

# ANALYSIS OF BIOPHARMA RAW MATERIALS BY ELECTROPHORESIS MICROCHIPS WITH CONTACTLESS CONDUCTIVITY DETECTION

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## ABSTRACT

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. We demonstrate the application of electrophoresis microchips with capacitively-coupled contactless conductivity detection (C<sup>4</sup>D) to the analysis of water-soluble vitamins and metal cations in raw material solutions that are subsequently fed into bioreactors for the production of biologics.

**KEYWORDS:** Electrophoresis microchip, Contactless conductivity detection, Biopharma raw materials, Vitamins, Metal cations

## INTRODUCTION

The analysis of vitamins in industry is typically performed using high-performance liquid chromatography (HPLC) with single-wavelength UV detection (or a photodiode array, PDA, spectrometer), whereas for the analysis of metal cations, inductively-coupled plasma (ICP) methods are used with various detection systems such as atomic emission spectrometry (AES). Compared to those methods, the use of electrophoresis microchips represents a cost-effective and promising tool for application in the analysis of raw materials in biologics: analysis times can be reduced to seconds and high separation efficiencies can be achieved using extremely small volume samples (a few  $\mu\text{L}$ ), and minimal reagent consumption/waste generation. Additionally, the use of C<sup>4</sup>D offers a rather simple and sensitive method for detection of ionic species with no need for sample derivatization. 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.

## EXPERIMENTAL

A commercially available C<sup>4</sup>D detector (TraceDec, Austria) was integrated with a hybrid PDMS/glass microchip and copper sensing electrodes (Figure 1). The copper electrodes were fabricated in-house using a commercially available printed circuit board (PCB) (Figure 1D). This fabrication procedure is simple, inexpensive and

enables the construction of very robust sensing electrodes that can be reused multiple times.

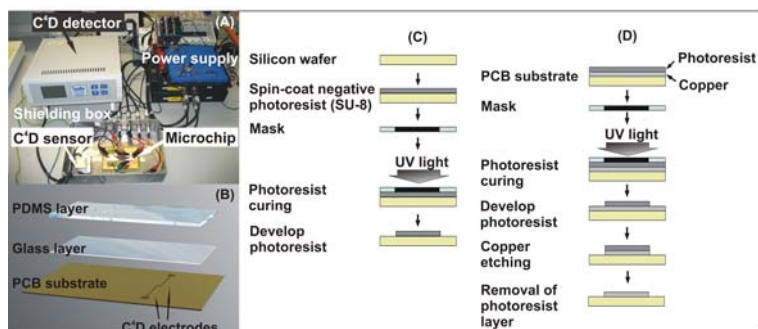


Figure 1. System setup (A) and layout of the hybrid PDMS/glass microchip and the  $C^4D$  electrodes (B). Process schemes for the fabrication of the master used in the production of the PDMS layer with embedded microchannels (channel dimensions:  $45\ \mu\text{m} \times 50\ \mu\text{m}$ ) (C), and the fabrication of the  $C^4D$  electrodes (D).

## RESULTS AND DISCUSSION

Separation of six metal cations in commercially available biopharma raw materials is demonstrated in Figure 2. Commercially available chemically-defined Chinese hamster ovary (CD-CHO) media samples were analyzed after sample dilution of 1:10,000.

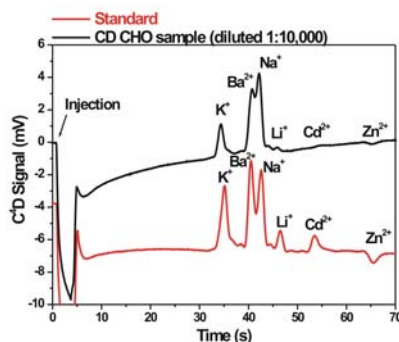


Figure 2. Electropherogram for 6 metals in a commercial CD-CHO sample. Electropherogram obtained with a standard solution ( $50\ \mu\text{M}$  each) is shown for comparison. Run buffer:  $10\ \text{mM}$  MES/His (pH 6.0). Effective length:  $4.0\ \text{cm}$ . Injection voltage:  $0.8\ \text{kV}$  ( $2\ \text{s}$ ). Separation voltage:  $1.2\ \text{kV}$ . MES = 2-(*N*-Morpholino)ethanesulfonic acid, His = Histidine.

A solid-phase extraction method (SPE) was also developed for the extraction of water-soluble vitamins from CD-CHO media by using a SPE cartridge based on a polymeric sorbent. The vitamins retained in the SPE cartridge were eluted with a

mixture of 30 % acetonitrile in water after a washing step. Eluting solvents were then evaporated and the resulting sample was reconstituted in water. The analysis of four water-soluble vitamins in standard mixtures is presented in Figure 3A before and after spiking the standards with vitamin B<sub>12</sub>. Application of the SPE method and the separation method developed for vitamins to a commercial CD-CHO sample is presented in Figure 3B. Prior to analysis, the vitamin extract, reconstituted in water after SPE, was further diluted 1:10.

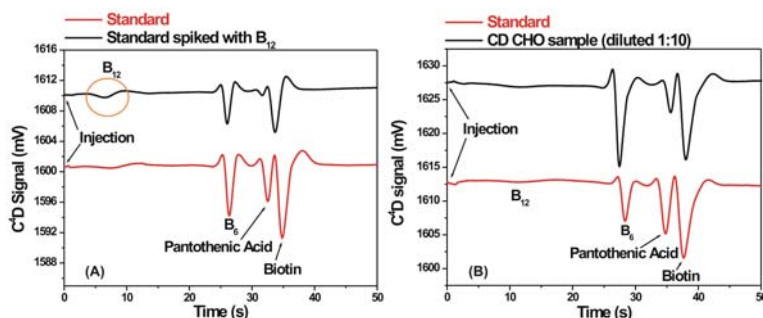


Figure 3. Electropherograms showing the separation of vitamins in a standard solution (10  $\mu$ M each) before and after spiking with B<sub>12</sub> (A) and in a commercial CD-CHO sample (B). Run buffer: 30 mM lactic acid (pH 2.6). Effective length: 3.7 cm. Injection potential: 0.8 kV (1.5 s). Separation potential: 1.2 kV.

## CONCLUSIONS

The application of electrophoresis microchips with C<sup>4</sup>D 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 was achieved in less than 70 s. Likewise, separation of four water-soluble vitamins was achieved in less than 40 s.

## ACKNOWLEDGEMENTS

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