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Capillary electrophoresis of methylmercury with injection by sample stacking

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Abstract

A procedure for separation and quantitation of methylmercury by capillary electrophoresis using sample stacking as the injection technique is presented. The CE conditions have been optimized in order to separate the methylmercury from the excess cysteine peak and to concentrate large volumes of sample obtaining a low detection limit. Under the proposed operational conditions, the detection limit (S/N=3) was 12 ng g^{-1} and the limit of quantitation (S/N=10) was 20 ng g^{-1} with a linear range of 20–100 ng g^{-1} (as methylmercury in samples). The method was tested using different reference materials with a certified methylmercury content.

Keywords: Capillary electrophoresis; Sample stacking; Injection methods; Methylmercury; Organometallic compounds; Cysteine

1. Introduction

The application of capillary electrophoresis (CE) in the separation and determination of environmental contaminants, such as organometallic compounds, has been studied by various authors (see e.g. Ref. [1]), however no low detection limits were obtained.

On-column sample concentration in a single continuous support buffer, also known as sample stacking, was first used in capillary zone electrophoresis (CZE) by Mikkers et al. [2]. Sample stacking results from the movement of sample ions across a stationary boundary that separates the region of the injected sample buffer from the rest of the capillary containing the support buffer. Because of the matrix difference between the two regions, the ions experience a lower electric field in the support buffer region than in the sample region. Thus, the velocity of the ions decreases as they cross the stationary boundary. Therefore, the slower moving ions will 'stack up' into a smaller volume, thereby increasing the concentration in the sample zone.

The matrix differences that generate the different electric fields can be a change in pH and/or the concentration of support buffer [3–6] for a continuous buffer system. In the simplest form of sample stacking, a large plug of sample dissolved in water is introduced hydrodynamically into the capillary. The sample ions form narrow bands when they migrate into the region containing the concentrated support electrolyte. Moring et al. [4] have reported an increase by a factor of 10 in detection in CZE with sample stacking, although other authors have reported larger increases by as much as several hundred-fold [7,8].

This procedure, recently accomplished by Chien and Burgi [9,10], involves the following steps:

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(a) Introduction of the analyte in a low-conductivity buffer or water.

(b) Application of a high voltage which is reversed in polarity to that which will be used for the separation and causes the stacking of ions.

(c) Reversal of the polarity of the high voltage, reverts the conditions back to normal for the analysis.

(d) Separation and detection of the stacked ions.

Using this procedure, we evaluated the utility of the sample stacking technique for the analysis of methylmercury–cysteine complex [11] by CE. Following optimization of the method for performance and sensitivity, we evaluated the practical applicability using different Certified Reference Materials (CRMs).

Table 1
Optimal CE parameters

Applied voltage	30 kV
Pressure	50 mbar
Capillary temperature	35°C
Vial temperature	18°C
Buffer sodium borate 0.2 *M*	pH 8.24
Injection time	15 min
Sample volume	3.6 μl
Wavelength detection range	200–400 nm
Run time	20 min
Pre-conditioning	
Flush capillary (0.1 *M* NaOH)	5 min
Flush capillary (Milli-Q water)	3 min
Flush capillary (buffer)	3 min
Post-conditioning	
Flush capillary (Milli-Q water)	5 min

At the begining of each experimental session, the capillary was flushed with 0.1 *M* NaOH solution for 10 min.

2. Experimental

2.1. Instrumentation

An HP^{3D}CE capillary electrophoresis system (Hewlett-Packard, Palo Alto, CA, USA), equipped with a diode array detector was used in the experiments. The absorbance was recorded from 200 to 400 nm (using 200 nm as the monitoring channel and 280 nm as the reference channel).

Polyimide-coated fused-silica capillaries with an inner diameter of 75 μm and a total length of (a) 64.5 cm and (b) 100 cm were purchased from Composite (Tecknocroma, Barcelona, Spain). On-column detection was carried out at a position of (a) 56 cm and (b) 91.5 cm from the injection end.

The optimum parameters used for sample stacking summarized in Table 1, were obtained with the 100-cm capillary.

2.2. Procedures

Preparation of standards

Standards for calibration injections were prepared by mixing the appropriate amounts of the diluted methylmercury stock solution (0.5 $\mu g\, g^{-1}$ methylmercury), with 25 μl of 0.025% cysteine acetate and 75 μl of Milli-Q water.

Preparation of sample extracts

The appropriate amount of freeze-dried sample (usually between 1 and 5 g as a function of the methylmercury content expected) has to be submitted to a cleanup stage prior to methylmercury extraction. To this end 5 ml of water and 20 ml of acetone are added. Shake and centrifuge for 5 min. Repeat this step three times, then add 20 ml of toluene, shake and centrifuge for 5 min. The clean residue is now treated with 5 ml of hydrochloric acid–water (1:1). Add 15 ml of toluene, shake for 2 min and centrifuge for 5 min. Repeat this extraction stage twice and combine the toluene extracts. Back-extract the toluene phase with 5 ml of 0.00125% aqueous cysteine. Shake for 2 min and leave for approximately 15 min to obtain a clean phase separation. The aqueous extract can now be injected into the capillary electrophoresis system.

3. Results and discussion

3.1. Optimization of CE conditions

The CE conditions using sample stacking injection were adapted from the parameters previously optimized for CE by direct injection [12]. The following items were studied: (a) separation of the methylmer-

Table 2
Effect of operating buffer pH

Buffer	pH	Current (μA)	Apparent mobility (peak area/migration time) (cm^2/V s)$\times 10^6$	Area	Height
0.2 *M* Sodium borate[a]	7.49	9	7.21	1.017[c]	6.728[c]
0.2 *M* Sodium borate[a]	7.92	15.4	9.72	0.569	3.958
0.2 *M* Sodium borate[a]	8.24	27	6.02	1.086	5.471
0.2 *M* Sodium borate[a]	8.40	28	8.00	0.471	1.029
0.2 *M* Sodium borate[a]	8.75	40.8	7.40	0.233	0.763
0.2 *M* Sodium borate[a]	9.35	55	–	–	–
0.4 *M* Sodium borate[b]	8.24	27.8	–	–	–
0.2 *M* Sodium borate[b]	8.24	15	2.70	0.208	1.177
0.1 *M* Sodium borate[b]	8.24	6	3.17	0.156	0.903
0.04 *M* Borax[b]	8.25	53	–	–	–
0.2 *M* Sodium borate/0.04 *M* borax[b]	8.24	16	2.48	0.164	1.015

[a] 1 ppm Me–Hg; 30 kV; 40 mbar; 3-min. injection; capillary, 64.5 cm$\times$75 μm.
[b] 0.1 ppm Me–Hg; 30 kV; 50 mbar; 15-min. injection; capillary, 100 cm$\times$75 μm.
[c] Quantitation of the methylmercury peak overlapped with a peak of excess cysteine.

cury from the excess cysteine peak; (b) concentration of large volumes of sample to obtain a low detection limit.

The first experiments were carried out using a capillary of 64.5 cm$\times$75 μm I.D. and the optimum conditions were later adapted to a capillary of 100 cm$\times$75 μm I.D..

pH of the operating buffer

During sample stacking of analytes in a sample matrix of low ionic strength, the pH of the operating electrolyte can greatly affect the efficiency and resolution.

Preliminary experiments were conducted in order to separate the organomercury compound at different pH values. The results obtained are shown in Table 2. As can be seen with an increase in pH (pH 9.35) the peak corresponding to methylmercury was not detected. This could be due to overlapping of the methylmercury peak and of one of the excess peaks of cysteine and by-products. A hypothetical decomposition of the methylmercury cysteine complex could also be produced at pH>9 [12,13]. The best separation and area of the methylmercury peak was obtained at pH 8.24. At this fixed pH, different buffer solutions were tested. Using 0.4 *M* sodium borate and 0.04 *M* borax methylmercury could not be detected. A 0.2 *M* sodium borate–0.04 *M* borax buffer behaved as the 0.2 *M* sodium borate buffer.

Applied voltage

The electroosmotic flow-rate, as well as the velocity of a migrating ion, is proportional to the applied voltage used for separation. Therefore, the analysis time can be shortened by increasing the applied voltage. In this work, the voltage was varied

Table 3
Sample volume injected using a capillary of 64.5 cm$\times$75 μm

Injected volume (μl)	Injection time (min)	Area (peak area/migration time)	Capillary filling (%)
0.34	1	0.142	13
1.01	3	0.536	40
1.68	5	0.726	68
2.0	6	0.810	82
2.1[a]	5	0.992	85

[a] Pressure, 50 mbar.

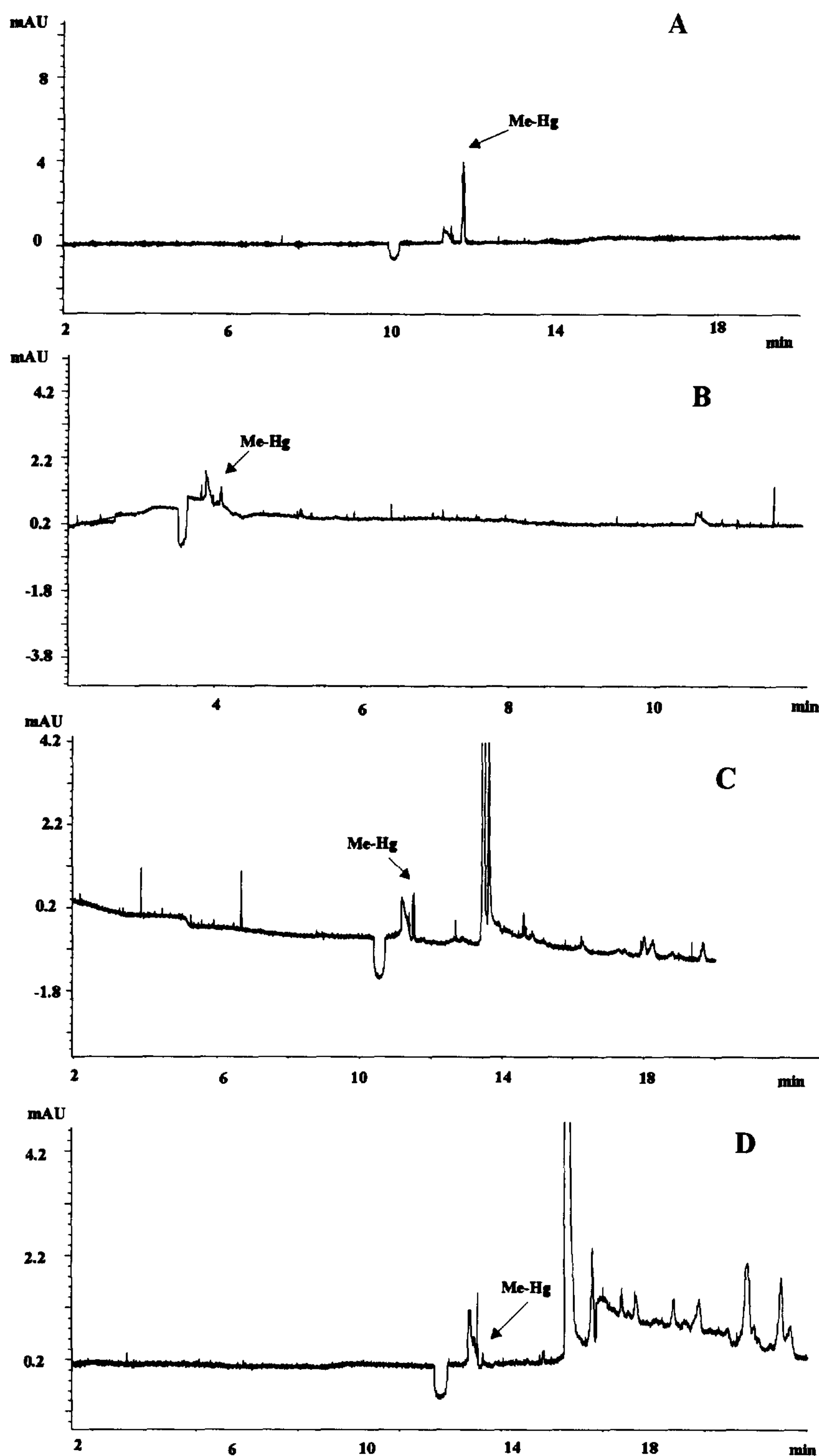

Fig. 1. Electropherograms for the separation of methylmercury for different injection modes. Run conditions: (A) Without sample stacking injection, 10 $\mu g\ g^{-1}$ Me–Hg; capillary, 100 cm×75 μm I.D.; voltage, 30 kV; pressure, 40 mbar; injection, 25 s. (B) Without sample stacking, 1 $\mu g\ g^{-1}$; capillary, 64.5 cm×75 μm I.D.; voltage, 30 kV; pressure, 40 mbar; injection, 12 s. (C) Sample stacking injection, 0.1 $\mu g\ g^{-1}$ Me–Hg; capillary, 100 cm×75 μm I.D.; voltage, 30 kV; pressure, 50 mbar; injection, 15 s. (D) As in C, 12 $ng\ g^{-1}$ Me–Hg.

between 15 and 30 kV. The optimum value was fixed at 30 kV producing a current of 15–20 μA.

Sample volume by sample stacking injection

To increase the amount of analytes injected into the column, while retaining high resolution, we applied a negative high voltage at the injection end. When the current was within 95% of the support buffer current, the sample support was almost completely out of the column, and the polarity of the electrodes was reversed.

Different sample volumes were studied and the data obtained are summarized in Table 3. The experiments were carried out with a 40 mbar pressure on the 64.5 cm×75 μm I.D. capillary at 30 kV.

The peak-area signal increased with the injected volume and no loss of resolution was observed. These optimal conditions were adapted to the 100 cm×75 μm capillary to give the final operating conditions summarized in Table 1.

Fig. 1 shows a comparison of four electropherograms obtained when a standard MeHg–cysteine complex was analyzed without sample stacking injection (Fig. 1A,B) and with the working conditions selected for sample stacking injection (Fig. 1C,D) .

3.2. Quantitation

Calibration lines used in this paper were drawn at four concentration levels in the range 20–100 ng g^{-1} obtaining a correlation coefficient of 0.998 with a residual standard deviation of 0.0499, giving a line $y=mx+b$, where $m=19.7081$ and $b=-9.4832$ E-02, x being the concentration of methylmercury in ng g^{-1} and y the peak area/migration time ratio. The limit of quantitation was 20 ng g^{-1} for a signal-to-noise ratio of 10:1, whereas the detection limit was 12 ng g^{-1} for a signal-to-noise ratio of 3:1.

Repeatability data for five successive injections of a methylmercury standard (100 ng g^{-1}) were: average migration time=13.64±0.23; coefficient of variation=1.7% and average peak area=2.57±0.33; coefficient of variation=12.9%.

Reproducibility data were determined on different days ($n=11$): average migration time=13.80±0.47; coefficient of variation=3.43% and average peak area=2.58±0.35; coefficient of variation=13.8%. It was observed that coefficients of variation were similar to those of within-day repeated injections.

3.3. Analysis of real samples

The method was tested using different Reference Materials with a certified methylmercury content. DORM-1 was supplied by the National Research Council of Canada and tuna CRM no. 464 and CRM no. 463 by Community Bureau of Reference (BCR). A human hair sample, spiked with methylmercury, was obtained from the International Atomic Energy Agency of Vienna, Austria during an intercalibration exercise in 1994, however certified values were not yet available at the time these measurements were performed. The results obtained are given in Table 4. Fig. 2 shows the electropherogram obtained after injecting an extract of DORM-1 under optimum conditions for sample stacking.

4. Conclusions

The presented results show that this sample stacking injection technique for CE could be used to improve detectability in the separation and determi-

Table 4
Results of methylmercury determination

Sample	Sample mass (g)	n	Me–Hg content (μg g^{-1})	Me–Hg found (μgg^{-1})
DORM-1	0.500	6	0.785±0.060[a]	0.777±0.061
CRM-463	0.09	4	3.04±0.16[a]	2.83±0.28
CRM-464	0.06	4	5.50±0.17[a]	5.20±0.13
Human hair	0.02	4	24.7±2.1[b]	22.9±1.7

[a] Certified values.
[b] Provisional value obtained by GC-ECD in our laboratory.

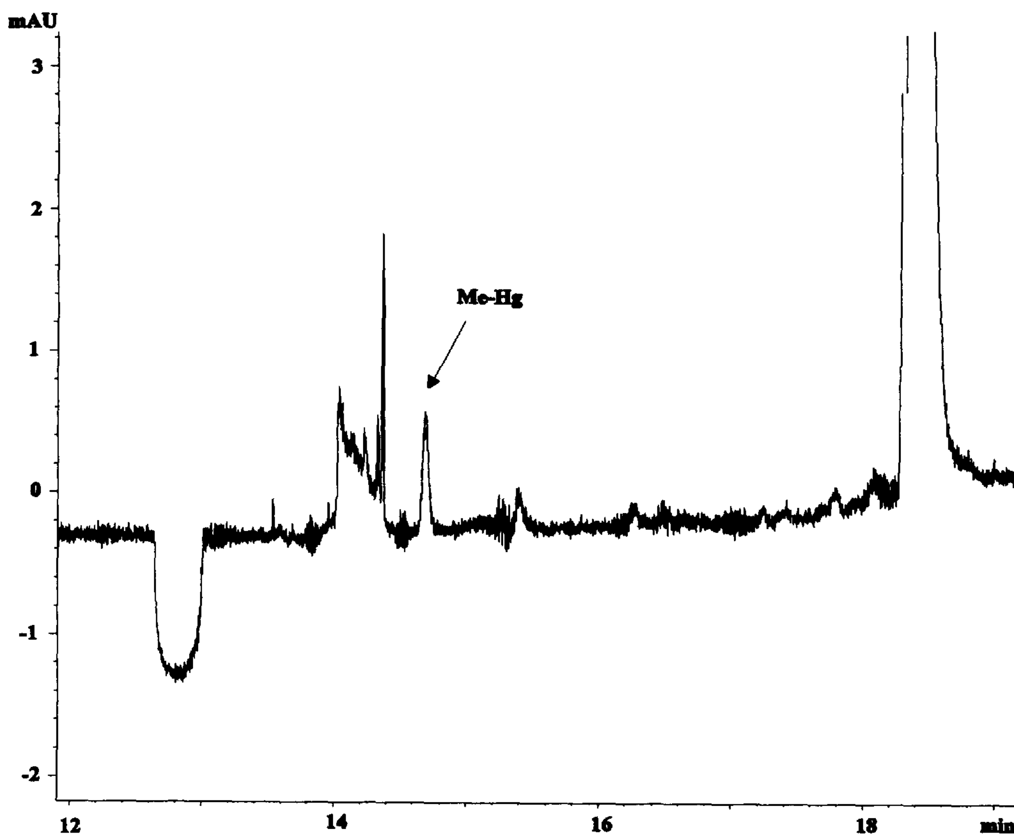

Fig. 2. Electropherogram obtained after extraction of Me–Hg from a Certified Reference Material (DORM-1). Electrophoretic conditions: As in Fig. 2C.

nation of methylmercury from biological and environmental samples. It has been shown that the detection limit is lowered by a factor of 10 [12–16] as compared with a conventional CE injection technique where the sample matrix was conditioned to a lower ionic strength than the support buffer. With the proposed conditions CE could be advantageously applied in the analysis of real samples and speciation studies

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