Analysis of Water-soluble Vitamins in Biopharma Raw Materials by Electrophoresis Micro-chips with Contactless Conductivity Detection

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INTRODUCTION

Micro-chip-based electrophoresis represents a 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**, **minimal reagent consumption and waste generation, low cost/disposability, portability and ease of mass-production** [1].

Capacitively Coupled Contactless Conductivity Detection (C^4D) offers a rather **simple** and yet **sensitive** method for detection of ionic species [2]. 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. Furthermore, the **background noise** is significantly **reduced** leading to **lower detection limits** than the conventional contact conductivity detection.

Vitamins are present at very low concentrations in biopharma raw materials and are usually determined using HPLC and CE methods [3]. Electrophoresis micro-chips are a very good alternative to these techniques due to the shorter analysis time and yet very good resolution, among others.

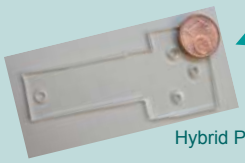
[1] J. West, M. Becker, S. Tombrink, A. Manz, *Anal. Chem.* (**2008**), 80, 4403-4419.
[2] P. Kubáň, P. C. Hauser, *Electroanalysis* (**2004**), 16, 2009.
[3] M. L. Marszall, A.Lebiedzinska, W. Czarnowski, P. Szefer, *J. Chromatogr. A* (**2005**), 1094, 91-98.

MICRO-CHIP FABRICATION

1. **MASK FABRICATION:** CAD design and high resolution mask printing.

2. **CLEAN ROOM:** master fabrication.

3. **MICRO-CHIP FABRICATION: PDMS** (polydimethylsiloxane) layer curing and reversible sealing of PDMS layer to a glass substrate.



Hybrid PDMS/glass micro-fluidic device

ACKNOWLEDGMENTS

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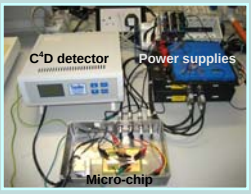
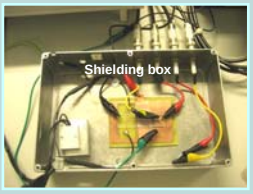
SET-UP



View of the micro-chip shielding box, the C^4D detector and the power supplies.

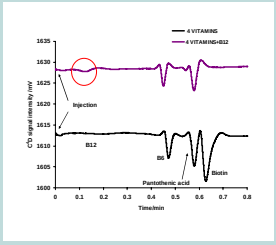


View of the micro-chip setup and C^4D sensor.

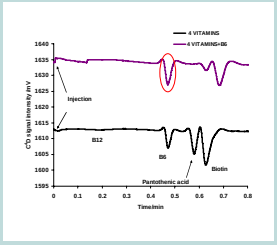
RESULTS

Electropherograms showing the separation of vitamins in a standard solution of B_{12}, B_6, pantothenic acid and biotin (10 µM each) after spiking B_{12} **(a)**, B_6 **(b)**, pantothenic acid **(c)**, or biotin **(d)**. Run buffer: 30 mM Lactic acid (pH 2.6). Micro-chip channels: effective length 3.7 cm, depth 45 µm, width 50 µm. Injection potential: 0.8 kV for 1.5 s. Separation potential: 1.2 kV.

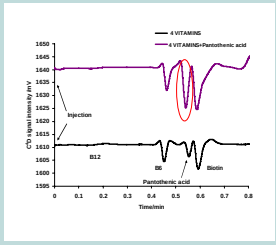
(a)



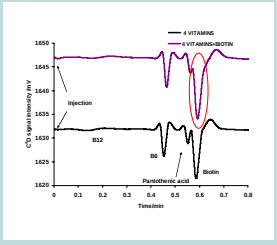
(b)

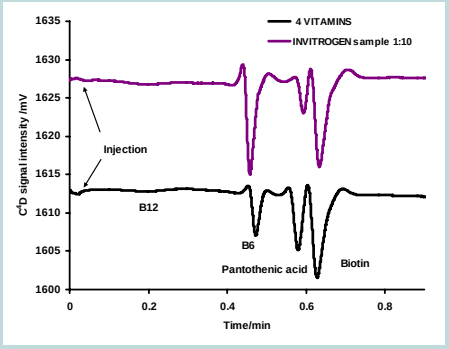


(c)

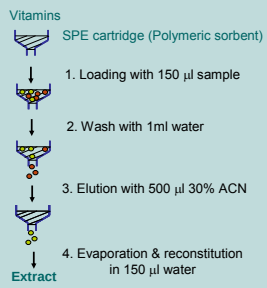


(d)





Solid phase extraction (SPE) of a real sample (INVITROGEN)



C^4D parameters: frequency, 300 kHz; excitation voltage, 20 $V_{peak\text{-}to\text{-}peak}$.