CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY EQUIPMENT

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Abstract

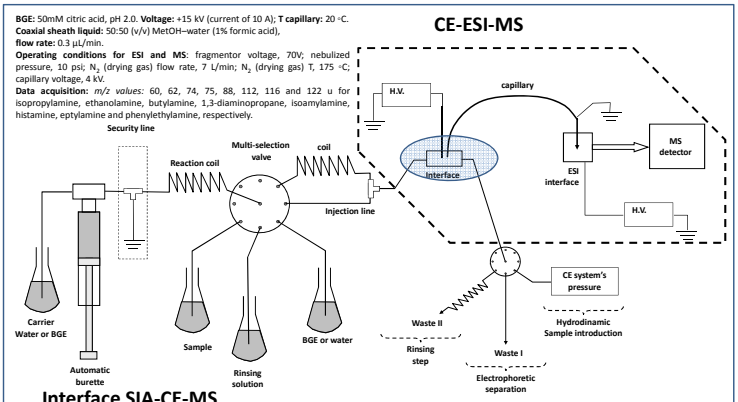
On-line coupling of an automated flow system with a commercially available **capillary electrophoresis** (CE) system with an **electrospray interface** (ESI) for **mass spectroscopic** (MS) detection is described.

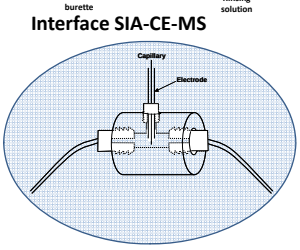
The peculiarities of CE–ESI-MS interfaces, in which a high electrical field must be applied to the capillary end where the sample is provided by the flow system, introduce significant difficulties for the appropriate work of the entire arrangement. Experimental strategies are proposed for achieving stable conditions for **on-line sample pre-treatment**, **conditioning of the separation capillary**, **sample injection**, as the **proper separation**. The versatility and robustness of the proposed arrangement is discussed, taken as example the separation of a variety of amines. Connection of the CE systems pressure to the automated flow system enables hydrodynamic introduction of sample with high precision.

The developed **hyphenated system** is of practical relevance as it opens an avenue for the simplification and automation of the whole analytical process required when using powerful CE–ESI-MS equipments.

Experimental Set-up

BGE: 50mM citric acid, pH 2.0. **Voltage:** +15 kV (current of 10 A); **T capillary:** 20 ºC.
Coaxial sheath liquid: 50:50 (v/v) MetOH–water (1% formic acid),
flow rate: 0.3 µL/min.
Operating conditions for ESI and MS: fragmentor voltage, 70V; nebulized pressure, 10 psi; N_2 (drying gas) flow rate, 7 L/min; N_2 (drying gas) T, 175 ºC; capillary voltage, 4 kV.
Data acquisition: *m/z values:* 60, 62, 74, 75, 88, 112, 116 and 122 u for isopropylamine, ethanolamine, butylamine, 1,3-diaminopropane, isoamylamine, histamine, eptylamine and phenylethylamine, respectively.



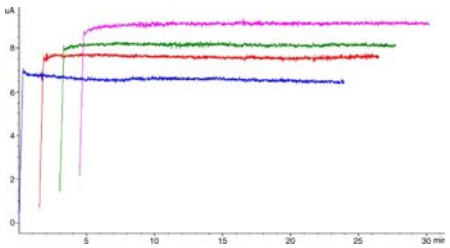


The automated flow system was coupled to a CE-MS equipment using a laboratory-made polyether ether ketone (PEEK)T-piece. To achieve this coupling the hardware configuration of the Agilent HP3D CE system had to be slightly modified because of the instrument needs to detect a vial in the sample position and immerse the capillary end and the surrounding electrode into the vial. After this movement the high voltage can be applied.
Moreover, the commercial system counts with a second elevator that allows to immerse the other capillary end into an outlet vial when only UV/vis detection but not MS detection is used.
To solve this conflict the cabling of the two elevators was interchanged. As a consequence the ChemStation detects a vial at the inlet position which in fact is located at the other position.

Results and discussion

Use of deionized water plugs for avoiding dissipative currents

One approach to avoid dissipative currents comprised insertion of deionized water between the interface and the automated flow system.



The intensity was stable and similar to that obtained working with the same capillary but without the automated flow system. The reproducibility of the current was in all cases lower than 0.8%.

For this configuration the amount of BGE located between the water and the interface must be enough for a complete CE separation. Considering the small volume of the interface, a constant flow of the BGE must be maintained to avoid possible changes in its composition.
It means that long separation times require long coils. The BGE must be introduced into the coil 2 in several steps to avoid that the BGE reaches the automatic burette due to dispersion in the coils.

The length of the reaction coil is determined by the following criteria:
(i) separation time, (ii) regeneration of the BGE in the interface (flow required during the electrophoretic separation) and (iii) water required to cut the electrical contact, which is a function of the nature of the BGE and the flow used.
Typically we use a coil of 300 cm for separation times of 20 min. To facilitate introduction of a new buffer and to reduce the analysis time, it is interesting to use the same BGE electrolyte as the carrier in the flow system. In this case, the cleaning and conditioning of the reaction coil will be faster.

Using water as carrier and placing the BGE for CE separation in the second coil several BGE frequently used in CE–MS were tested to check the stability and robustness of the experimental set-up.

Background electrolyte	Length (cm)	Flow rate (µl/min)	Separation time (min)
Citric acid at pH 2.0	100	10	20[a]
	150	0	5[b]
	150	10	30[a]
	200	10	40[a]
***Formic* acid at pH 2.0**	150	0	7[b]
	150	10	30[a]
Ammonium acetate at pH 5.0	150	0	1[b]
	150	10	4[b]
	150	50	10[a]
Ammonium acetate at pH 9.0	150	0	2[b]
	150	10	5[b]
	150	50	10[a]

[a] Time to observe a interruption of current
[b] Time to observe a diminution of 5% in the current

Use of air plugs for avoiding dissipative currents

An interesting alternative to insulate the electrode from the safety line is insertion of an air plug between the interface and the valve of the flow system in the reaction coil. We found that insertion of 100 µL of air is enough; however, for sake of robustness and safety we recommended to introduce a segment of 200 µL.
The most remarkable characteristic is the dramatic reduction in the length of the reaction coil from 300 cm (minimum) to 50–100 cm, which permits an increase in the length of the coil between the valve and the interface, but in turn resulting in longer separations.
With tubes of 0.5mm of diameter, the maximum separation time was established according to the flow, coil length and the nature of the BGE. The length of the coil must be longer than 150 cm when working at 10 µL/min in order to achieve CE separation in 30 min.

Injection modes

Most on-line continuous flow–CE studies reported so far use electrokinetic injection (EI) despite the fact that hydrodynamic injection (HI) is the generally preferred mode as it avoids discrimination problems.
For HI one of the positions of the selection valve is connected to the equipment's air system. The sample plug is located at the interface and it is HI into the capillary. Afterwards, the sample is removed from the interface by BGE and the potential is applied to the equipment to perform the CE separation.

The possibility of performing HI using the syringe pump is also evaluated. However, the precision of the results expressed as relative standard deviation (RSD) are higher than 5%.

	Hydrodynamic introduction				Electrokinetic introduction	
	Using flow system		Using CE system's pressure			
	% RSD (area)	% RSD (time)	% RSD (area)	% RSD (time)	% RSD (area)	% RSD (time)
Histamine	5.4	0.8	1.5	0.5	2.3	0.8
Phenylethylamine	6.7	0.7	1.9	0.6	3.1	1.5
Butylamine	5.0	1.1	1.1	0.5	3.4	1.3

Analytical figures of merit

The capability of the automated flow system to perform on-line sample pre-treatment has been used for preparation of samples and standards has been tested.

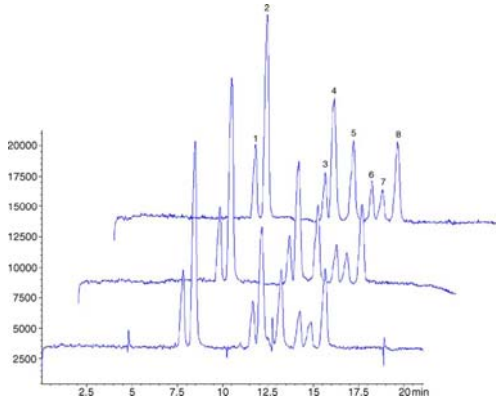
Standards at concentrations from 0.25 to 15 mg/L have been prepared by dilution of the most concentrated with water at different ratios, preparing a total volume of 500 µL of each diluted standard. The mixture was pumped to the interface and the injection was performed using HI.

The analytical features of the calibration curves demonstrate the high efficiency of the proposed alternative for coupling automatic flow systems to CE–MS equipment

	Y = a + bx	r	Sy/x	LOD (µg/L)	LOQ (µg/L)
Isopropylamine	a =–0.023 b = 0.4031	0.997	0.091	25	83
Ethanolamine	a = 0.0021 b = 0.6451	0.999	0.020	30	100
Butylamine	a = 0.0003 b = 0.7865	0.998	0.018	23	76
1,3-Diaminopropane	a =–0.0032 b = 0.4317	0.999	0.021	51	162
Isoamylamine	a =–0.0021 b = 0.7612	0.998	0.015	19	66
Histamine	a =–0.0003 b = 0.6541	0.999	0.013	20	66
Heptylamine	a = 0.0021 b = 0.5472	0.999	0.017	31	103
Phenylethylamine	a = 0.0011 b = 0.6420	0.999	0.011	17	57

Calibration graphs (n=8). *a*: intercept; *b*: slope; *r*: correlation coefficient; *Sy/x*: standard deviation of residual

Electropherogram obtained with the on-line coupling of an automated flow system with a CE–ESI-MS system. The peaks are: (1) 1,3-diaminopropane; (2) histamine; (3) ethanolamine; (4) isopropylamine; (5) butylamine; (6) isoamylamine; (7) phenylethylamine; (8) heptylamine.



Analysis of synthetic samples

The reliability of the method was evaluated by the analysis of several synthetic water samples. The recoveries of target analytes ranged from 98 to 105%. A paired t-test revealed the absence of statistical difference between the concentrations added and those found.

Sample	Analyte	Amount added (µg/L)	Amount found (µg/L)	Recovery (%)
1	Isopropylamine	250	245	98.0
	Ethanolamine	250	261	104.4
	Butylamine	250	247	98.8
	Diaminopropane	250	259	103.6
	Isoamylamine	250	250	100.1
	Histamine	250	253	101.2
	Heptylamine	250	254	101.6
	Phenylethylamine	250	247	98.8
2	Isopropylamine	300	302	100.7
	Ethanolamine	300	297	99.0
	Butylamine	300	298	99.3
	Diaminopropane	300	306	102.1
	Isoamylamine	300	301	100.3
	Histamine	300	299	99.7
	Heptylamine	300	296	98.7
	Phenylethylamine	300	294	98.0
3	Isopropylamine	400	396	99.2
	Ethanolamine	400	399	99.8
	Butylamine	400	405	101.2
	Diaminopropane	400	405	101.3
	Isoamylamine	400	394	98.5
	Histamine	400	403	100.8
	Heptylamine	400	393	98.3
	Phenylethylamine	400	410	102.5

Conclusions

The robust on-line coupling of an automated flow system with a CE–MS system introduces a new powerful integration of different analytical techniques.
Sample pre-treatment, including reaction and sample clean-up steps, can be performed in an automated fashion. The treated sample can then be separated in its components taking advantage of the high resolving power of modern CE. Finally, important advantages in the detection stage in terms of selectivity and sensitivity can be achieved by MS. Therefore, it may be expected that this hyphenation can be applied for the solution of a broad variety of different analytical problems.

Acknowledgements: S. Armenta acknowledges Spanish Ministry of Education and Science for the postdoctoral grant Ref. EX-2007-1257.