SEPARATION OF LEAD AND BARIUM ON ELECTROPHORESIS MICROCHIPS COUPLED WITH CONTACTLESS CONDUCTIVITY DETECTION

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Abstract: *The main metals found in gunshot residue are lead, barium and antimony, originated from the primer of the cartridge. Their detection is an important field of study in forensic sciences. Therefore, this study proposed the detection and separation of barium and lead by electrophoresis glass microchips coupled with contactless conductivity. The best conditions to separate the metals were 30 mM lactic acid and 10 mM histidine as a running buffer, 1200 kHz and 20 V$_{peak-to-peak}$. The separation was obtained within ca. 120 s, and initial studies showed the presence of barium in a primer´ sample, demonstrating to be a promising tool for the analysis of gunshot residues. The proposed method can be useful for forensic studies.*

Key-Words*: microchip electrophoresis, gunshot residue, forensic, metals*

Introduction: Gunshot residue (GSR) is produced during the discharge of a firearm. It is used for criminal investigation and its analysis is one important test in forensic science. Lead, barium and antimony are originated from the primer of the cartridge, and they are the main metals found in the GSR. Recently, some studies have demonstrated the analysis of organic and inorganic ions[1,2] and the metals[3] from GSR by capillary electrophoresis and inductively coupled plasma-optical emission spectroscopy, respectively. The main goal of this current study is to detect and to separate the metals Ba^{2+} and Pb^{2+} using microchip electrophoresis (ME) devices coupled with capacitively coupled contactless conductivity detection (C^{4}D) to achieve a cheaper and faster alternative for ballistics analysis.

Experimental: Experiments were performed using a Quad HV microchip electrophoresis system (model ER455) supplied by eDAQ (Denistone East, NSW, Australia). The electrophoretic separations were carried out on a commercial glass microchip model ET190 from Micronit Microfluidcs (Enschede, Netherlands). The electrophoresis chip layout was composed of two channels arranged in a double-T geometry with gap of 100 µm. The sampling and separation channels were 7.0 mm and 85 mm, respectively. The width and height of both channels were 100 and 10 µm, respectively. Two pairs of sensing electrodes (200 µm wide × 500 µm long × 200 nm thick spaced by 250 µm) were positioned at 70 mm from the channel intersection. All channels were preconditioned with 0.1 M NaOH (15 min) followed by rinsing steps with ultrapure water and running electrolyte during 5 min each. Sample was introduced inside microchannels through gated injection protocol. For this purpose, voltages of 1.2 kV and 1.8 kV were applied to the sample and buffer reservoirs, respectively, keeping the other reservoirs grounded. To introduce discrete sample zones, the voltage applied to buffer reservoir was floated during 2 s and immediately reestablished to avoid sample leakage. For contactless conductivity measurements, the operational frequency and the excitation voltage were optimized and the best response was achieved applying a 1200-kHz sinusoidal wave with excitation voltage of 20 V$_{peak-to-peak}$.

Results and discussion: The running buffer composition and operational parameters of C^{4}D system were initially optimized to achieve the best analytical response. The separation of barium and lead was performed using a running buffer composed of lactic acid and histidine. The optimum condition for the separation of both compounds was found using 30 mM lactic acid and 10 mM histidine. For contactless conductivity measurements, the operational frequency and the excitation voltage were optimized and the highest signal-to-noise ratio was achieved applying a 1200-kHz sinusoidal wave with excitation voltage of 20 V$_{peak-to-peak}$. In a preliminary test, mixtures of both metals in different concentrations were injected and analyzed as mentioned. As it can be seen in Figure 1A, the signal intensity raised proportionally to the concentration, as expected. For barium, the proposed approach offers linear behavior for a concentration range from 120 to 360 µM. For lead, the linear range varied from 200 to 600 µM. To demonstrate the feasibility for applications in ballistic studies,

the residue of a primer was collected, diluted in the running buffer and analyzed on ME-C^{4}D system. As displayed in Figure 1B, barium was successfully detected in the model sample. Its presence was confirmed by spiking a standard solution. On the other hand, lead was not detected using the optimized conditions. However, further experiments are required to improve the detectability levels or to assure its solubility prior to analysis.
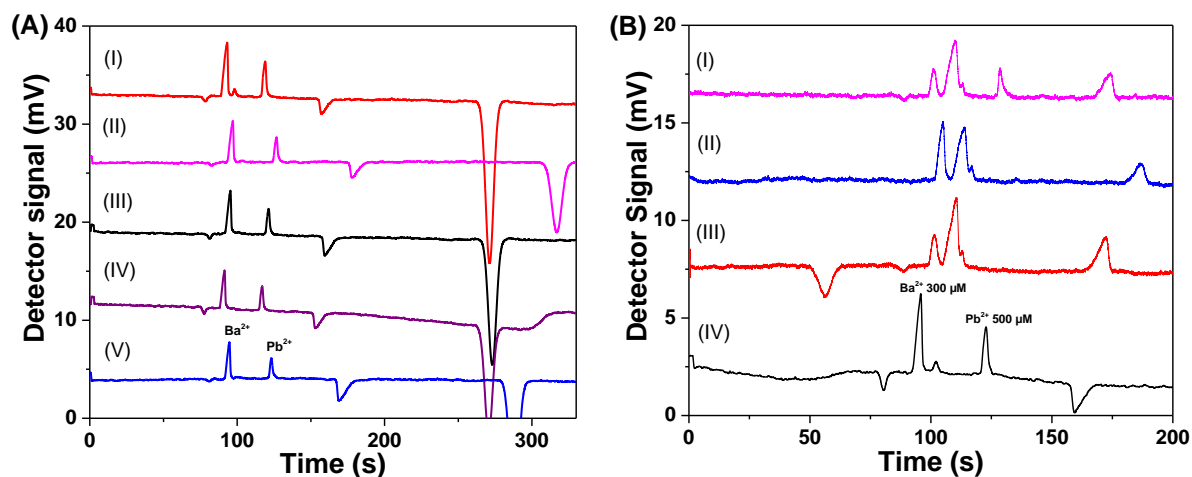

Figure 1. Electropherograms showing the separation and detection of Ba^{2+} and Pb^{2+}. **(A)** Ba^{2+} and Pb^{2+} at different concentrations: (I) 360 µM Ba^{2+} and 600 µM Pb^{2+}, (II) 300 µM Ba^{2+} and 500 µM Pb^{2+}, (III) 240 µM Ba^{2+} and 400 µM Pb^{2+}, (IV) 180 µM Ba^{2+} and 300 µM Pb^{2+} and (V) 120 µM Ba^{2+} and 200 µM Pb^{2+}. **(B)** Primer´ sample analysis: (I) Primer´s sample with 500 µM Pb^{2+}, (II) Primer´ sample with 300 µM Ba^{2+}, (III) Primer´ sample and (IV) Standard solution. Running buffer: 30 mM lactic acid and 10 mM histidine. Electrokinetic control was performed applying 1.2 kV and 1.8 kV. Detection conditions: 1200 kHz, 20 V$_{pp}$.

Conclusion: ME-C^{4}D devices has demonstrated to be a simple and efficient tool for forensic analysis. The metals can be easily separated within ca. 120 s. The next steps will be the detection of lead in primer´ sample, and the analysis of real GSR samples.

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