plasma. This liquid sample, which in elemental speciation studies usually is either an HPLC column or CE capillary effluent, is introduced as an aerosol through the center tube of the torch into plasma by means of a nebulizer connected to a spray chamber. This spray chamber separates and removes the larger droplets of the aerosol. The ions produced in the plasma enter the mass detector through the sampler and skimmer cones and then they are focused (through a series of lenses) into the mass analyzer, where they are separated based on their mass to charge ratio and then are detected [37,40]. With most ICP-MS commercial instruments, the response for the majority of elements is linear over 4–11 [41] orders of magnitude with excellent precision in count rates near the signal baseline (0.2–3% RSD) [41]. Usually, the chemical interferences in ICP-MS are less pronounced than in other techniques such as ICP-AES [41]. Moreover several approaches for overcoming these interferences are found in the literature. These include the use of high-resolution mass spectrometers [42–44], cryogenic desolvation [45], different plasma sources or cooled plasmas (plasma generated with low power) and more recently a shield torch. Recently, a new approach called collision/reaction cell technology has been developed which reduces the problems associated with polyatomic species before they enter the mass analyzer [46].

Although the application of ICP-MS as a detection system for CE seems to offer obvious advantages of low detection limits and interference-free, element-specific response, the instrumental design of such a technique is not straightforward. Several design issues need to be addressed including the small sample volumes introduced by CE (typically in the range of nanoliters) with very low liquid flow-rates (around 1 μl/ml) and the problems in transferring efficiently the capillary effluent to the nebulizer. Furthermore, the nebulizer/spray chamber introduces dead volume (extra-capillary volume) to the detector system which can lead to additional peak broadening.

4. Interfacing capillary electrophoresis to inductively coupled plasma-mass spectrometry

As previously mentioned, the challenge for the successful application of the hybrid CE–ICP-MS technique is the development of a suitable interface. The main factors that need to receive special attention in coupling these techniques are: (1) maintaining effective electrical contact at the outlet end of the CE capillary; (2) countering or minimizing laminar flow generated from the suction-effect of the operating nebulizer [47]; (3) minimizing band broadening; and (4) obtaining high transport efficiency. In typical instrumental design, the outlet end of the capillary from CE is introduced into the central tube of a nebulizer while maintaining an electrical connection to the end of the capillary. Many of the reported CE–ICP-MS interfaces have a tee union, through which the buffer is introduced to make-up the capillary flow-rate (μl/min range) that is too low for the normal uptake rate of the nebulizer. A typical tee union interface is shown in Fig. 2. The outlet of the CE capillary is taken out of the system and threaded through the collinear ends of the cross and it is tightly sealed with the ferrules. The other arms of the tee are connected to a tube for the make-up/sheath solution flow and to a platinum electrode. An alligator clip is used to connect the platinum electrode at the tee interface to the power supply, thus providing a complete electrical circuit by conduction through the make-up buffer to the tip of the CE capillary [48]. The other important parameter is the generation of an aerosol with high transport efficiency so that a large quantity of the sample will reach the plasma. Low flow nebulization systems are, therefore, essential for the CE–ICP-MS interface.

4.1. Practical considerations of interfacing

The CE–ICP-MS interface based on the sheath flow and concentric nebulizers described by Lu et al.

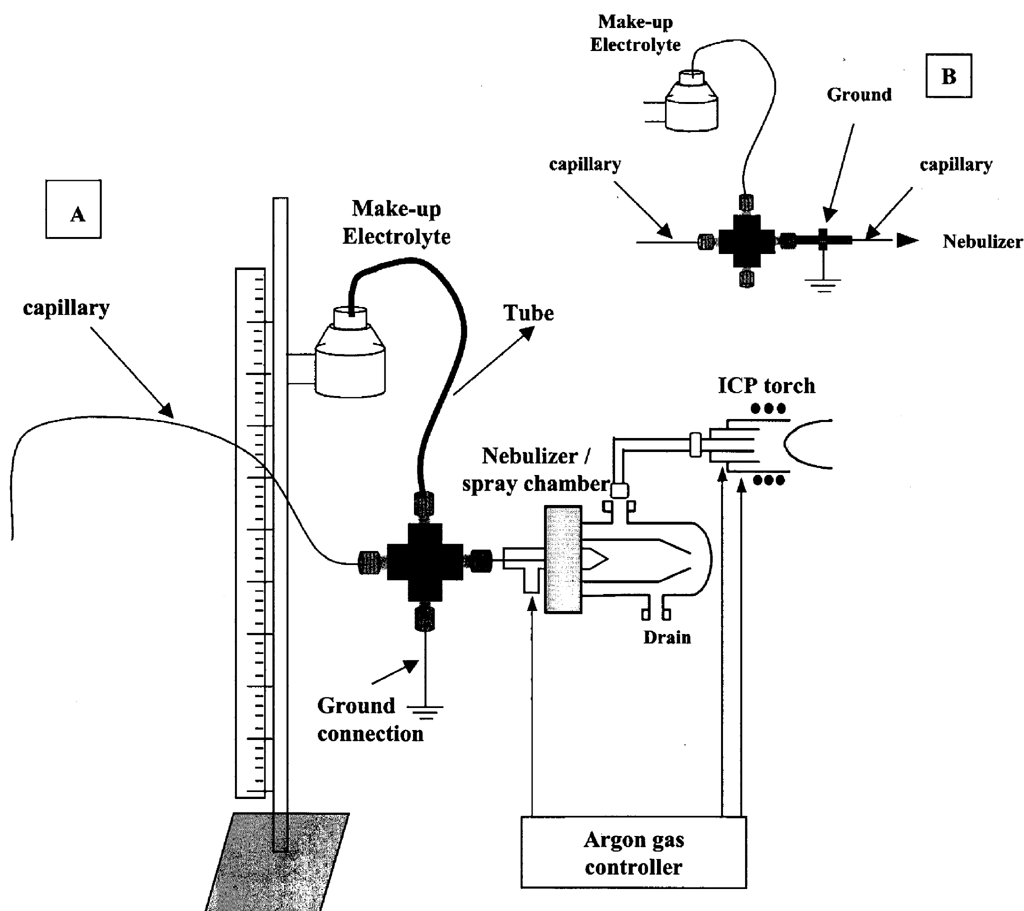

Fig. 2. CE interface to ICP-MS.

[35] is the most widely used interface at present for elemental mass spectrometry and has also been used previously for the electrospray MS [49]. The sheath flow makes an electrical connection to the outlet end of CE capillary, grounding being solved by placing a metal tube at the connector to the nebulizer (Fig. 2) or by coating the capillary with silver [36]. The second function of sheath flow is the compensation for the suction effect, which is caused by the low pressure created at the tip of the nebulizer due to the gas flow. This suction induces a laminar flow inside the capillary and can impair the separation profile of analytes. However, the required sheath flow-rate can be as high as 50 μl/min or more, which in-turn can decrease the sample transport efficiency to the plasma [25]. Sheath flow can be introduced into the nebulizers either through self-aspiration or a peristaltic pump, the latter presenting the use of flow control. In self-aspiration, the uptake is automatic, and there will be a variation in the uptake rates based upon run or make-up buffer liquid viscosities. By the use of a peristaltic pump to provide the sheath flow, the optimization of the flow-rate is necessary. A low flow-rate would not compensate for the suction effect, while an excessive flow would cause a backpressure to the CE capillary and thus affect the separation of analytes. The use of controlled make-up/sheath flow-rates to give equivalent CE–MS and CE–UV eletropherograms has been reported by Day et al. [50]. Some studies have also shown that the sheath flow should be kept as low as possible to minimize dead volume and to reduce band broaden-

ing as well as to minimize the loss of sensitivity [47]. In addition to the sheath flow-rate, the geometric position of the CE capillary inside the nebulizer is very important; one case in point being microconcentric nebulizers, where the center tube of the nebulizer is more narrow than standard nebulizers. If the CE capillary is too far inside the nebulizer, greater laminar flow can be created from suction at the tip of the nebulizer. If the CE capillary is placed too far back from the nebulizer's tip, the resolution of analytes can be impaired from an increase in the effective liquid dead volume (the extra-capillary volume). The position of the CE capillary within the nebulizer is, thus, another important parameter for adjustment which can affect band broadening and control laminar flow within the CE capillary. The choice of the make-up buffer also plays an important role. If the make-up buffer is different from the run buffer, then a change in the entire separation conditions can occur. Creation of a pH gradient across the CE capillary or isoelectric focusing from the use of a dissimilar make-up buffer may cause undesirable results. Another issue, to be considered, is run buffer depletion; it is best to replace the buffer solution after a few runs, which is normally a good general practice with CE.

The elimination of a make-up or sheath flow has been reported by several research groups. Olesik et al. [36] described a sheathless interface, in which a negative pressure was introduced at the CE capillary inlet and compensated against laminar flow through the capillary. The limiting feature of this design was in the difficulty to keep the nebulizer end wet, thus assuring electrical connection to the CE capillary. The important advantage of this system was that it effectively counter-balanced the suction generated by the nebulizer's operation without any apparent loss in sensitivity of the CE system [51]. Recently, Deng and Chan [52] reported a modified concentric glass nebulizer used as an interface of CE to ICP-MS, where the sheath flow also was eliminated. Finally, the commercial CEI-100 capillary electrophoresis interface for ICP-MS from CETAC has to be noted [53]. It was constructed to eliminate secondary laminar flow within a capillary, to reduce dead volume and to minimize dilution factor. In this interface, the make-up buffer flow-rate operates below 10 μl/min.

A significant advantage of CE–ICP-MS over HPLC–ICP-MS is that the chemical composition used for the separation step is usually more compatible with the inductively coupled plasma. Sometimes in the applications of HPLC, conditions are compromised for plasma/detector compatibility rather than optimized for separation. The concentration of salts and/or organic modifiers must be kept relatively low to maintain plasma stability and minimize signal drift of the mass spectrometer. However, owing to low solution loading with the CE effluent, its chemical composition can minimally affect the nebulization process, plasma stability or MS signal drift. Thus, the optimal separation conditions usually can be used when coupling CE to ICP-MS unlike HPLC to ICP-MS. The influence of CE experimental parameters on migration time, band dispersion and resolution was recently reviewed by Olesik [25]. With the interfaces using sheath flow, the make-up solution becomes the effective matrix for ICP-MS, which is constant over time and from sample to sample. The composition of the sheath flow solution should be selected carefully and with consideration of not disturbing the nebulization process or the plasma stability.

There is a potential loss of sample in CE capillaries, by either chemical adsorption on the walls of the capillary or the analyte's capture in the electrical double layer near the walls of the capillary. This may reduce the sensitivity, as well as increase the background signal due to continuous elution of the trapped ions over time. Proper washing of the capillary before an injection is necessary and can easily be facilitated when interfaced to an ICP-MS by hand using a Luer lock syringe adapted to the CE capillary [48]. Also, care must be taken to see that the heights of both ends of the capillary are equal during the run to prevent siphoning effects in the capillary which would create induced laminar flow.

In ICP-MS, the detection is based on the mass of the analytes rather than on their concentration in the solution. Since CE injection volume is in the nanoliter range and capillary flow is usually complemented by a sheath flow, high transport efficiency is required to reduce the loss of analyte from the aerosol introduced into the plasma. High transport efficiency is an important characteristic of low liquid flow high efficiency nebulizers that will briefly be discussed in the next section.

4.2. Nebulizers

Nebulization is a process to form an aerosol, that is, to convert a liquid sample into a cloud of droplets. A suspension of liquid in air or a gas, an aerosol, can be introduced to flame or plasma with a lower risk of disturbing its stability. High plasma stability leads to stable and constant vaporization, atomization, excitation and ionization processes. There are numerous nebulizers available commercially for the introduction of liquid sample to ICP-MS systems and their detailed description can be found elsewhere [54]. In brief, pneumatic (concentric and cross-flow) and ultrasonic nebulizers have been used in different studies and Fig. 3 shows some typical designs. In the concentric pneumatic nebulizer, both the gas stream and the liquid flow in the same direction as shown in the Fig. 3A, while in the cross flow nebulizer the two streams are at right angles to each other (Fig. 3B). When the gas travels at a high flow-rate, it creates a drop in pressure near the tip, which draws the liquid through the inner tube and the sample emerges as an aerosol. A general scheme of ultrasonic nebulizer is shown in Fig. 3D; a thin film of the solution flows over the surface of a piezoelectric transducer and is converted to the aerosol by the high frequency vibrations from the transducer. The typical solution intake rates for a conventional concentric nebulizer are 1–2 ml/min with 1–2% transport efficiency [55], while in the ultrasonic nebulizers the transport efficiency is usually higher (10–30% range). This important parameter depends on the mean droplet size (known as Sauter mean droplet diameter) of the aerosol as well as on the droplet size distribution. Much of the sample in aerosol is lost by condensation in the spray chamber and only small droplets enter the plasma. On the other hand, the particle size of the aerosol has a very high impact on the ionization capabilities of the plasma and indirectly affects the signal detection. The "quality" of the aerosol thus depends on the nebulizer efficiency. Montasar et al. [56] give a detailed picture on droplet size characterization, droplet size distribution, aerosol droplet distribution, typical droplet sizes with various nebulizers and gases and modeling of nebulizer systems. With the use of conventional nebulizers, the transport efficiency can be increased (up to 20%) by lowering the sample flow-rate (below 0.1

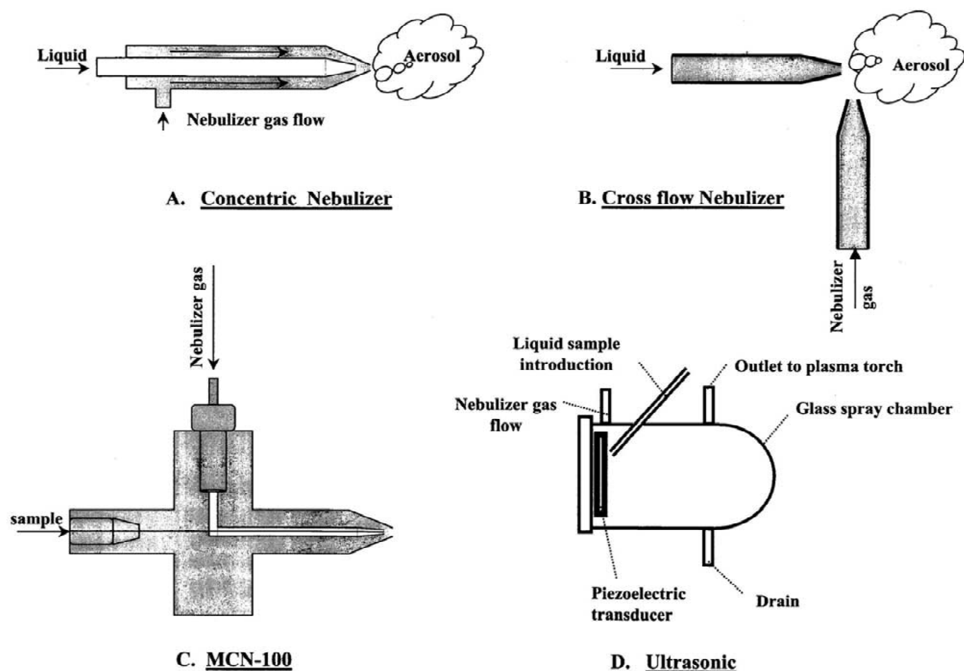

Fig. 3. Different types of nebulizers.

ml/min) [57], but this involves poorer precision of the instrumental response. This led to the development of new nebulizers that operate at low flow-rates and provide increased transport efficiency as well as an improvement in the limits of detection [55]. These low flow devices are the nebulizers of choice when coupling CE or micro bore HPLC separations to ICP-MS. In Table 1, different nebulizers are listed and compared in terms of flow-rate, approximate transport efficiency and applicability. Three commercially available, low flow microconcentric nebulizers are the microconcentric nebulizer (MCN) from CETAC (Fig. 3C), the Meinhard® high efficiency nebulizer (HEN) [47,58] and the MicroMist nebulizer [59]. Also, the DIHEN [60] from Meinhard is a fourth choice, and it has the design advantage of minimizing dead volume.

A number of laboratory-made microconcentric nebulizers have been developed and evaluated for possible applications in coupling HPLC or CE to ICP-MS [58,61–66]. Houk et al. [67] described the direct injection nebulizer (DIN), in which the spray chamber was eliminated and, at the flow-rates 30–120 μl/min, the transport efficiency was close to 100% [68,69]. The oscillating capillary nebulizer (OCN), was used for HPLC coupling to ICP-MS by Browner et al. [70]. The OCN was also adapted by B'Hymer et al. [71], but the detection limits were not as good when compared to the performance of the HEN or a standard concentric nebulizer in an

Table 1
Comparison of nebulizers used in CE–ICP-MS applications

Nebulizer	Gas flow-rate (l/min)	Sample uptake	Transport efficiency	Comments	References
Operating at conventional flow-rates					
Conventional concentric	normally 1	0.5–4 ml/min	1–2%	Worst short term stability (%RSD) at low solution uptake (<100 μl/min)	[35,36,101]
Cross flow	0.5–1.0	1–3 ml/min	1–4%	Less prone to salt build up	[112]
Ultrasonic	0.5–1.5	0.5–2.5 ml/min	20% with 1 ml/min, almost 100% with low flow	Better transport efficiency when spray chamber is heated or if the solution uptake is reduced	[73,77]
Operating at low sample flow					
HEN	1	10–1200 μl/min	10–20% (50–60% at 11 μl/min)	Not suitable for high solute concentrations	[47,58]
DIN	1	100 μl/min	close to 100%	No spray chamber, good efficiency for volatile analytes and organic solvents	[68,69]
DIHEN	0.25	85 μl/min	close to 100%	Good precision, risk of high oxide ratios	[112]
OCN	1	1–2000 μl/min	close to 100% at lowest flow-rates	Undisturbed by the composition of the solvent, the use of sol–gel frits to minimize suction effects [65]	[65,71,72]
MCN	1	10–50 μl/min	close to 50%	HF resistant, offers desolvation	[50,51,75,79]
Modified, laboratory made	0.58	5.6 μl/min		The uncertainty in the position of the capillary in nebulizer did not affect significantly the nebulization efficiency	[61]
micro concentric	0.8	1–15 μl/min		I.D. = 200 μm of the gas introduction capillary assured best sensitivity, internal standard recommended for quantitative analysis	[62]
	0.9–1.1	50 or 100 μl/min		A cyclone spray chamber (v = 4 ml), Critical parameter: relative position of the concentric tubes	[63]
	0.6–1.2	6 μl/min		Self-aspiration mode, fluid mechanical properties optimized	[64]
		Coaxial sheath flow 4 μl/min		Possibility of an exact and optimized positioning of the capillary end	[66]

interface experiment with small bore HPLC to ICP-MS. Kirlew and Caruso [72] used a modified oscillating capillary nebulizer (MOCN) for a CE–ICP-MS interface. The ultrasonic nebulizer has also been used and was able to avoid the suction effect usually generated by a pneumatic nebulizer. Increased sensitivity was observed using the USN, however, there was also an effective increase in the noise levels, hindering the improvement of S/N ratio [73]. The use of sol–gel frits to minimize the suction effect through the CE capillary while using a pneumatic nebulizer has also been studied [65]

4.3. Interfaces

Successful coupling of CE to ICP-MS requires both high transport efficiency and the establishment of electrical connection between the CE capillary outlet and the nebulizer. To fulfill these two requirements, researchers usually construct the interfaces. Tangen et al. [62] observed problems with the grounding of the CE capillary at the tip of DIN when coupling CE to ICP-MS, due to interference of the high voltage applied across the capillary with the RF power supply of the ICP-MS. An interesting design for the electrical contact was reported by Deng and Chen [52]; they used a platinum electrode (thin wire of 50 μm diameter) wrapped across the opening of the outlet end of CE capillary instead of inserting it into the capillary [61]. In the interface described by Prange and Schaumloffel [74], an MCN from CETAC was used in the self-aspiration mode with dilute nitric acid used as the make-up sheath solution (flow-rate approximately 6 μl/min). The suction effect was minimized by optimizing the fluid mechanical properties of the interface. The interface proposed by Mei et al. [75] was a Y-shaped quartz connector with tapered arms, which was compatible with various types of pneumatic concentric nebulizer. In other studies, several modifications of the nebulizer and spray chamber were designed in order to minimize the dead volume as well as to remove large droplets from the aerosol [36,51,52,63,76]. Most of the modified spray chambers were of conical or cyclonic shape with the volume varying from 20 to 120 ml. Kirlew et al. [77] evaluated the feasibility of using an ultrasonic nebulizer as a CE–ICP-MS interface; they compared the laboratory built design with the commercial device and finally refined their interface to improve the gas flow pattern within the spray chamber. The importance of the interface dimensions (length, internal diameter of the transfer capillary) has also been described in detail [76].

The materials used to construct an interface also play a part in the accurate determination of elemental species. The material of CE–ICP-MS interfaces has to be chosen carefully, because the materials themselves may affect the distribution of elemental species in the capillary effluent [78]. Presently, PEEK interfaces are widely used to counter this problem [50,79]. The tip of the capillary also plays a major role in the peak shape. When cutting the CE capillaries, the ends must be cleaned and all debris removed; both ends of the CE capillary must be evenly cut and not have jagged edges.

Another interesting approach with the coupling of CE with MS detection is in the use of an electrospray ion source (ES). ES generally operates within the same flow-rate range as CE and has high transport efficiency. ES can be described as an electrophoretic-like process, in which charged droplets are formed when a high voltage is applied between a capillary containing the sample and the front plate of the mass spectrometer [28]. If a positive potential is applied to the capillary with respect to the entrance slit, the positive ions are attracted towards the capillary tip and the filament solution breaks off to form a charged droplets. Cao and Moini [80] reported an ES-type interface in which a 25 μm diameter platinum wire was introduced into the CE capillary to maintain the electrical contact. Schrammel et al. [81–83] developed a device, which allowed infinitely variable adjustment of the CE capillary inside the ES tip. Other interfaces of CE and ES have been reported using membrane or frit junctions [84]. Since ES is a soft ionization source, the direct introduction of electrospray to the mass spectrometer offers excellent possibilities for characterizing elemental species composition. One drawback of this hybrid technique is the limitation of CE using only volatile background electrolytes in relatively low concentrations (below 10^{-4} M) [85]. On the other hand, ES can be considered an aerosol source in which small droplets are produced with a relatively uniform size distribution in contrast to the larger droplet size distribution produced by pneumatic nebulization.

Electrospray MS is more common in interfaces with HPLC; Gotz et al. [86] reported the technique in use with a micro-bore HPLC separation.

Other techniques are available to side step the low transport efficiency generally encountered with nebulizers. A CE–ICP-MS interface, which used post-column hydride generation, has been shown to circumvent the transport efficiency nebulization problem [87,88]. In these two cited works, both arsenic and selenium compounds were separated by CE, and gaseous hydride species of the analytes were formed and introduced into the ICP-MS by means of a membrane gas–liquid separator. Also, a movable reduction bed hydride generation system has been designed and studied [89,90]. This system enabled the generation of hydride at micro-level sample volumes without the use of a gas–liquid separator. Only preliminary results were reported with this micro-level system, and a number of questions still have to be addressed. All hydride generation detection approaches are limited to the analysis of elements or compounds which will readily form volatile hydrides. Therefore, this approach is somewhat limited in applicability.

5. Applications in elemental speciation analysis

The interfacing of CE to ICP-MS requires continued development. There is a need for sensitive and selective detection for CE separations and the known advantages of ICP-MS seem to meet these requirements. CE to ICP-MS interfacing is difficult due to the small sample volume introduced by CE. Low flow-rates within the capillary combined with the necessity to maintain electrical contact with the outlet of the CE capillary only adds to the level of difficulty in interface design and construction. Another limitation is the possible effect of sample matrix and/or chemical conditions used in CE separation on the elemental speciation results [27]. The CE conditions may change the composition or affect the stability of the analytes during the run. Thus, there are fewer publications in the literature on CE–ICP-MS applications in contrast to other speciation applications which used either gas or high-performance liquid chromatography. The majority of studies using CE for elemental speciation have been focused on the evaluation of laboratory-made coupling devices and used elemental species standard solutions at mg/l concentration levels. The applications of CE in elemental speciation analysis to real-problem studies are still limited. The outstanding potential advantage of CE is the possibility to separate a variety of species that possess different charge and/or size within a single run. Thus, the interest in analyzing the biological and environmental materials, containing an abundance of metallic or metalloid compounds is high. A number of studies have focused on arsenic and selenium speciation, while much less work was done on chromium, cobalt, iron, iodine and other elements. These studies will be briefly discussed further in this review.

5.1. Arsenic and selenium

Arsenic and selenium speciation analysis are the two most prominent elements used in conjunction with CE separation. The growing interest in selenium speciation is due to both its toxic and essential properties that depend on total element quantity and, more importantly, its actual chemical form. In order to elucidate the specific biological function(s) of selenium, studies have been carried out in different experimental systems and several selenoamino acids have been identified and characterized [91]. Selenium incorporation into proteins was demonstrated by a specific pathway, as well as, following sulfur analog pathways. Selenium can be found in the active site of antioxidant enzymes which protect cells against the effects of free radicals that are produced during normal oxygen metabolism [92]. Thus, elemental speciation analysis of selenium in environmental and biological samples has been focused on selenium in different oxidation states (selenite is potentially more toxic than selenate), on identification and quantitation of selenoamino acids, and other species that can be generally classified as selenium metabolites. The toxicity of arsenic is also species and compound dependent. As (III) is more toxic than As (V), and the inorganic forms are several fold more toxic than the organic compounds [93]. In studies of arsenic pathways in the environment and in the living organisms, both the inorganic forms and a variety of methylated forms, such as monomethylarsonic acid (MMA), dimethylarsenic

acid (DMA), arsenocholine (AsC) or arsenobetaine (AsB) are of primary importance [94–96]. For the needed analytical task of elemental speciation, CE can be the technique of choice. Due to the large possible number of species present in a sample, and because these chemical species possess different charge and size, CE often can offer the perfect separation mechanism. In addition, minimum disturbance in species composition can be expected with these arsenic and selenium compounds. Table 2 contains much in the literature available on arsenic and selenium speciation by CE–ICP-MS and provides an excellent summary.

In the early work of Liu et al. [68], the proposed CE–ICP-MS interface based on the direct injection nebulizer was evaluated using various test solutions, including a solution of four arsenic compounds (As (III), As (V), MMA and DMA, 500 μg/l each) and a solution containing inorganic forms of selenium and arsenic (2 μg/l each). Baseline separation was achieved using an electrophoretic buffer containing a cationic surface modifier (pH 7.7) within 24 and 15 min, respectively. In another study, the experimental conditions for CE–ICP-MS analysis of Se (IV), Se (VI), As (III), As (V) and DMA were performed by Kirlew et al. [77]. Different interface designs were used, based on ultrasonic nebulization. Using optimized conditions, a separation was accomplished within 10 min; the best sensitivities were obtained using electrokinetic injection and pH 8 electrophoretic buffer. In the Kirlew study, the As (III) peak did not appear ($pK_a = 9.4$) and the migration times of As (V) and Se (IV) were very similar (around 4 min), the latter two analytes being easily resolved by the ICP-MS multielement capability. In the work by Day et al. [50], a self-aspiration microconcentric nebulizer interface for CE–ICP-MS was constructed and tested for four anionic arsenic species (As (III), As (V), MMA, DMA) and one neutral species (AsB). In order to compare the analytical performance of the hyphenated technique with the procedure based on the common UV detection under the same experimental conditions, an electrophoretic buffer containing strong UV chromophore was selected (4.4 m*M* sodium chromate, pH 8.2 with sodium hydroxide). A baseline separation of the five arsenic species was achieved in 15 min with significantly lower detection limits obtained using ICP-MS detection system (53–280 μg/l vs. 440–1900 μg/l for UV).

In other work by Day et al. [48], results of a CE–ICP-MS application to chiral speciation of selenomethionine obtained from selenized yeast after enzymatic digestion were reported. Marfey's reagent (1-fluoro-2,4-dinitrophenyl-5-L-alanine amide) was used for the derivatization of the free selenoamino acids and the separation of the derivatives was achieved (Fig. 4A and B) using a phosphate buffer (pH 3.3). A self-aspiration interface with a microconcentric nebulizer was applied for coupling CE to ICP-MS. The migration time reproducibility was comparable between ICP-MS and UV on-column detection, while the detection limit was lower using ICP-MS detection (50 vs. 250 μg/l).

A good example of the high resolution provided by CE as applied to speciation studies with mass spectrometry detection was presented by Prange and Schaumloffel [74]. By using a laboratory-constructed CE–ICP-MS interface, they obtained baseline separation of 12 compounds, including seven arsenic species (As (III), As (V), MMA, DMA, AsC, AsB and phenylarsonic acid), two inorganic selenium forms (Se (IV), Se (VI)), one antimony species (Sb (V)) and two inorganic forms of tellurium (Te (IV), Te (VI)) within a 10 min run time along with excellent peak shapes (Fig. 5). Differences in migration times was less than 3% and the detection limits were around 1 μg/l (somewhat poorer detection limits were obtained for AsB, Se (IV) and Se (VI)).

Two other active researchers in selenium speciation analysis by CE–ICP-MS are Michalke and Schramel [97]. Their chief interests are in analysis of inorganic selenium forms, selenoamino acids, as well as selenium carrying glutathione (GSSeSG) in biological samples. Using a capillary zone electrophoretic method (CZE) with alkaline background electrolyte (pH 11.5), six selenium species (selenocystamine, Se (VI), selenocystine, Se (IV), selenomethionine and GSSeSG) were separated in 10 min (Fig. 6). The evaluated detection limit for inorganic and organic species were 10–20 and 35–50 μg/l, respectively [97]. Shifts in migration times occurred with increasing the number of compounds loaded on the CE system relative to the single standard runs. The cross interferences were attributed to changes in sample conductivity and ion concentration. In a

Table 2
Applications of CE–ICP-MS in elemental speciation analysis

Element	Species analyzed	Sample matrix	Instrumentation	Capillary dimensions (Fused silica) I.D., μm ×length, cm	Electrolyte/buffer	Sheath solution applied voltage, injection type	L.O.D.	Ref.
As	As(III), As(V), DMA, MMA	Test solution	ISCO 3850 CE HP4500 ICPMS	50×75	2.3 m*M* pyromellitic acid, −6.5 m*M* NaOH −1.6 m*M* TEA −0.75 m*M* hexamethonium hydroxide (pH 7.7)	2% HNO_3, 50 μg/l Y(III), −30 kV, Hydrostatic injection	20–100 ng/l	[68]
As, Se	As(III), As(V), Se(IV), Se (VI)	Test solution	ISCO 3850 CE HP4500 ICPMS	50×75	2.3 m*M* pyromellitic acid −6.5 m*M* NaOH −1.6 m*M* TEA −0.75 m*M* hexamethonium hydroxide (pH 7.7)	2% HNO_3, 50 μg/l Y(III), −30 kV, Hydrostatic injection	80–300 ng/l	[68]
As, Se	AS(III), As(V), DMA, Se(IV), Se(VI)	Test solution	Isco 3850 CE, PlasmaQuad II Turbo (VG Elemental)	50×103	4.4 m*M* sodium tetra borate −0.49 m*M* CIA-PAK OFM Anion-BT (pH 8 with NaOH)	2.2 m*M* borate buffer −30.6 kV, Electro migration	0.2–2 μg/l	[77]
Se	Se(IV), Se (VI), Selenocystamine Selenomethionine, Selenocystine	Test solution	BioFocus 3000 CE, Elan 5000 ICPMS	50×130	10 m*M* Na_2CO_3 (pH 11.50 with KOH)	Buffer, +18 kV, Pressure injection	10–50 μg/l	[97]
Se	Se(IV), Se(VI), GSSeSG, Selenomethionine, Selenocystine, Selenocystamine	Human milk, serum	BioFocus 3000 CE, Elan 5000 ICPMS	CZE: 50×100	10 m*M* Na_2CO_3 (pH 11.50 with KOH)	10 m*M* Na_2CO_3, +18 kV, Pressure injection	10–50 μg/l	[98]
				cIEF: 50×120	100 m*M* NaOH (catholyte) 100 m*M* H_3PO_4 (anolyte)	100 m*M* H_3PO_4, −18 kV, Pressure injection	10–30 μg/l	
Se	Selenomethionine, Selenocystine, Selenocystamine	Test solution	BioFocus 3000 CE, Elan 5000 ICPMS	50×150	5% acetic acid	Buffer, +18 kV, Pressure injection	30 μg/l	[97]
Se	Se(IV), Se(VI), Selenomethionine, Selenocystine	Test solution	Waters Quanta 4000 CE, Elan 6000 ICPMS	75×75	25 m*M* HNO3 –0.5 m*M* cetyltrimethyl ammonium hydroxide (pH 9.25)	10 m*M* HNO_3, with 10% MeOH, −30 kV, Hydrostatic injection	2–10 μg/l	[69]
Se, As	Se(IV), Se(VI), As(III), As(V), MMA, DMMA	Drinking water matrix	Dionex CES-1, Hydride generation, HP-4500 ICPMS	75×85	20 m*M* potassium hydrogenphthalate– 20 m*M* boric acid (pH 9.03)	DI water or dil. HCl, −22 kV, Pressure injection	10–24 pg of Se	[88]

Table 2. Continued

Element	Species analyzed	Sample matrix	Instrumentation	Capillary dimensions (Fused silica) I.D., μm×length, cm	Electrolyte/buffer	Sheath solution applied voltage, injection type	L.O.D.	Ref.
As	As(III), As(V), MMA, DMA	Test solution	Lab built CE, Baird PS-4 ICPOES	100×66	50 m*M* phosphate buffer (pH 6.0)	Buffer, +15 kV, Hydrostatic injection	0.32–0.35 mg/l	[89]
As	As(III), As(V), MMA, DMA, AsB, AsC	Urine, sewage sludge	BioFocus 3000 CE Elan 5000 ICPMS	50×150	20 m*M* phosphate buffer (pH 5.6)	Buffer, +18 kV, Pressure injection	15–65 μg/l	[97]
As	As(V), MMA, DMA, As(III), AsB	Test solution	Waters Quanta 4000 CE, VG plasma Quad II STE	75×77	4.4 m*M* sodium chromate (pH 8.2 with NaOH), 1:50 Waters OFM anion BT	0.1 m*M* sodium borate (pH 8.2 with NaOH), −25 kV, Hydrostatic injection	53–280 μg/l	[50]
As	AsC, AsB, As(III), DMA, MMA, As(V)	Mineral water, human urine and soil leachate	HP ^{3D} CE, HP4500 ICPMS,	75×88	20 m*M* borate–2% OFM (pH 9.4)	0.14 *M* HNO_3+1% MeOH, −25 kV, Electro migration and Hydrostatic injection	1–2 μg/l	[101]
As, Sb, Se, Te	AsC, AsB, As(III), DMA, PA, MMA, As(V), Se(IV), Se(VI), Sb(V), Te(IV), Te(VI)	Test solution	HP ^{3D} CE, Element, Finnigan MAT (SFMS),	75×55	20 m*M* CAPS–40 m*M* β-cyclodextrin (pH 10), 4 μg/l Cs as marker	1% HNO_3, 1 μg/l In, 30 kV, Hydrostatic injection	3–9.5 μg/l	[74]
Cr	Cr(III), Cr(VI)	Test solution	Brandenburg alpha III 3807(High Voltage Power supply), Elan 5000 ICPMS	20×40	6.5 m*M* HIBA–5.0 m*M* UVCAT-1	+30 kV, Electro migration	10 μg/l	[62]
Cr	Hydrolytic polymerization products of Cr (III)	Test solution	Spellman CZE-1000R (High Voltage Power supply), Elan 6000 ICPMS	50×60	5 m*M* $Lacl_3$	5 m*M* $Lacl_3$ –dilute HNO_3, +30 kV, Hydrostatic injection		[102]

Hg	Hg(II), methyl- and ethyl mercury	Test solution	Unicam crystal 300, HP4500 Finnigan Mat (DF)	75×100	20 m*M* sodium tetraborate decahydrate (pH 9.3)	5% HNO_3, +20 kV, Electromigration	4–7 μg/l	[103]
Hg	Hg(II), methyl- and ethyl mercury	Contact lens solution	Spellman CZE-1000R (High Volatge Power supply), Elan 5000 ICPMS	100×110	50 m*M* $NaHCO_3/Na_2CO_3$, –0.2%(m/v) L-Cysteine– 20 m*M* SDS (pH 11)	2% HNO_3, 17 kV, Hydrostatic injection	80–70 μg/l	[79]
Au	Au(III)–histidine complexes	Test solution	Bertan high voltage power supply CE, Elan 6000, ICPMS	70×110	50 m*M* Tris–HCl (pH 7.5)			[106]
Cd, Zn	Metal complexes with Metallothionein Isoforms I & II	Test solution	Prince Crystal 310 CE, VG Plasma Quad (PQ II Turbo Plus)	50×77	20 m*M* Tris–HCl (pH 7.8)	10 m*M* NH_4NO_3, 25 kV Hydrostatic injection	1 μg/l for Cd 70 μg/l for Zn	[51]
I	Iodide, iodate, thyroxine	Human milk	Biofocus 3000(Bio-Rad), Elan 5000 ICPMS	50×150	Borate buffer (pH 8.3), 25 m*M* (BioRad 148-5023)	2% acetic acid, 20 kV, Electro migration	0.04–1.2 μg/l	[109]
I	Iodide, iodate, thyroxine, triiodothyronine	Human serum, urine	Biofocus 3000 (Bio-Rad), Elan 5000 ICPMS		Sandwich of phosphate (2.3), NaOH, SDS and Borate buffer (pH 8.3)		0.08–3.5 μg/l	[109]
Pt	Pt(II), Pt(IV)	Highway tunnel dust, soil	BioFocus 3000 (Bio-Rad), Elan 5000 ICPMS	50×150	100 m*M*, phosphate buffer (pH 6)	50 m*M* phosphate buffer (pH 6)		[111]
Co	Cyano, hydroxo, methyl, 5′ deoxy-adenosyl cobalamin species and cobinamide dicyanide	Commercial Vitamin B12 tablets	Bertan high voltage power supply, Elan 6000 ICPMS	50×80	20 m*M* phosphate buffer or 20 m*M* formate buffer (pH 2.5)	30 kV, Electrokinetic injection	50 μg/l	[104]
Sb	Sb(III) tartarate, Sb(V), $(CH3)_3\ Sb^{2+}$		Biofocus 3000 (Bio-Rad), Elan 5000 ICPMS	50×150	1% acetic acid (pH 2.0)	20 m*M* phosphate buffer (pH 5.6) −18 kV		[27]

HIBA, α-hydroxyisobutyric acid; UVCAT, 4-methylbenzyl amine; TEA, triethanolamine; Tris, Tris(hydroxymethyl)aminomethane; DF, double focusing; MMA, monomethylarsonic acid; DMA, dimethylarsinic acid; AsB, arsenobetaine; AsC, arsenocholine; PA, phenylarsonic acid; GSSeSG, Se-glutathione.

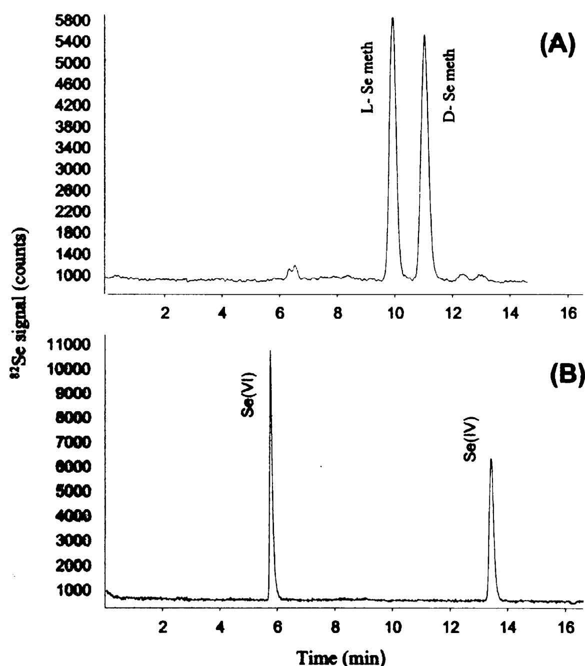

Fig. 4. CE–ICP-MS electropherograms of standards (A) Marfey's derivatized DL-selenomethionine; and (B) 1 ppm mixture of selenate and selenite. Conditions: 30 m*M* $(NH_4)_2HPO_4$, pH 3.3, −18 kV [48].

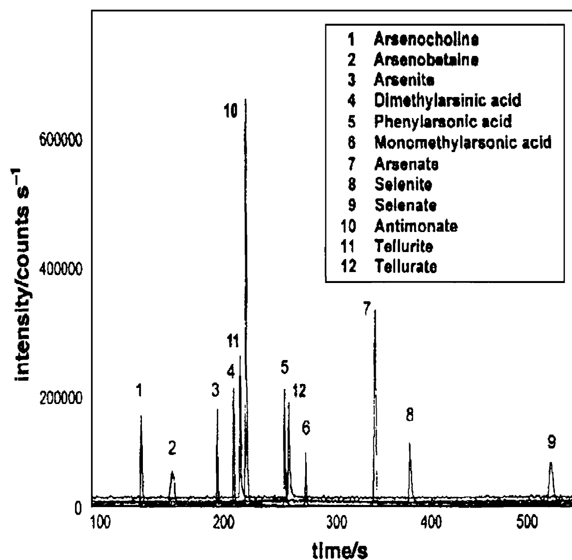

Fig. 5. Simultaneous separation of 12 species of four elements. Concentration of elements: As, Sb, Te 100 μg/l each, Se 1000 μg/l [74].

further development of this work, the method was applied to the analysis of human milk and serum [98]. The pretreatment procedure for milk consisted of defatting and deproteinization, followed by lyophylization and re-dissolution in the electrophoretic buffer. After a 10-fold preconcentration, GSSeSG, selenocystamine and selenocystine were found at levels close to the detection limit. The serum samples were diluted with electrophoretic buffer and several selenium species were observed. However, the results of a standard addition procedure were misleading (several peaks were increased in height after single standard addition) and the identification of species was postponed to future studies. In the same work, the feasibility of capillary isoelectric focusing (cIEF, pH range 2–10) was explored for organic selenium species. Slightly lower detection limits (10–30 μg/l) as compared to CZE were shown.

Recently, some of the advantages and improvements in selenium speciation using CE–ICP-MS were presented by Michalke [99]. Biological samples (urine and sewage sludge supernatant) were analyzed by these same authors for arsenic speciation [100]. The six species (As (III), As (V), MA, DMA, AsB and AsC) were separated in less than 2 min with a detection limit of about 15 μg/l (65 μg/l for AsC and AsB). The $^{40}Ar^{35}Cl^{+}$ interference in ICP-MS detection was not observed, but a broad signal appeared using the ^{35}Cl channel that was explained by the high sample conductivity. The sensitivity of the procedure was too low when compared with urine selenium levels, hindering speciation analysis in this material. In the liquid phase of a sewage sludge, MMA and As(V) were partially identified. In another study, Van Holderbeke et al. [101] investigated arsenic speciation in three different chemical matrices, namely, drinking water, human urine and soil leachate. All were run under basic conditions by CE (20 m*M* borate buffer, pH 9.4) and in the presence of cationic surfactant as the osmotic flow modifier. The separation of AsC, AsB, As (III), DMA, MMA and As (III) was obtained; excellent detection limits for all species (around 2 μg/l) were observed in this study (Fig. 7).

In a few studies of arsenic and selenium speciation, the hydride generation technique was used for interfacing CE to ICP-MS [87,88] or ICP-OES

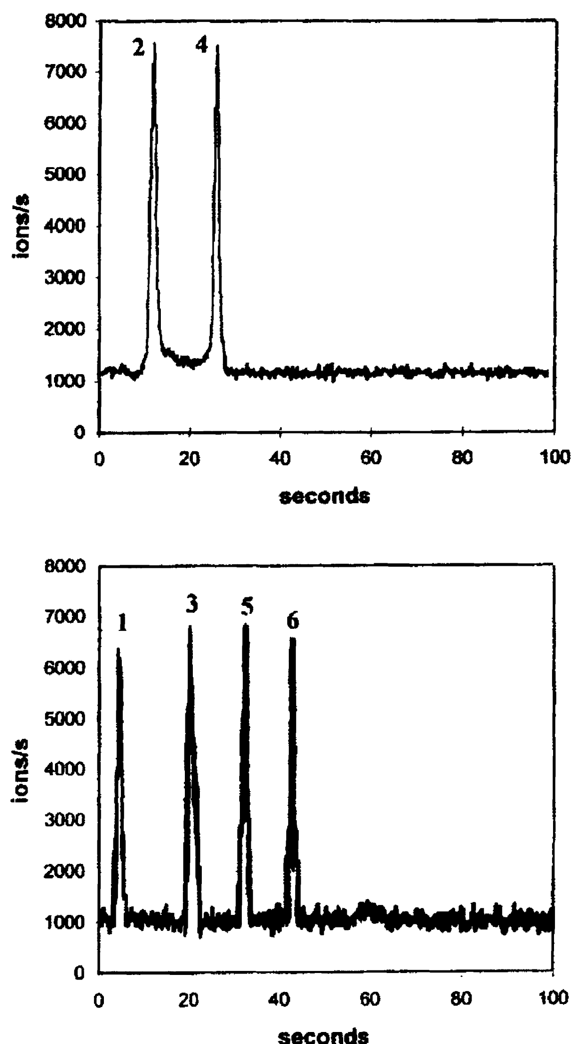

Fig. 6. Separation of inorganic (upper) and organic (lower) Se species, each 150 μg Se/l. Only the [77]Se isotope is shown, as the [78]Se isotope is interfered by polyatomic interferences more frequently. This results in "pseudo-Se-signals" and decreases the signal-to-noise ratio. The species are monitored at (1) 6 s (SeCM), (2) 13 s (Se (VI)), (3) 20 s (SeC), (4) 26 s (Se (IV)), (5) 33 s (SeM) and (6) 45 s (GSSeSG) [97].

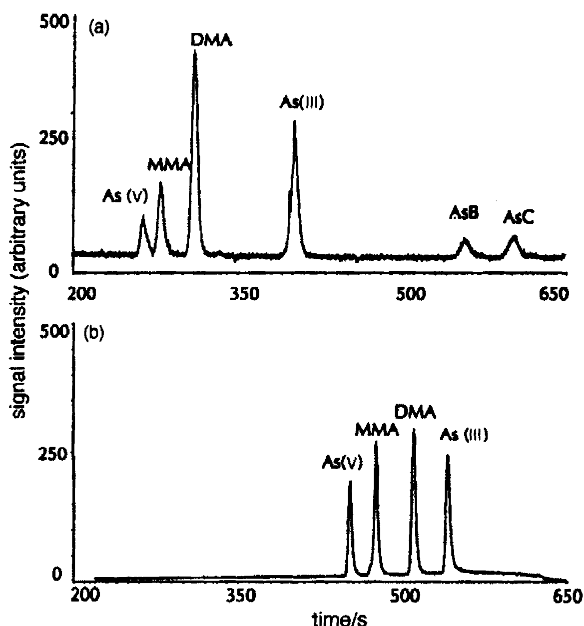

Fig. 7. (a) Electropherogram showing the separation of As (V), MMA, DMA, As (III), AsB and AsC, obtained with CE–ICP-MS before optimization, (b) Electropherogram of about 20 μg/l As(V), MMA, DMA and As (III) obtained after optimization of the CE–ICP-MS system. Conditions: 20 m*M* borate (pH 9.4), 2% OFM, 75 μm I.D. capillary, total length 88 cm, 5 kPa for 40 s plus 5 s post-injection, −25 kV [101].

(optical emission spectroscopy) [89]. Magnuson et al. [87] investigated As(V), DMA, MMA and As(III) speciation under the conditions of electrokinetic injection (pH 9.03), hydrodynamically modified EOF and microporous membrane gas–liquid separator. In a further development of this work, the simultaneous speciation of the two elements was used in a similar experimental design, with reduction of Se (VI) prior to hydride generation [88]. A movable reduction bed hydride generation system was applied in coupling CE to an ICP ion source in arsenic speciation (As (III), As (V), MMA, DMA). A CZE separation method was used (pH 6.5), and the hydride generation device enabled hydride formation at micro-level sample volumes without the use of a gas–liquid separator.

The on-line coupling of CE to electrospray ionization mass spectrometry (ES-MS) for selenium speciation has been studied. Various problems related with both technical and methodological aspects, have been described [85]. A similar approach was used for the analysis of six arsenic species (As (III), As (V), MMA, DMA, AsC and AsB) [87]. An acetate buffer for pH stacking was used, and baseline resolution was achieved for As (V), DMA, AsC and AsB. The detection limits for these arsenic compounds ranged

from 60 to 480 μg/l in this study [82]. Both hyphenated techniques, CE to ES-MS and CE to ICP-MS, were used to maximize analytical information in the speciation of selenium [83]. Because of the limitation of the electrospray process, acetic acid (2-5%) was used as the electrophoretic buffer and the separation of three selenoamino acids (selenocystamine, selenocystine and selenomethionine) was achieved by both CE–ESI-MS and CE–ICP-MS with total analysis time of 28 and 8 min, respectively.

5.2. Miscellaneous elements

Table 2 shows summarizes a variety of species of elements that have been studied using CE–ICP-MS, in addition to arsenic and selenium speciation previously discussed in this review. The investigated species listed within Table 2 and the literature noted cover a wide range of compounds of different physicochemical properties; from the element oxidation states through methylated element compounds, and from inorganic and organic complexes to elements contained within biomolecules [74,97,102–104]. The separation conditions, as well as the details of instrumental design are summarized, and hopefully, will enable a further understanding of the potential advantages and limitations of elemental speciation using capillary electorphoresis.

5.2.1. Studies of model solutions

Model solution studies of elements have often been noted in the literature. Two chemical systems, Cr (III)/Cr (VI) and Cu (II)/Cu [EDTA], have been often used [52,61,62,75] in studies using either CE–ICP-OES or CE–ICP-MS. The speciation of Cr (III) hydrolytic polymerization products was undertaken by Stewart and Olesik [102]. In spite of chemical lability of the species, it was possible to separate and detect monomeric and polymeric species in solutions at different pH's and after aging. Electrospray mass spectrometry was applied to confirm the presence of the species expected in the solutions. In work by Michalke [27], the problems in speciation analysis related to the possible effect of separation conditions on species stability, their inter-conversion or new species formation, appearance of "ghost" peaks and adherence to the capillary walls were addressed while analyzing compounds of antimony, Sb (III), Sb (V) and $(CH_3)_3Sb^+$. Three chemical species of mercury (inorganic, methyl- and ethyl-mercury) were separated as cysteine complexes at alkaline pH and introduced on-line to ICP-MS [79,103]. Two isoforms of rabbit liver metalloproteins which contain cadmium and zinc were used to evaluate the instrumental design for CE–ICP-MS coupling by Taylor et al. [51]. Cuadrado et al. [105] presented an interesting application of CE–ICP-MS to investigate the kinetics of gold (III)–L-histidine complex formation.

In another study, off-line ES-MS species characterization in the CE effluents was studied by Schramel et al. [81]. This was done in order to assess the suitability of this ES-MS technique; three types of compounds were selected for the Schramel study. A free metal ion (Cu (II)), metal–ion containing complexes (CuEDTA, $(CH_3)_3SbCl_2$) and covalent organometallic compounds (selenocystamine, selenomethionine) were evaluated.

5.2.2. Studies of complex samples

Complex sample matrices have also been studied and reported in the literature using CE elemental speciation. The analysis of different iodine chemical forms in biological fluids was addressed in different speciation schemes [106,107] because such information is useful in evaluation of the thyroid gland function [93]. Michalke [27] developed a CE–ICP-MS procedure for three iodine species (iodide, iodate and thyroxine), in which low detection limits (0.04, 0.05 and 10 μg/l, respectively) were achieved by sample stacking. This procedure was then applied to analysis of human milk, serum and urine samples. The iodide and thyroxine were the two species found in pooled milk and the quantitative results (14 and 27 μg/l) showed good species recovery relative to total iodine concentration [27]. In human serum, the three species (iodide, thyroxine and triiodothyronine) were identified by the method of standard addition and the iodine distribution among these species correlated with the health status of the individuals.

The predominant iodine species found in urine was iodide [108], in agreement with earlier literature data [106]. This same research group used the CE–ICP-MS technique for platinum speciation in soils [66,109,110], where fast transformations of the soluble platinum species occur [111]. They demonstrated that neither the CE conditions nor the pressure during the detection step significantly affected species stability [66]. They then applied an orthogonal speciation scheme involving reversed-phase HPLC–IC-MS and CE–ICP-MS to maximize information on platinum speciation and on the processes of species transformation in tunnel dust/soil samples [109].

Other applications of CE–ICP-MS to ''real world'' samples was in the speciation of cobalamins and the potentially harmful corrinoid analog (cobinamide dicyanide) in vitamin B12 supplements by Baker et al. [104]. The cobalamines studied were cyanocobalamin, hydroxocobalamin, methylcobalamin and 5′-deoxyadenosylcobalamin. In this study, the separation was carried out by a CZE method using phosphate and formate buffers (pH 2.5) and by micellar electrokinetic chromatography (Tris–HCl buffer with sodium dodecyl sulfate (SDS), pH 8). All separation run times were under 30 min. The excellent selectivity of these CE–ICP-MS procedures was demonstrated with detection limits ranging from 30 to 70 μg/l. This easily enabled compound quantification in pharmaceutical formulations. However, the authors stressed that the possible applicability to food or clinical samples would depend on the success of clean-up and preconcentration procedures because of the low concentrations of analytes within these complicated sample matrices.

6. Conclusions and future outlook

There is no doubt that CE and ICP-MS are techniques of separation and detection that are well suited for elemental speciation analysis. The outstanding advantages of CE are the high peak plate number (peak efficiency) and its separation efficiency which allow for resolving a variety of compounds, including small metal ions to large neutral or electrically charged biomolecules, within a single run. Moreover, the technique has been successfully used for the separation of relatively labile complexes and to study the kinetics of complex formation. An important limitation of this technique is the influence of electrophoretic buffer composition. The conditions that assure optimum separation often involve low or high pH values, or the presence of a ''complexing'' reagent. These conditions could affect the natural elemental speciation of the sample analyzed. Therefore, care is needed while selecting the experimental conditions for the CE separation. On the other hand, there is little limitation regarding the CE effluent for ICP-MS detection, which often is the case when interfacing other chromatographic techniques to the ICP-MS. A second limitation of CE with interferences occurs when the sample conductivity is different from that of electrophoretic buffer. In many cases, sample pretreatment will be needed to simplify the sample's chemical composition. ICP-MS detection seems to be a technique of choice for CE, since it offers high detection power, element specificity, multielement capabilities and enables isotope analysis. It should be stressed, that ICP-MS detection response is based on the quantity of analyte (mass) rather than the concentration of the solution. Because of the small sample load in CE analysis, the advantage of ppt or lower detection levels is lost in CE–ICP-MS. Studies have shown only a few fold decrease in the detection limits (on the single element basis) as compared to UV detection. An area still undergoing improvement is interface design. The construction of the laboratory built interfaces must be done carefully to assure high transport efficiency without affecting the separation process. Hopefully, more commercially built units will become available in the near future to minimize these difficulties.

In summary, the actual use of CE–ICP-MS in elemental speciation analysis has had somewhat limited applicability. Most of the applications could have been done with HPLC methods, which often provide lower detection levels. Currently, cases where CE can be very useful is when only nanoliter sample volumes can be used. Limited sample quantities or radioactive samples are ideal examples and

certainly should be analyzed by CE elemental speciation procedures. Future analytical developments for CE elemental speciation analysis need to include: (a) improvement in sample pretreatment procedures (clean-up, preconcentration and the preservation of the natural elemental species distribution); (b) exploration of different operating modes of capillary electrophoresis (CZE, MECC, isoelectric focusing and isotachophoresis); (c) enhancement of sample loading to CE (stacking); (d) the use of ICP-MS detectors with the lowest possible detection levels (the use of sector-field ICP-MS has already been reported [103]); and (e) improvements of the transport efficiency of the interface. The use of complementary detectors enabling the identification/confirmation of species is also desirable. Electrospray mass spectrometry is one such detector and has already been applied to selenium and arsenic speciation by CE. Low sensitivity of ES has been an important limiting factor in its use. Finally, the future looks very promising for CE in its continued use for elemental speciation analysis.

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