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Short communication

Comparative study on conventional and low-flow nebulizers for arsenic speciation by means of microbore liquid chromatography with inductively coupled plasma mass spectrometry

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Abstract

The performance of conventional and low-flow nebulizer systems with liquid chromatography in differentiating four arsenic species in urine was evaluated. Two low-flow (DIN and MCN) chamber assemblies and a conventional (CFN) nebulizer-spray chamber assembly were compared in the hyphenation of anion-exchange microbore liquid chromatography with inductively coupled plasma mass spectrometry. Under optimal analytical conditions, the detection limits of the four arsenic species were 0.2–0.6 ng ml^{-1} for all the nebulizer systems tested. The chromatographic resolution was best in the case of DIN due to its minimal off-column dead volume and superior transport efficiency. Four arsenic species were determined in the certified reference materials NIST SRM 2670E and 2670N.

Keywords: Low-flow nebulizers; Inductively coupled plasma mass spectrometry; Speciation; Nebulizers; Interfaces, LC–ICP-MS; Arsenic

1. Introduction

The combination of a powerful separation technique such as liquid chromatography (LC) with a selective detector such as inductively coupled plasma mass spectrometry (ICP-MS) enables the speciation of arsenic compounds in various biological samples [1]. Microbore LC provides the advantage over conventional LC separation techniques [2,3], that significantly less undesired organic material and salt buffers are introduced into the detector. In addition to the lower flow-rate and small sample volume associated with microbore column separation, the transport efficiency of the analyte through the sample introduction systems into the ICP detector is also important in ensuring successful chromatographic measurement. Several different low-flow nebulizers have been employed to provide finer primary aerosols and higher solution transport rates, thus improving the sensitivity at a low sample uptake rate [4].

Various low-flow nebulizers, including DIN [5], MCN [6], OCN [7], HEN [8] and DIHEN [9], have been developed to introduce a small amount of

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aerosols at a low flow-rate into ICP systems. However, according to Falk et al. [10], not only the overall analytical performance, such as the detection limit and repeatability, of various nebulizers for interfacing LC and ICP-MS may be different, but also the transport behavior of different arsenic species may be changed when a hydraulic high pressure nebulizer (HHPN) was used. Additionally, separation on a column into the single species always yields very low concentrations of arsenics to be determined, so the selection of an appropriate nebulizer becomes very critical in interfacing chromatographic separation and subsequent ICP determination.

DIN is the first low-flow nebulizer developed for interfacing low-flow LC columns with ICPs [11,12]. Using a DIN as interface improves the analyte transport efficiency by approximately 30-fold over that associated with a conventional pneumatic nebulizer, because of minimal off-column dead volume of the former and its better transport efficiency. However, incomplete analyte vaporization and solvent-induced plasma cooling [13] are suspected to explain why only a 2.5-fold improvement in detection limits is observed. Unlike DIN, an MCN can provide a nebulization efficiency of approximately 50% [14]. However, MCN may suffer from the problems of long wash-out times and large dead volumes, because of the use of a spray chamber, possibly resulting in an unsatisfactory chromatographic separation with an unsatisfactory resolution. To the best of our knowledge, DIN and MCN-Scott spray chamber assemblies have not yet been compared as interfaces in a μLC–ICP-MS system. The actual advantages of coupling DIN or MCN with μLC–ICP-MS must be clarified, since the inevitable solvent loading and off-column dead volume, resulting from the use of DIN and MCN, respectively, may negate the inherent advantages of the analytical system.

This work was aimed at evaluating the analytical characteristics of two low-flow nebulizers, namely DIN and MCN, as interfaces in the μLC–ICP-MS system. In order to investigate the effect of off-column dead volume and transport efficiency on the separation efficiency of chromatograms, a conventional nebulizer-spray assembly, such as cross-flow nebulizer and Scott spray chamber, was also used for comparison. The utility of the proposed method is demonstrated in determining the quantity of arsenic in certified urine samples.

2. Experimental

2.1. ICP-MS device and conditions

The ICP-MS used was the Elan Model 5000 (Perkin-Elmer Sciex, Thronhill, Ontario, Canada). The outer, auxiliary and nebulizer gas flow rates were set at 15, 0.6 and 0.8 l min^{-1}, respectively. The plasma forward power was 1150 W. The sampling position and the ion lenses for the optimum arsenic signal at m/z 75 were adjusted using a 10-ppb arsenous acid standard solution. Three different nebulizers were utilized in this work; a cross-flow nebulizer (Perkin-Elmer), a direct injection nebulizer (Micro-Neb 2000, Cetac Technologies, Omaha, NE, USA) and a microconcentric nebulizer (MCN-100, Cetac Technologies, Omaha, NE, USA). The MCN nebulizer was fitted to a Scott-type Ryton double-pass spray chamber. The make-up gas selected for the direct injection nebulizer was 0.15 l min^{-1}.

2.2. Column preparation and chromatographic separation

An LC pump (Hitachi L-6220, Japan) with a metal-free on-line-injector (Rheodyne 9010, Cotati., CA, USA) equipped with a 5-μl polyetheretherketon (PEEK) sample loop was employed. The column was connected to the nebulizer using PEEK tubing. The filtrate of urine samples was separated using a microbore column, which was packed with silica-base anion-exchange resin (150×1 mm I.D., Nuceleosil SB 100-5; particle size 5 μm). Chromatographic separation was conducted at ambient temperature. The flow-rate of the mobile phase was adjusted at 100 μl min^{-1}.

The mobile phase for separating arsenic species was comprised of 20 mM of monoammonium dihydrogen phosphate acid and diammonium hydrogen phosphate buffer (Merck, Darmstadt, Germany). The pH was adjusted to 5.25 using dilute ammonia solution and nitric acid. Mobile phase solutions were degassed by sonication prior to use.

Stock standard solutions (1000 μg ml^{-1}) of arsenous acid, arsenic acid, monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) were prepared by dissolving sodium arsenite, disodium hydrogenarsenate, sodium dimethylarsenite and disodium monomethylarsenate (Chem Service, West Chester, PA, USA), respectively, in 0.2% v/v sulfuric acid and were stored in Pyrex bottles at 4 °C until required.

2.3. Sample analysis

The urine samples were stored in polyethylene bottles at 4 °C in the dark. Prior to analysis, the urine was diluted to 1:5 with water and filtered through a 0.45-μm filter.

Calibration curves based on peak areas were calculated. Detection limits were estimated using peak area data, based on three times the standard deviation of the baseline noise divided by the slope of the calibration curves.

Two certified reference materials were used to test the performance of the established analytical methods. NIST SRM 2670E and SRM 2670N urine were obtained from the National Institute for Standards and Technology (Gaithersburg, MD, USA).

3. Results and discussion

The analytical performance of the LC–ICP system depends critically not only on the characteristics of the LC separation, but also on the interface used in the hyphenation of μLC and ICP-MS system. A conventional cross-flow nebulizer (CFN) and a low-flow microconcentric nebulizer (MCN) coupled with a Scott spray chamber and a direct injection nebulizer (DIN) were employed as interfaces to evaluate the effect of different nebulizer-spray chamber combinations on the chromatograms. Figures of merit comparisons including detection limits and chromatographic separation were evaluated.

3.1. Coupling of nebulizers with μLC–ICP-MS

Using an aqueous 20 m*M* ammonium phosphate solution at pH 5.25, the four arsenic species of interest could be successfully separated with the μLC within 11 min at a flow-rate of 100 μl min^{-1}. Fig. 1 gives the chromatograms of the four arsenic species of interest, where CFN and MCN coupled with a Scott spray chamber and DIN were, respectively, used as interfaces for hyphenating μLC separation and ICP-MS measurement. In the hyphenation system in which ICP was coupled to a chromatographic separation, the off-column dead volume that resulted from the spray chamber and the transfer line sometimes led to broadening of the resultant peaks [15].The peak shapes shown in Fig. 1 indicate that the nebulizer-spray chamber assemblies with larger dead volumes (CFN≈MCN>DIN) detrimentally affect the shape of the resultant peaks. In other words, band broadening, and thus loss of peak efficiency, might be attributed to the off-column dead volume and transport efficiency of different nebulizer-spray chamber combinations.

The low-flow-rate nebulizers that provide finer primary aerosols and higher transport rates through the spray chamber cause a higher sensitivity than the CFN-Scott spray chamber assembly [16]. As shown in Fig. 1, fully resolved chromatograms are observed with MCN and DIN. These nebulizers were used for arsenic speciation in the following study.

3.2. Analytical performance

The performance of the μLC–ICP-MS system with DIN or MCN as an interface was investigated for arsenic speciation. Table 1 shows the results in terms of the linear dynamic range and detection limits of four arsenic species. All calibration curves were linear with regression coefficients of 0.997 or better. The precision based on three replicate injections of 20 ng ml^{-1} of each species and measurement of the peak areas was better than 4% RSD for all analytes. The MCN showed better precision than the DIN, which may be attributed to the lower solvent loading of the MCN. As to the detection limits, there is little difference between MCN and DIN; but the MCN is seen to be slightly superior. The detection limits are between 0.2 and 0.6 ng ml^{-1} for both DIN and MCN. Since the injection volume in the present work is very low (5 μl), the detection limits achievable are comparable to those obtained with conventional nebulizers in which a larger injection volume is used [17,18].

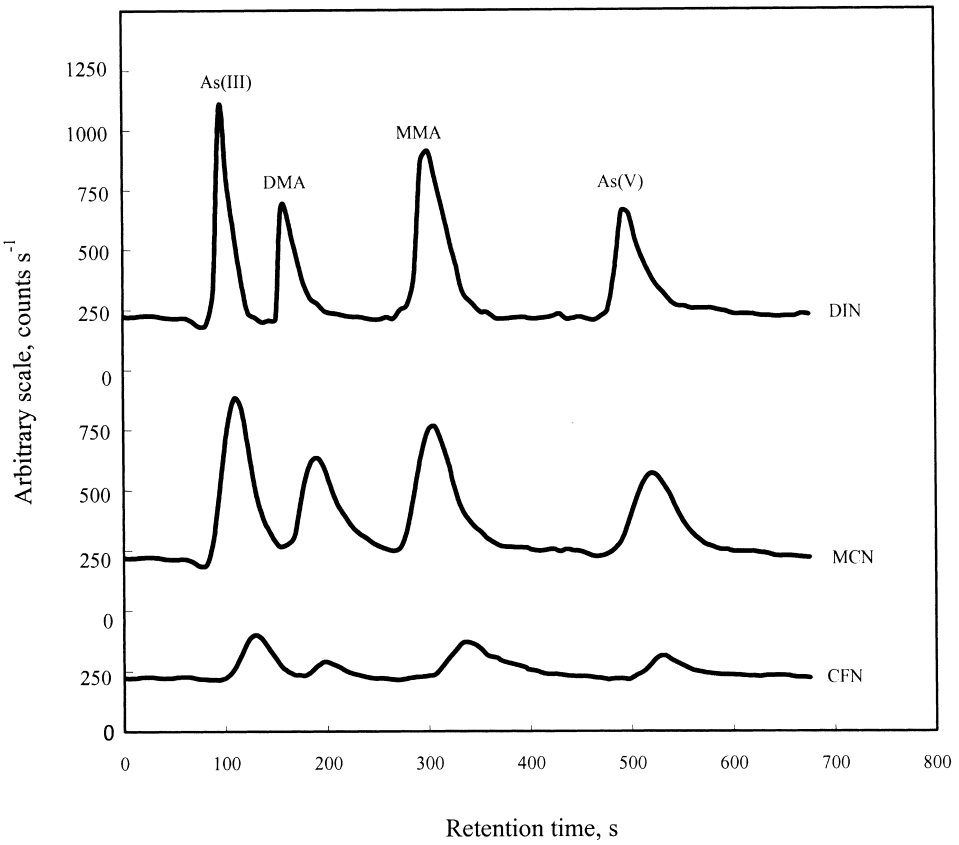

Fig. 1. The chromatograms obtained with use of different nebulizers as interface for the hyphenation of chromatographic separation and ICP-MS.

3.3. Real sample analysis

The potential interference of $ArCl^+$ with As, caused by an extremely high chloride content must be checked by applying this hyphenation system to identify the arsenic species in urine samples. Fig. 2 shows the chromatogram of four arsenic species of interest and $ArCl^+$ ($m/z=77$) interference in a urine sample (SRM 2750E). Obviously, no significant interference can be seen in the determination of the desired arsenic species in the urine sample, although the $ArCl^+$ peak appears somewhat close to the peak of arsenic acid.

The applicability of the established μLC–MCN-

Table 1
Analytical characteristics of proposed μLC–ICP-MS systems

	As(III)	DMA	MMA	As(V)
μLC–DIN-ICP-MS				
Linearity (*r*-value)	0.9995	0.9995	0.9983	0.9980
Detection limit (ng ml^{-1})	0.4	0.3	0.4	0.6
μLC–MCN-ICP-MS				
Linearity (*r*-value)	0.9998	0.9989	0.9988	0.9976
Detection limit (ng ml^{-1})	0.2	0.3	0.2	0.3

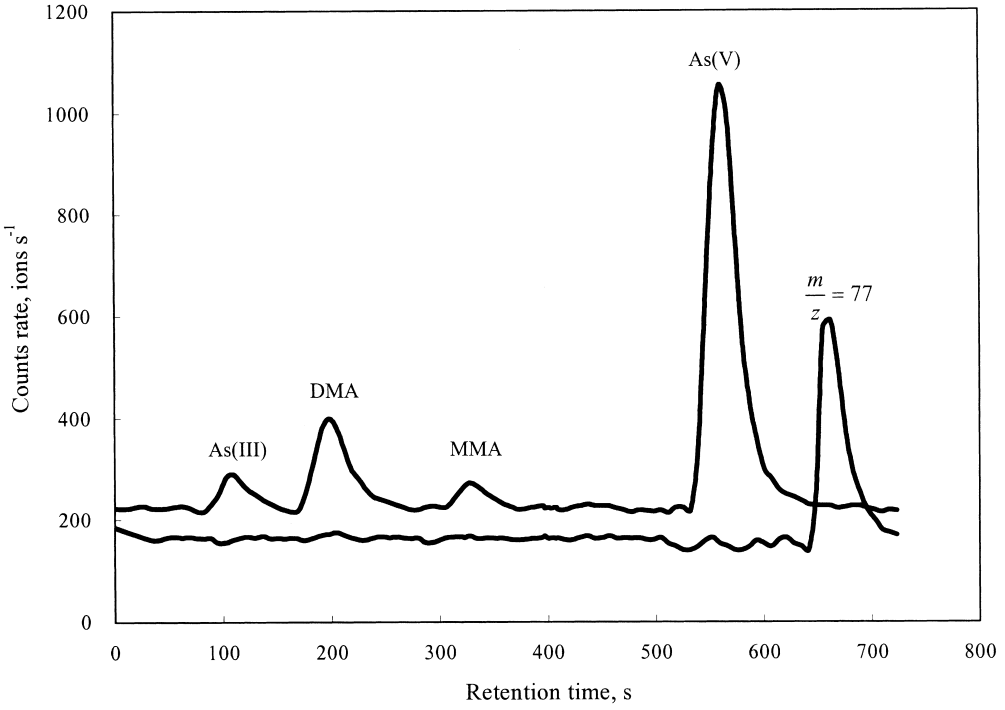

Fig. 2. Chromatogram for the separation of four arsenic species in urine sample (SRM 2670N) using microconcentric nebulizer as interface.

ICP-MS and μLC–MCN-ICP-MS systems to determining arsenic species in urine samples was investigated. Table 2 shows the results obtained by analyzing two standard urine samples, in which only the total arsenic concentration is provided (NIST SRM 2670E and 2670 N). No certified concentration of each individual arsenic species in the urine standards is available, so the analytical reliability of the proposed methods can only be validated either by comparing the total arsenic concentration obtained by summing the respective arsenic species with that certified in the standard, or by comparing the speciation data of the standard sample, which have been reported in the literature. As shown in the table, the certified total arsenic concentration agrees quite closely with the analytical results. The arsenic speciation data seem to show substantial discrepancies, especially for the normal level sample (SRM 2670N) compared to the elevated one (SRM 2670E), among different analytical laboratories. According to the literature [19], some of the discrepancies may be partially explained by matrix-induced incomplete chromatographic resolution and/or misidentifying and quantifying Cl^- as MMA or As(V). Besides, a redox transformation between arsenous acid and arsenic acid during the handling of the sample and/or on-column oxidation/reduction are also suspected to cause inconsistency between reported values. Table 2 may indicate that the proposed methods (μLC–MCN-ICP-MS and μLC–DIN-ICP-MS) can be used to analyze the ng ml^{-1} level of arsenic species in urine samples.

4. Conclusion

For purpose of establishing a miniaturized speciation technique, an anion-exchange μLC and ICP-MS hyphenation system with different nebulizers as interfaces was investigated for the differentiation of four arsenic species in urine samples. Experimental results reveal that DIN and MCN yielded a higher chromatographic resolution in arsenic speciation than obtained using a conventional nebulizer system. DIN is the best among the nebulizer systems tested, perhaps because of its minimal off-column dead

Table 2
The analytical results of certified reference materials

Samples	Certified values (ng ml^{-1})	Observed values (ng ml^{-1})				Sum
		As(III)	DMA	MMA	As(V)	
SRM2670N	60	–	–	–	–	
This work[a]		10.7±0.8	48±3	3.21±0.37	1.57±0.21	63±5
[20]		<4	48±2	18±4	<4	102.3
[21]		15±3	48±2	9.5±3.0	2.9±0.7	97±14
[22]		53±4	46±4	9.9±1.4	0.8±0.5	109±6
[23]		<2.5	52±2	16.0±0.6	ND	84±3
[24]		ND	49±5	11±3	ND	60±7
[25]		ND	48±2	7.4±0.7	ND	56±3
[26]		ND	48±2	9.8±0.3	1.3±0.2	56±3
SRM2670E	480±100	–	–	–	–	
This work[b]		11.6±0.7	48±5	3.77±1.31	427±14	490±13
This work[a]		14±2	43±4	4.01±0.38	426±16	486±12
[20]		<2	52±10	13±4	417±64	482.2
[21]		13±5	52±3	11±2	386±51	
[22]		44±9	35±9	5.0±3.6	406±153	489±154
[24]		ND	49±2	8.1±0.7	403±8	487±10
[25]		15±3	49±3	7±2	443±20	514±23
[26]		ND	52±4	16±4	430±56	487±54

[a] μLC–MCN-ICP-MS.
[b] μLC–DIN-ICP-MS.

volume and better transportation efficiency. Low-flow nebulizer assemblies (DIN and MCN) do not have better detection limits than the conventional assembly. The detection limits achievable with DIN were found to be comparable to those obtained with MCN. The applicability of the established systems to determine arsenic species in urine samples was tested as well.

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References

[1] H. Garraud, A. Woller, P. Fodor, O.F.X. Donard, Analusis 25 (1997) 25.
[2] W.R.L. Cairns, L. Ebdon, S.J. Hill, Fresenius J. Anal. Chem. 355 (1996) 202.
[3] K. Sutton, R. Sutton, J.A. Caruso, J. Chromatogr. A 789 (1997) 85.
[4] J.L. Todolí, V. Hernandis, A. Canals, J.M. Mermet, J. Anal. At. Spectrom. 14 (1999) 667.
[5] G. Zoorob, M. Tomlinson, J. Wang, J. Caruso, J. Anal. At. Spectrom. 363 (1995) 490.
[6] A. Waller, H. Garraud, J. Boisson, A.M. Dorthe, P. Fodor, O.F.X. Donard, J. Anal. At. Spectrom. 13 (1998) 141.
[7] L. Wang, S.W. May, R.F. Browner, S.H. Pollock, J. Anal. At. Spectrom. 11 (1996) 1137.
[8] H. Liu, A. Montaser, S.P. Dolan, R.S. Schwartz, J. Anal. At. Spectrom. 11 (1996) 307.
[9] J.A. McLean, H. Zhang, A. Montaser, Anal. Chem. 70 (1998) 1012.
[10] K. Falk, H. Emons, J. Anal. At. Spectrom. 15 (2000) 643.
[11] S.C.K. Shum, R.S. Houk, Anal. Chem. 35 (1993) 2972.
[12] J.F.B. Alder, G.F. Kirkbright, Spectrochim. Acta, Part B 35 (1980) 163.
[13] J.C. Fister, J.W. Olesik, Spectrochim. Acta, part B 16 (1991) 869.
[14] MCN-100 Micro-Concentric Nebulizer User's Manual, Cetac Technologies, Omaha, NE.
[15] A. Tangen, W. Lund, J. Chromatogr. A 891 (2000) 129.
[16] B.S. Sheppard, J.A. Caruso, D.T. Heitkemper, K.A. Wolnik, Analyst 117 (1992) 971.
[17] B.S. Sheppard, J.A. Caruso, D.T. Heitkemper, K.A. Wolnik, Analyst 117 (1992) 971.
[18] P. Thomas, K. Pereira, D. Koller, Analusis 25 (1996) 19.

[19] X. Wei, C.A. Brockhoff-Schwegel, J.T. Creed, J. Anal. At. Spectrom. 16 (2001) 12.
[20] D. Tsalev, M. Sperling, B. Welz, Analyst 123 (1998) 1703.
[21] W. Goessler, D. Kuehnelt, K.J. Irgolic, Determination of arsenic compounds in human urine by HPLC–ICP-MS, in: C.O. Abernathy, R.L. Calderon, W.R. Chappell (Eds.), Arsenic Exposure and Health Effects, Chapman and Hall, New York, 1997.
[22] D. Heitkemper, J. Creed, J. Caruso, J. Anal. At. Spectrom. 4 (1989) 279.
[23] J. Zheng, W. Kosmus, F. Pichler-Semmelrock, M.R. Moore, Analyst 123 (1998) 929.
[24] X.C. Le, M. Ma, Anal. Chem. 70 (1998) 1926.
[25] X.C. Le, M. Ma, Clin. Chem. 44 (1998) 539.
[26] E. Crecelius, J. Yager, Environ. Health Perspect. 205 (1997) 650.