micrux
FOOD

Application Notes

www.micruxfluidic.com

TEA ANALYSIS ON MICROFLUIDIC ELECTROPHORESIS CHIPS WITH ELECTROCHEMICAL DETECTION

empresa innovadora de base tecnológica reconocida por ANCES e!bt

Tea analysis on microfluidic electrophoresis chips with electrochemical detection

» INTRODUCTION

Tea is the third most consumed drink in world after water and coffee. It is prepared from plant shoots or leaves from *Camellia Sinensis.* All the varieties of this drink, available in the market (white, green, red and black) come from this plant and the main difference between them is the oxidation degree of the shoots and leaves.

Main healthy properties of the tea are related with the **antioxidants content**, specific with polyphenols (flavonoids and phenolic acids, between others) responsible of the anti-inflammatory, anti-carcinogenic, etc… properties. Cathequines such as epicathequine are predominant in tea composition, but flavones as rutin (RUT) and quercetin (QUER), as well as the phenolic acids rosmarinic (RA) and caffeic (CA) could be also present in this drink.

Several **strategies** have been used for their analysis, such as: the Folin Ciocalteau method, HPLC and capillary electrophoresis (CE) with different detection systems.

Microchips electrophoresis (MEs) in combination with **electrochemical detection** (ED) could be a promising alternative to those methods because of their fast analysis time and low cost. Although separation and detection can be performed in MEs, pre-treatment of the sample use to take place *off-chip*.

A ready-to-use portable microfluidic system (MicruX® iHVStat) can be a powerful solution for using **microchips electrophoresis** (ME) and electrochemical detection in the analysis of different commercial teas: Green (TG), *Ceilan* (green) (TC), *Hornimans* (red) (TH) and Black (TNFB). Thus, it has been proposed a strategy to the identification of those compounds in teas in less than 85 s using a fast and simple sample pre-treatment carried out *off-chip*.

» EXPERIMENTAL

Samples: Standard solutions of rutin, quercetina, rosmarinic acid, caffeic acid and gallic acid. Commercial teas: Green, Ceilan, Hornimans and Black.

Sample volume: <100 pL.

Instrumentation: MicruX® HVStat / iHVStat. Holder DC series.

Microfluidic device: SU8/Pyrex microchips with integrated Pt electrodes (*MCE-SU8-Pt001T*).

Conditioning: 0.1 M NaOH – 30 min.
Deionized water – 15 min.
Buffer solution – 10 min.

Sample pretreatment: 2 g of each tea were used to prepare the corresponding infusion in 200 mL of water with the aid of a hot plate. These infusions were filtered through filter paper and then with a syrengin Nylon filter. A dilution of the tea was made previous to the injection in the microchip (Tea:H_2O (1:7, v/v)). A scheme of that procedure is shown in ***Figure 1***.

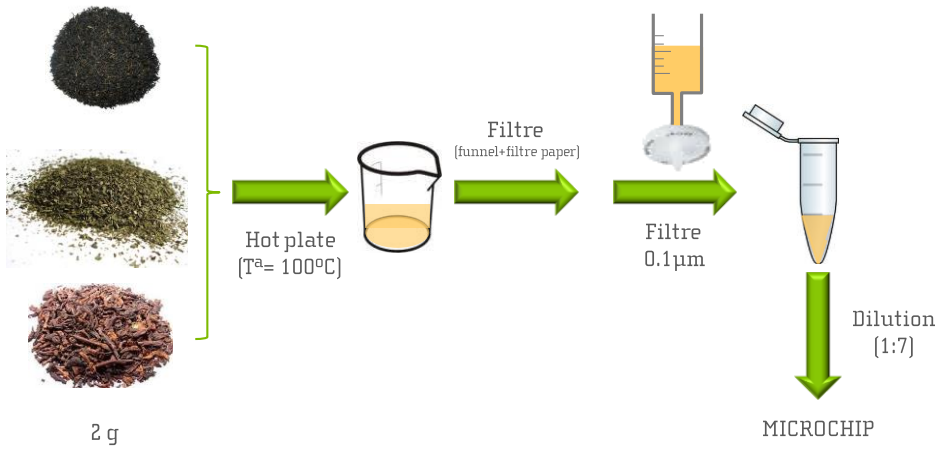

Figure 1. Off-chip pretreatment of the tea samples

» RESULTS & DISCUSSION

Before tea samples analysis, the microfluidic electrophoresis system has been used for the identification of the different compounds of a sample mixture containing the flavones rutin (RUT) and quercetin (QUER), and the phenolic acids: rosmarinic (RA), caffeic (CA) and gallic (GA).

A sample mixture consisting on RUT 50 µM, QUER 100 µM, RA 100 µM, CA 100 µM and GA 100 µM has been analyzed. For the injection, separation and detection of these compounds an injection voltage of +850 V was applied during 5 seconds, the separation voltage was +900 V and the detection potential was +0.90 V. In ***Figure 2,*** the electropherogram for the separation of that mixture is showed.

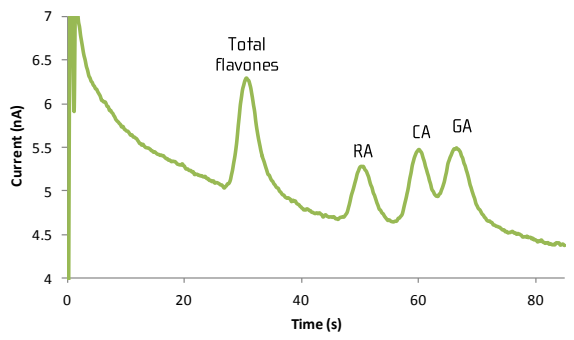

Figure 2. *Electropherograms for a sample mixture of 50 µM RUT and RA and 100 µM QUER, CA and GA. Conditions: V_{inj} = +850 V, t_{inj}= 5 s, V_{sep} = +900 V, E_D=+0.90 V (vs. Pt) and 20 mM MES-NaOH, pH =5.0 as running buffer*

In the working buffer solution, the flavones RUT and QUER are neutral compounds (pk_a 6.74 and 6.62 respectively) and they cannot be separated. For that reason, just one peak is observed for the two compounds with a migration time of 32s, whereas the separation of phenolic compounds (anionic) RA, CA and GA, was possible in less than 85 s.

Some of these compounds are present in tea samples, so that, these experimental conditions have been used for the identification of these compounds in four commercial teas.

As it was mentioned in the introduction, all the teas come from the same plant and the main difference between them consist on the antioxidants content. White tea comes directly from the flowers and it has not been subjected to any fermentation process, so antioxidants content is expected to be the highest one followed by green, red and black tea.

In ***Figure 3***, the electropherograms registered for the four varieties of tea and the sample mixture are presented. Sample preparation for each tea was made following the procedure described in ***Figure 1***. This step do not last more than 20 min.

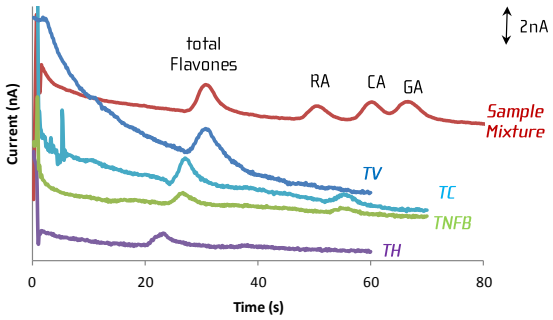

Figure 3. *Electropherograms for different varieties of tea: Green tea (TV), Ceilan tea (TC), Black tea (TNFB) and Hornimans tea. Conditions Sample mixture of 50 µM RUT and RA and 100 µM QUER, CA and GA, V_{inj} = +850 V, t_{inj}= 5 s, V_{sep} = +900 V, E_D=+0.90 V (vs. Pt) and 20 mM MES-NaOH, pH =5.0 as running buffer*

The four teas analyzed presented one peak corresponding to the total flavones content with a migration time of 30 s.

As it was expected, the highest concentration of total flavones corresponds to the two varieties of green tea: TV (1.36±0.04 nA) and TC (1.1±0.2 nA). Hornimans tea (TH) and black tea (TNFB) yield to an exhaustive fermentation process. For that reason total flavones decrease in comparison with the two varieties of green tea (TH: 0.4±0.1 nA; TNFB: 0.43±0.04 nA).

For TC and TNFB a second peak was detected in the phenolic acids region with a migration time of 55 s. By comparing this peak with the electropherogram of the sample mixture, it could be due to the presence of rosmarinic (RA) or caffeic (CA) acid; however, no experiments have been carried out for its identification yet.

Thus, it has been demonstrated the viability of the portable instrument iHVStat in combination with MEs for the easy, rapid and low consuming identification of flavones and phenolic acids at real samples.

micrux
TECHNOLOGIES

Severo Ochoa Building · Floor -1 – Room 4 & 6
Julián Clavería s/n · 33006 · Oviedo (Asturias) · SPAIN

Phone/FAX: +34 984151019

E-mail: info@micruxfluidic.com
Web: www.micruxfluidic.com

Smart Microfluidic and Electrochemistry
olutions for Research Science