Use of a parallel path nebulizer for capillary-based microseparation techniques coupled with an inductively coupled plasma mass spectrometer for speciation measurements

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Abstract  
A low flow, parallel path Mira Mist CE nebulizer designed for capillary electrophoresis (CE) was evaluated as a function of make-up solution flow rate, composition, and concentration, as well as the nebulizer gas flow rate. This research was conducted in support of a project related to the separation and quantification of cobalamin (vitamin B-12) species using microseparation techniques combined with inductively coupled plasma mass spectrometry (ICP-MS) detection. As such, Co signals were monitored during the nebulizer characterization process. Transient effects in the ICP were studied to evaluate the suitability of using gradients for microseparations and the benefit of using methanol for the make-up solution was demonstrated. Co signal response changed significantly as a function of changing methanol concentrations of the make-up solution and maximum signal enhancement was seen at 20% methanol with a 15 μl/min flow rate. Evaluation of the effect of changing the nebulizer gas flow rates showed that argon flows from 0.8 to 1.2 l/min were equally effective. The Mira Mist CE parallel path nebulizer was then evaluated for interfacing capillary microseparation techniques including capillary electrophoresis (CE) and micro high performance liquid chromatography (μHPLC) to inductively coupled plasma mass spectrometry (ICP-MS). A mixture of four cobalamin species standards (cyanocobalamin, hydroxocobalamin, methylcobalamin, and 5’ deoxyadenosylcobalamin) and the corrinoid analogue cobinamide dicyanide were successfully separated using both CE-ICP-MS and μHPLC-ICP-MS using the parallel path nebulizer with a make-up solution containing 20% methanol with a flow rate of 15 μl/min.

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1. Introduction  
In recent years there has been growing interest in the field of speciation determinations [1–3]. Researchers understand that total elemental concentrations are no longer always appropriate for assessment of toxicity or absorption and bioavailability of elements important to human health and the environment. The chemical form must be considered and often times levels of interest may be at the part-per-billion level. Speciation analyses typically require hyphenation of separation techniques with a sensitive element specific detector.

The primary prerequisite for the successful hyphenation of techniques is the assurance of good transport efficiencies of the effluent (sample components) exiting the separation column and entering the plasma. A variety of devices have been designed for the introduction and transport of the sample into the plasma. Most consist of a nebulizer connected to a spray chamber but a direct injection nebulizer (DIN) which is connected directly to the ICP torch central channel has also been used. Capillary electrophoresis (CE) and micro high performance liquid chromatography (μHPLC) have been successfully interfaced to plasma detection systems such as inductively coupled plasma atomic emission spectrometry (ICP-AES) and, more recently, inductively coupled plasma mass spectrometry (ICP-MS) [4]. The advantages of capillary-based microseparation techniques include the following: low sample and stationary phase consumption, possible higher separation efficiency, and
increased flexibility with gradient programming using organic solvents with ICP-MS [5,6].

Designing an interface for the nebulization of microseparation technique effluents into a fine aerosol and ensuring efficient transport into the plasma is not an easy task [7]. The extremely low flow rates associated with capillary techniques require a sample introduction device which can handle flows in the nl to μl range and sometimes a make-up solution is required to provide the necessary minimum flow. Several commercially available, low flow microconcentric nebulizers have been studied and used for interfacing with ICP-MS [8–10]. One major limitation of these nebulizers is the suction effect they provide which can lead to degradation of the separation. Kinzer et al. [11] and others have postulated that laminar flow could alternatively be beneficial producing faster analysis times. The primary requirement is that the integrity of the separation is maintained so the peaks remain resolved. The laminar flow can be minimized or completely eliminated by the use of a self-aspirated sheath flow or a pumped make-up solution to satisfy the flow requirements of the nebulizer. Depending on the material selected, the primary potential disadvantages of using a make-up solution is plasma instability leading to poor precision and, more importantly, ICP-MS performance may be degraded over time due to accumulation of salts on the lens, sampler and/or skimmer cones. All of these factors, in the long run, can affect instrument response.

Transient effects are time-dependent changes in aerosol properties resulting in changes in transport efficiency as a function of changes in the solution matrix. These effects prove to be problematic when nebulizing samples of varying composition (e.g. changing acid concentrations) during typical solution nebulization experiments. Transient effects may result in a significant time delay to obtain a steady-state signal. Stewart and Olesik [12] characterized these effects in both ICP-OES and ICP-MS by systematically changing nitric acid concentrations over time and recording the signals. He determined that with increasing nitric acid concentrations, analyte signals were depressed. Todoli and Mermet [13] confirmed these effects and highlighted that transient effects were more significant when working at low flow rates. He also demonstrated the benefit of using a cooled spray chamber to minimize transient effects as a result of minimizing solvent evaporation from the spray chamber walls. Bjorn and Frech [14] highlighted the fact that transient effects are largely a function of the design of the nebulizer and/or spray chamber and demonstrated that the DIHEN nebulizer did not suffer from transient effects.

With increased interest in speciation determinations where microseparation techniques are combined with ICP-MS detection, transient effects must be considered. In particular, when separation conditions utilize an organic solvent for the elution gradient, transient effects may significantly affect the time-resolved chromatographic signal. A literature review revealed that although transient effects have been studied extensively using acids, organic modifiers have not been considered. Yang et al. [15] and others have reported that organic solvents may generally prove problematic with the ICP-MS because they can lead to increased or decreased analyte sensitivity, an increase in the number of carbon containing polyatomic ions, and deposition of carbon on the sampling orifice which results in degradation of both measurement precision and accuracy. These considerations along with concerns related to transient effects lead to conventional wisdom dictating that only isocratic conditions using aqueous eluents or only a small amount of organic modifier will be suitable for speciation determinations when using ICP-MS detection with conventional pneumatic nebulization.

In this study, a low flow parallel path, Mira Mist CE nebulizer was evaluated for the interface of microseparation techniques and inductively coupled plasma mass spectrometry. Suction effects are not of concern with this system because it is a self-aspirating nebulizer. A very low flow of make-up solution may still be used with this nebulizer to provide the necessary electrical connection for CE. According to the manufacturer’s specifications, the flow rate may be as low as a few microliters per minute. Another interesting feature is that the parallel path design avoids problems with nebulizer clogging, making maintenance very simple. In this study, a Mira Mist CE parallel path nebulizer was characterized using cobalt standards in preparation for research to be conducted related to cobalamin speciation [16–18]. The following parameters were studied: (1) ICP-MS transient effects; (2) effect of make-up solution flow rate and concentration; (3) effect of nebulizer gas flow rates.

2. Experimental

2.1. ICP-MS instrumentation

The inductively coupled plasma mass spectrometer used in this work was an Elan 6000 (Perkin Elmer Scieix, Thornhill, Ontario, Canada). The ICP-MS was operated at 1.0 kW with a coolant gas flow rate of 15 l/min and an intermediate gas flow rate of 0.86 l/min. For the nebulizer characterization studies as well as the cobalamin separation studies, the $^{59}$Co isotope was monitored. The dwell time was 1 s. When changing conditions during the nebulizer characterization studies, a 10-min equilibration period was used to ensure stable operating conditions. Data acquisition for the cobalamin separation study was initiated immediately after the sample was injected into either the CE or μHPLC system. The data were exported into Microcal Origin (Northampton, MA, USA) for processing.

A routine daily performance test recommended by the manufacturer was done for the ICP-MS after optimization. Tuning procedures included continuous nebulization of a multielement 10 ppb solution containing Mg, Cu, Rh, Cd, In, Ba, Ce, Pb and U as well as Co standard. The multielement solution was introduced by using a syringe
pump (KD Scientific, New Hope, PA, USA) through a Teflon tubing attached to the cross which is part of the nebulizer.

2.2. Nebulizer and spray chamber

A Mira Mist CE parallel path nebulizer (Burgener Research, Mississauga, Ontario, Canada) coupled with a cooled cyclonic spray chamber (Glass Expansion, Camberwell, Australia) was used. The Mira Mist CE nebulizer (shown in Fig. 1) is a low flow, parallel path nebulizer with specified sample flow rates ranging from 1 to 2500 μl/min depending on the composition of the solution. The nebulizer was designed specifically for interfacing CE with ICP-MS but can also accommodate other capillary-based separation techniques. Fig. 1 shows the four-way cross connected to the liquid inlet of the nebulizer. The capillary column which is inserted from the left through the four-way cross extends all the way to the tip of nebulizer. The tip of the capillary column is recessed 1 mm from the tip of the nebulizer, which is enough to allow the make-up solution to cover the capillary column and keep it wet. The capillary is held in place with a finger tight fitting. The make-up solution is introduced through the lower port on the four-way cross with a syringe pump (KD Scientific). The fourth port on the cross holds a platinum wire electrode needed for the electrical connection for CE or capillary electrokinetic chromatography (CEC).

The argon gas flow was varied from 0.6 to 1.2 l/min keeping the nebulizer pressure at 85 psi for the nebulizer characterization studies, as recommended by the manufacturer. The ICP-MS mass flow controller was used to control all gas flows.

A jacketed Glass Expansion cyclonic spray chamber used was to provide cooling. The cooled spray chamber minimizes solvent loading and provides improved precision and sensitivity. The recirculating water temperature was set to 5 °C and maintained using a Model 911 recirculating chiller from PolyScience (Niles, IL, USA). A Miniplus peristaltic pump (Gilson, France) was used to drain the waste.

2.3. Micro-HPLC instrumentation

The chromatographic system used was a TriSep 2000GV CEC (Unimicro Technologies, Pleasanton, CA, USA). The system consists of two pumps for gradient elution, a four-port valve injector and an online UV/Vis detector. The system can be interfaced with an ICP-MS to provide element specific detection. In addition, The TriSep system provides a high voltage power supply to facilitate capillary electrophoresis separations. Packed microseparation columns are directly connected to the four port injector valve which has an internal sample rotor volume of 20 nl. The user-selected gradient flow enters the separation column through a valve injector and is maintained at a fixed pressure using a backpressure regulator. In this study, the instrument was modified to use an adjustable backpressure regulator to easily change the column pressure as necessary for separation optimization.

Micro-HPLC chromatographic separation was achieved using reverse phase conditions at room temperature. A C18 capillary column (Unimicro Technologies) with 2 μm particle size packing material was used. Selection of the capillary length was dictated by the amount needed to facilitate interfacing to the Unimicro system with the ICP-MS. The capillary column had a total length of 37 cm with a packed section of ~25 cm. It had an internal diameter of 75 μm and an outer diameter of 365 μm.

2.4. Capillary electrophoresis instrumentation

CE separations were carried out using a Beckman P/ACE MDQ system (Beckman Coulter, Fullerton, CA, USA). The instrument is an automated capillary electrophoresis system equipped with an UV/Vis and Diode Array detector and has the option to have an external detection system (ICP-MS or MS). To minimize temperature effects related to joule

![Fig. 1. Schematic of a Mira Mist CE parallel path nebulizer.](image-url)
heating, a column cooling adapter was used providing recirculated liquid cooling for the entire length of the capillary. In this application, cooling was provided to the capillary column inlet on the four-way cross as opposed to the outlet tip of the capillary. The Beckman P/ACE MDQ system provides reproducible injections using either electrokinetic, pressure or vacuum injection.

Fused silica capillaries (Polymicro Technologies, Phoenix, AZ, USA) were used for the electrophoretic separations. Capillaries were 80 cm in length and 50 μm i.d. and 360 μm o.d. A voltage of +30 kV was applied at the capillary inlet and the ground was at the cross (as shown in Fig. 1). The capillary temperature was kept at 20°C. Hydrodynamic injections were done by applying a pressure of 0.5 psi for 30 s.

2.5. Reagents and standards

Analytical reagent grade chemicals were used throughout without further purification. Deionized distilled water (18 MΩ) was used for all solution preparation and dilutions. Ammonium acetate was obtained from Fluka (Ronkonkoma, NY, USA). Methanol, acetonitrile, sodium hydroxide and sub-boiling distilled nitric acid were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Sodium formate and formic acid were purchased from Sigma (St. Louis, MO, USA).

For chromatography, acetate buffers were prepared by dissolving 25 mM ammonium acetate in deionized, distilled water (or in 50% methanol) and then adjusting the pH to 4 with nitric acid. For gradient elution μHPLC separations, two mobile phases were prepared: mobile phase A was 25 mM ammonium acetate in water and mobile phase B was 25 mM ammonium acetate in 50% methanol. For capillary electrophoresis separations, 20 mM sodium formate was dissolved in deionized, distilled water and the pH was adjusted to pH 2.5 with pure formic acid. All buffer solutions were filtered through a 0.45 μm Whatman filter paper (Clifton, NJ, USA) and de-gassed using an ultrasonic bath. New fused silica capillaries were conditioned by flushing with 1 M NaOH for 10 min, de-ionized, distilled water for 3 min, followed by 20 mM sodium formate for 10 min. Between runs, capillary regeneration was achieved by flushing with 0.2 M HNO₃, for 2 min, followed by de-ionized, distilled water and sodium formate for 1 min each.

Cobalamin standards including cyanocobalamin (vitamin B12, CN-), hydroxocobalamin (vitamin B12a, OH-), methylcobalamin (methylcoenzyme B12, Me-), 5’-deoxyadenosylcobalamin (coenzyme B12, Ado-), and cobinamide dicyanide [CN₂-Çbn], Cob) were obtained as crystalline materials from Sigma and were continuously stored in either the freezer or refrigerator as required to ensure the stability of the material. Stock solutions were prepared under low-light conditions by dissolving 10 mg of the substances in 10 ml of deionized, distilled water and stored in dark bottles at 2°C. Working standard mixtures were prepared before analysis by appropriate dilution of the stock standard solutions and were also stored in the refrigerator.

3. Results and discussion

3.1. Parallel path nebulizer characterization

The ultimate goal of this nebulizer characterization study was to evaluate the use of the Mira Mist CE parallel path nebulizer when interfacing capillary microseparation techniques and ICP-MS. Nebulizer performance was characterized by introducing a fixed flow of Co solution into the four-way cross of the nebulizer through the capillary column. The eluent exiting the capillary column is combined with the make-up solution at the tip of the nebulizer. A 50 ppb Co solution was introduced through the capillary column (see Fig. 1) using a syringe pump set a flow rate of 333 nl/min using a 3 ml syringe. Make-up solution was introduced into the four-way cross using a second syringe pump equipped with a 10 ml syringe. The make-up solution composition and flow rates were varied depending upon the experiment. This approach of introducing the analyte solution via the capillary instead of via the make-up solution inlet of the four-way cross was chosen because it most closely simulates the effect seen when microseparation-ICP-MS techniques are performed. The result was that the effects of changing separation and nebulization parameters could be systematically evaluated using the transient Co signal. This approach is particularly important when looking at the effect of the make-up solution on the analyte signal.

3.1.1. ICP-MS transient effects

The purpose of this study was to evaluate transient effects associated with capillary-based microseparation systems. Such effects would be important with the use of a gradient where the amount of organic modifier is changing with time. Ackley et al. [5] hypothesized that microbore systems coupled with ICP-MS might offer an advantage as compared with conventional HPLC since the amount of organic solvent reaching the plasma is reduced due to the smaller diameter and decreased flow rate. Our hypothesis was that capillary-based separation systems might offer an even greater advantage because the amount of organic solvent is further reduced with the very low flows associated with this capillary-based technique. Transient effect studies done with acids typically vary the acid concentration with time. In this study, the organic solvent was added to the make-up solution and the flow rate of that solution was systematically changed to study the transient effect. The cobalt standard was pumped through the capillary to simulate effects seen when using a gradient for the chromatographic separation. Prior to the start of the experiment, the spray chamber was conditioned with 20% methanol at a flow rate of 5 μl/min for several minutes and then data acquisition was started. After 5 min the flow rates were
rapidly changed to 15 μl/min. This experiment was repeated three times after allowing enough time for signal re-equilibration between runs.

Fig. 2 shows the transient effects of aqueous methanol when make-up solution flow rates were changed from 5 to 15 μl/min (a) and from 15 to 5 μl/min (b). This resulted in the volume of methanol to the spray chamber changing from 1 to 3 μl and vice versa. In Fig. 2 trace a, a drastic increase in the signal is observed reaching a steady-state signal in less than 2 min. Fig. 2 trace b shows that 6 min is needed to achieve signal stability when decreasing the methanol concentration. Two conclusions can be made from these data: (1) increasing quantities of methanol lead to signal enhancement; (2) signal equilibration takes three times longer when decreasing the quantities of methanol as compared to increasing the amount of methanol. It is important to note that changes in the amount of methanol reaching the ICP-MS with gradient μHPLC will be very small (nl volumes) and have virtually no impact on the signal if 20% methanol is used for the make-up solution (μl volumes) as shown in Fig. 3. This highlights the benefit of using a make-up solution which contains the same organic solvent as being used for the gradient μHPLC separation. This approach of utilizing the organic make-up solution flow to “overwhelm” the influence of the organic solvent used for the gradient separation would not be feasible if conventional HPLC was used due to the much greater flow rates.

3.1.2. Effect of make-up solution flow rate and concentration

To establish operating conditions for microseparation-ICP-MS, it was necessary to study the effect of flow rates on the nebulizer and to study the effect of methanol concentration on Co signals. Liquid flow rates into the nebulizer consist of the make-up solution flow (μl flows) plus the eluent from the capillary (nl flows). These experiments were conducted using 333 nl/min flows of 50 ppb Co through the capillary combined with varying flows of make-up solution as a source of liquid to the nebulizer. Fig. 4a shows the Co signal as a function of flow rate of a make-up solution containing 20% methanol. Methanol (20%) was selected because it is suitable for the separation of cobalamin compounds for this project. The Co signal

Fig. 2. Methanol transient effects study: (a) 5 to 15 μl/min; (b) 15 to 5 μl/min.

Fig. 4. Effect of make-up solution concentration and flow rate. (a) Dependency of 50 ppb Co signal on make-up solution flow rates (20% methanol); (b) Dependency of 50 ppb Co signal on methanol concentration (15 μl/min flow rate).
increases when increasing the make-up flow rate from 5 to 15 μl/min and then plateaus and slightly decreases when liquid flow rates increase from 15 to 30 μl/min. Original manufacturer’s specifications indicated that the Mira Mist CE parallel path nebulizer could be operated at flows as low as 1 μl/min but at very low flows the nebulizer is “starved” and precision will be poorer. In fact, one experiment was done with zero make-up flow rate, in which the nebulization process relied solely on the nl volumes of solution exiting the capillary and significant signal pulsing was observed. Based on these data, a 15 μl/min flow rate was used for further investigations.

Fig. 4b highlights the fact that 20% methanol provides nearly a 10-fold increase in Co signal intensity as compared to water or dilute acid. The generation of fine aerosols is directly dependent on the physical properties of the solvent including: surface tension, viscosity, and density of the sample [19–21]. Methanol has a low viscosity and high volatility resulting in signal enhancement due to improved nebulization and transport efficiencies. The observed decrease in the Co signal at higher flow rates and consequently higher methanol concentration in the plasma is most likely due to cooling of the plasma as a result of higher solvent loading.

Results shown in Fig. 4a and b may be compared since changes in the flow rate (Fig. 4a) result in changes in the total amount of methanol in the plasma (Fig. 4b). As expected, similar intensities values (ca 8500 cps) are obtained when introducing 40% methanol at a fixed make-up rate of 15 μl/min compared to introducing 20% methanol at 30 μl/min. In both cases, the calculated amount of pure methanol being introduced into the spray chamber is 6 μl/min. At the identified optimum flow rate of 15 μl/min, this is equivalent to 3 μl/min of pure methanol.

It is clear that the amount of methanol necessary for enhancement of ionization processes in the plasma must be optimized for each system. It is not meaningful to discuss the optimum conditions in terms of the percentage of methanol without talking about the flow rate as well. In a recent literature report by Björn and Frech [14] using a different nebulizer, it was found that 5% methanol produced better intensities for 8 elements studied as compared to 20% methanol. In that study a flow rate of 85 μl/min was used and maximum analyte intensity was seen with 5% methanol (equivalent to 4.25 μl/min of pure methanol). This amount is very similar to the amount determined to be optimum in this study (3 μl/min of pure methanol). Also, at high concentrations of methanol, Björn and Frech also observed a signal decrease due to plasma loading. In their studies minimum analyte response was seen with 85 μl/min of 20% methanol (equivalent to 17 μl/min pure methanol) which was in good agreement with our results achieved at 15 μl/min of 80% methanol (equivalent to 12 μl/min pure methanol). Although the amount of organic solvent being introduced into the system can have a profound effect on ionization efficiency and/or transport, other parameters such as nebulizer and spray chamber design as well as plasma power are also critical to the overall optimization of the system.

3.1.3. Nebulizer gas flow effects

Suction effects which could affect microseparations seen with self-aspirating nebulizers, are not expected to be an issue with the Mira Mist CE nebulizer. There was, however, an interest in evaluating the effect of nebulizer gas flow on Co. The reason is that it is well known that for a fixed solution flow rate, the nebulizer gas flow directly influences aerosol characteristics and their transport into the plasma. Several reports [20,22,23] have shown, depending upon nebulizer design, that high nebulizer gas flow rates decrease droplet size and improve transport efficiency, resulting in an increase in the analytical signal.

Fig. 5 shows the Co signal as a function of the nebulizer gas flow rate in the range from 0.6 to 1.2 l/min. At nebulization gas flow rates below 0.6 l/min insufficient aerosol was generated to provide a measurable signal. The Co signal increases as the nebulizer gas flow increases from 0.6 to 0.8 l/min. It is interesting to note that at nebulizer gas flows above 0.8 l/min, no significant changes in the Co signal are observed. The likely explanation of this steady-state phenomenon is that although more droplets are being formed with increasing nebulizer gas flow, the likelihood of droplets colliding and coalescing and not reaching the plasma is significant. Other possible explanations for the steady-state Co signal response over the range of 0.8–1.2 l/min nebulizer gas flow setting could be related to plasma cooling or may simply be the result of the actual gas flow reaching a maximum which cannot be exceeded. The conclusion was that a range of nebulizer gas flows could provide acceptable and reproducible performance. A nebulizer gas flow of 1 l/min was selected for the balance of the experiments. This is in agreement with the manufacturer’s recommended nebulizer gas flow rate for the Mira Mist CE nebulizer.
3.2. Capillary-based microseparations using a parallel path nebulizer

To demonstrate the usefulness of the Mira Mist CE parallel path nebulizer, both CE and μHPLC experiments were run using ICP-MS detection. Although a large majority of this project was related to optimization of Mira Mist CE nebulizer for use with μHPLC-ICP-MS, this nebulizer was originally designed for use with CE and it was anticipated that its application for CE-ICP-MS would be straightforward. The samples studied were a mixture of four cobalamin species: cyanocobalamin (CN-), hydroxocobalamin (OH-), methylcobalamin (Me-) and 5'-deoxyadenosylcobalamin (Ado-) as well as the corrinoid cobinamide dicyanide (Cob).

Fig. 6a shows the electrophoretic separation of the Co containing compounds using 20 mM sodium formate as the separation buffer and an aqueous mixture containing 1% nitric acid and 20% methanol as the make-up solution. Nitric acid was used to maintain an electrical connection while methanol was used to enhance the analytical signal. The sample was injected hydrodynamically by applying 0.5 psi for 30 s. In addition, 0.4 psi pressure was applied during the separation to reduce the sample analysis time.

Fig. 6b shows the reverse phase chromatographic separation of the same Co containing compounds using a C18 column with a particle size diameter of 2 μm and a gradient elution program using 2 mobile phases (25 mM ammonium acetate in water and 25 mM ammonium acetate in 50% methanol). The make-up solution contained 20% methanol. Despite the fact that some peak tailing was observed, this chromatogram shows good separation of the various cobalamin species. The peak tailing is the result of too large a dead volume and this subject is discussed in more detail in another manuscript [24].

As you may be seen, reasonable separations can be obtained using either CE-ICP-MS or μHPLC-ICP-MS using the Mira Mist CE nebulizer. In both cases, approximately 20 nl of the mixed standard solution was injected. Quantitative determinations were not the goal in this study so the detection limits of the two systems were not compared but CE-ICP-MS using a 20% methanol make-up solution appears to provide a measurable improvement in sensitivity as compared with μHPLC-ICP-MS.

4. Conclusions

The Mira Mist CE parallel path nebulizer combined with a jacketed cyclonic spray chamber has been successfully used for both CE-ICP-MS and μHPLC-ICP-MS separations of important cobalamin species. Historically, when coupling HPLC with ICP-MS, researchers have not used gradients and have limited themselves to the use of modest amounts of organic solvents because of expected problems with transient effects and/or plasma instability. In this study, signal enhancement was observed with increasing quantities of methanol and concerns related to transient effects were determined to be insignificant when using a make-up solution containing 20% methanol for μHPLC-ICP-MS determinations. This approach to compensating for gradient effects using the make-up solution would not be feasible with non-capillary microseparation techniques because the solution flow rates required would be prohibitively high. In addition, this study demonstrated that when discussing enhancement effects of organic solvents, it is important to talk in terms of the absolute amount of solvent going into the nebulizer (e.g. 3 μl/min pure methanol) rather than talking in terms of the percentage of solvent (e.g. 20% methanol). This is particularly important when comparing different nebulizers and may affect the selection of the most appropriate nebulizer for a particular speciation application. In this study, the Burgener Mira Mist CE parallel path nebulizer was determined to operate efficiently over a range of 0.8–1.2 l/min nebulizer gas flow highlighting its ruggedness. The make-up solution flow rate selected for μHPLC-ICP-MS and CE-ICP-MS of 15 μl/min was based on optimization studies using methanol. One might expect that different make-up solution flow rates might
be required for μHPLC-ICP-MS as compared to CE-ICP-MS but because both separation techniques provide nl/min flow rates, the 15 μl/min make-up solution flow ensures the use of a flow greater than the necessary minimum flow for the optimum performance of the nebulizer. A second, concurrent publication focuses on the optimization of separation conditions for μHPLC-ICP-MS for the quantification of individual cobalamin species [24]. Ongoing research is focused on the optimization of conditions for CE-ICP-MS as well as sample preparation and preconcentration strategies so that microseparation-ICP-MS methods can be successfully applied to the quantification of cobalamin species in foods and supplements.

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References