ANALYSIS OF BIOPHARMA RAW MATERIALS BY ELECTROPHORESIS MICORCHIPS WITH CONTACTLESS CONDUCTIVITY DETECTION

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INTRODUCTION

Detailed information concerning the composition of the raw materials employed in the production of biologics is important for the efficient control and optimization of bioprocesses. Vitamins are present at very low concentrations in biopharma raw materials and are usually determined by HPLC with a UV detector or a photodiode array spectrometer (PDA). For the analysis of metal cations, inductively-coupled plasma (ICP) methods are used with various detection systems such as atomic emission spectrometry (AES). Microchip-based electrophoresis represents a cost-effective and promising tool for application in the analysis of raw materials in biologics since analysis times can be reduced to seconds and high separation efficiencies can be achieved using extremely low volume samples (a few µL), minimal reagent consumption and waste generation, low cost/disposability, portability and ease of mass-production. additionally, Capacitively Coupled Contactless Conductivity Detection (C^4D) offers a rather simple and yet sensitive method for detection of ionic species with no need for sample derivatization. There is no physical contact of the detection electrodes with the electrolyte solution. Therefore, the integration of this detection mode within the analytical system is rather simple.

Though the analysis of real samples with electrophoresis microchips remains challenging, a significant number of publications dealing with real food samples has been published in recent years [1-2]. However, to the best of our knowledge, this is the first report of the analysis of vitamins and metal ions in real biopharma raw materials using electrophoresis microchips.

SET-UP

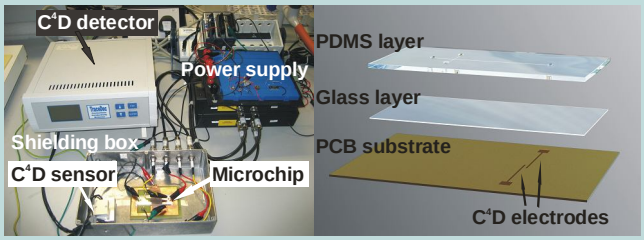

Figure 1. Picture showing the system setup (left). Layout of the hybrid PDMS/glass microchip and the C4D electrodes (right). Channel dimensions: 45 µm x 50 µm.

MICROHIP & ELECTRODE FABRICATION

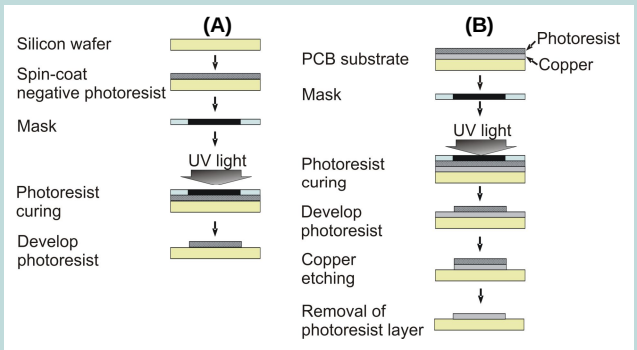

Figure 2. Scheme showing the fabrication of the master for the production of the PDMS layer with embedded microchannels (A) and the fabrication of the C4D electrodes (B).

RESULTS

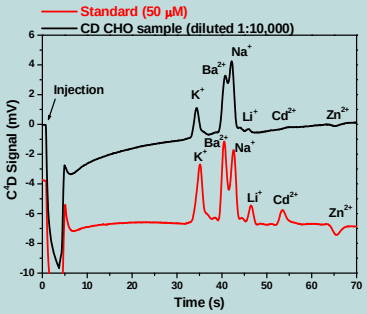

Figure 3. Electropherogram for 6 metals in a standard solution (50 µM each) and in a commercial CD-CHO sample. Run buffer: 10 mM MES/His (pH 6.0). Effective length: 4.0 cm. Injection voltage: 0.8 kV (2 s). Separation voltage: 1.2 kV. C^4D parameters: frequency, 300 kHz; excitation voltage, 20 $V_{peak-to-peak}$.

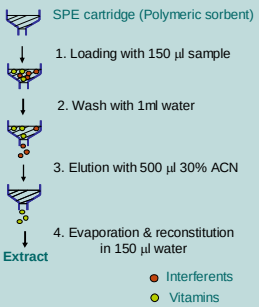

Figure 4. Solid Phase Extraction (SPE) method developed for extraction of water-soluble vitamins from biopharma raw-material samples.

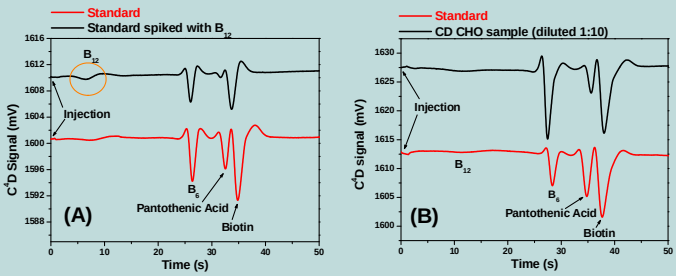

Figure 5. Electropherograms showing the separation of vitamins in a standard solution (10 µM each) before and after spiking with B_{12} (A) and in a commercial CD-CHO sample (B). Run buffer: 30 mM lactic acid (pH 2.6). Effective length: 3.7 cm. Injection potential:0.8 kV (1.5 s). Separation potential: 1.2 kV. C^4D parameters: frequency, 300 kHz; excitation voltage, 20 $V_{peak-to-peak}$.

CONCLUSIONS

The application of electrophoresis microchips with C^4D detection to the ultra-fast analysis of water-soluble vitamins and metals in raw materials used for the production of biologics was demonstrated. Successful separation of six metal cations in commercial CD-CHO samples, with no sample prep, was achieved in less than 70 s. Likewise, separation of four water-soluble vitamins was achieved in less than 40 s after SPE.

REFERENCES

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