SUPPLEMENTARY MATERIAL

Fast and reliable urine analysis using a portable platform based on microfluidic electrophoresis chips with electrochemical detection

Ana Fernández-la-Villa, Vanesa Bertrand-Serrador, Diego F. Pozo-Ayuso, Mario Castaño-Álvarez*

MicruX Fluidic S.L., Severo Ochoa Building, Floor -1 – Room 4 & 6, Julián Clavería s/n, 33006 Oviedo, Asturias, Spain

Phone/Fax: +34 984151019, e-mail: mcastano@micruxfluidic.com

Instrumentation

The main unit of the instrumentation used in this work is presented in ***Figure 1***. The instrumentation is provided in a suitcase containing all the tools (software, HV cable, Bipot cable…) required for interfacing the different parts of microfluidic platform.

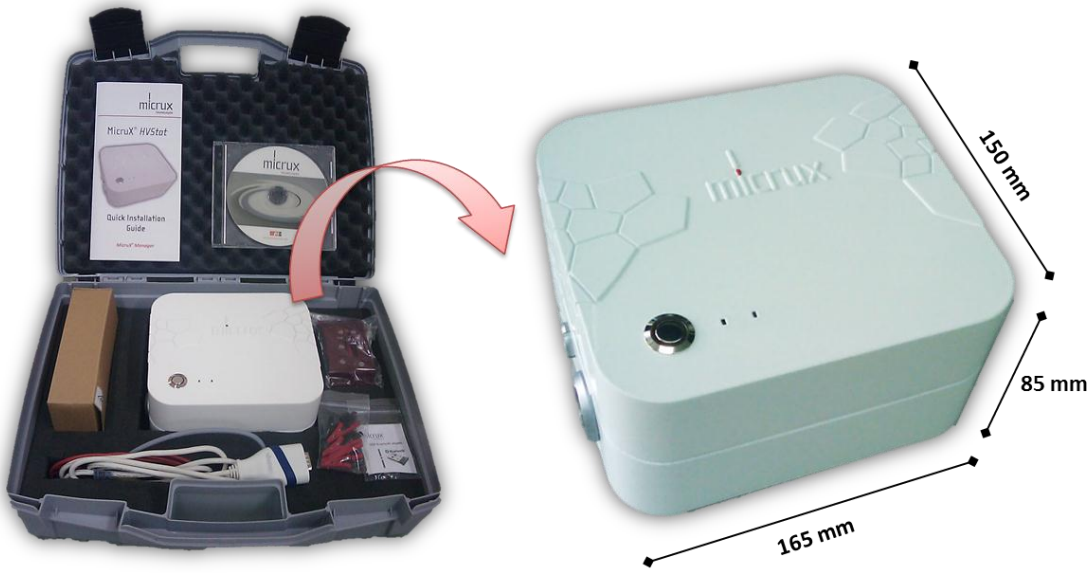

Figure 1. *Portable HVStat instrument.*

The first part of the instrumentation, HVStat, is a portable unit (*165x150x85 mm*) in which is integrated a high voltage power supply and a bipotentiostat for dual electrochemical detection.

The HV power supply consists of one channel with four outputs (one for each reservoir in the single-channel microchips). The HVPS has a maximum voltage of 3000 V with positive or negative polarity and the output current is limited to 0.34 mA. The HVPS was designed for unpinched injection and separation.

The bipotentiostat allows the work with single- (one working electrode) or dual- (two working electrodes) amperometric mode. In both case, DC amperometry or pulsed amperometric detection (PAD) can be performed. In this work only the DC amperometry with a single-mode was employed.

HVStat is a battery-powered instrument that can be connected to the computer by means of USB-RS232 cable or Bluetooth®. In all performed experiments in this work, Bluetooth® connection was always used.

The system is completed with a reusable microfluidic chip holder used for housing the microchip and containing the reservoirs for buffer and samples. The holder consists of two parts fixed with plastic screws. The bottom part includes a space to accommodate the microchip always in the same position. The top part includes the reservoirs for the buffer and samples as well as the electrodes (0.3mm platinum wires) for applying high voltage. The top part also includes the electrical connections for the electrodes of detection (integrated on the ME) and high voltage. The chip holder can be easily re-used changing the ME by a new one. ***Figure 2*** shows the different parts of the microfluidic analytical system used in this work.

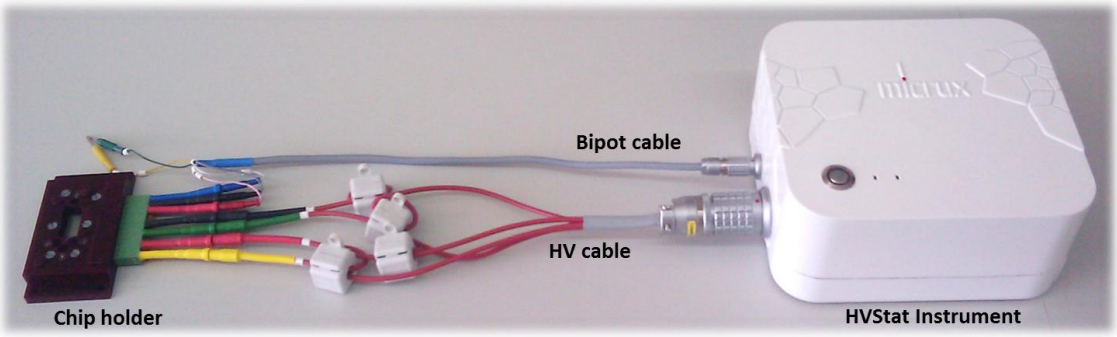

Figure 2. *Electrophoresis microsystem set-up.*

The portable system is controlled by a PC software (*MicruX Manager v2.0*) specially designed for using microchips electrophoresis with amperometric detection. The software incorporates all the tools necessaries for the control of the high voltage power supply and bipotentiostat. Moreover, the software allows the performance of different parameter studies (injection, separation and detection) in an automatic way. Thus, the microfluidic chip becomes a real tool for developing end-user applications.

Microfluidic platform performance

Different analytical parameters were studied and optimized for separation and detection of uric acid, epinephrine, *p*-aminophenol, acetaminophen and ascorbic acid.

Firstly, the hydrodynamic voltammogram (HDV) was performed in order to select the optimal detection potential. The detection potential was studied between 0.0 and +1.1V using a mixture of EP (100 µM), pAP (100 µM), APAP (200 µM), UA (250 µM), and AA (500 µM), an injection voltage of +750 V applied during 3 s and a separation voltage of +1000 V. Taking into account the hydrodynamic

curves, +0.8 V was chosen as optimal detection potential considering the best signal-to-noise ratio. Higher potentials than +0.9 V increased considerably the current of the background and noise level.

The effect of the separation voltage was achieved using the novel microfluidic platform. Thus, in order to get a better resolution of these compounds, the separation voltage was studied between +500 and +1500 V using a mixture of EP (50 µM), *p*AP (50 µM), APAP (100 µM), UA (125 µM), and AA (250 µM). ***Figure 3*** presents the electropherograms for the mixture using different separation voltages.

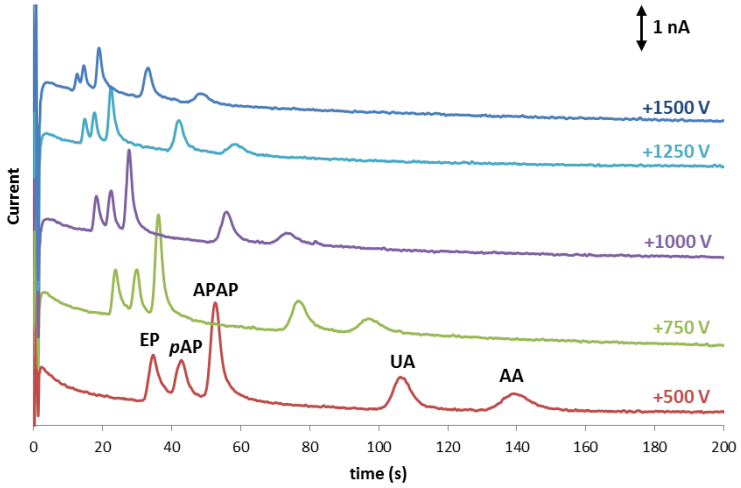

Figure 3. *Effect of the separation voltage for a mixture of EP (50 µM), pAP (50 µM), APAP (100 µM), UA (125 µM) and AA (250 µM) in a SU8/Pyrex microchip with platinum thin-film electrode. Conditions: 20mM MES, pH = 6.0;* V_{inj} *= +750 V,* t_{inj} *= 3 s,* E_d *= +0.8 V.*

When the separation voltage is increased, the migration time and half-peak width decrease. However, the peak current and resolution is going down. Therefore, the use of higher voltages than +1000V did not improve the analytical signals. Thus, the separation voltage where was achieved a good efficiency and resolution with the minimum migration time and good peak current was +1000V.

The effect of the injection time and voltage was also evaluated. Thus, when the injection time and voltage is increased, the sample plug loaded in the microchannel is also bigger and the peak current is improved. However, the peak width also grows up affecting the efficiency and resolution of the separations. The best peak current with good separation efficiency and resolution was obtained using an injection voltage of +750V applied during 3s.

Figure 4 shows several successive electropherograms for a mixture of EP (50 µM), *p*AP (50 µM), APAP (100 µM), UA (125 µM), and AA (250 µM) using the optimal conditions.

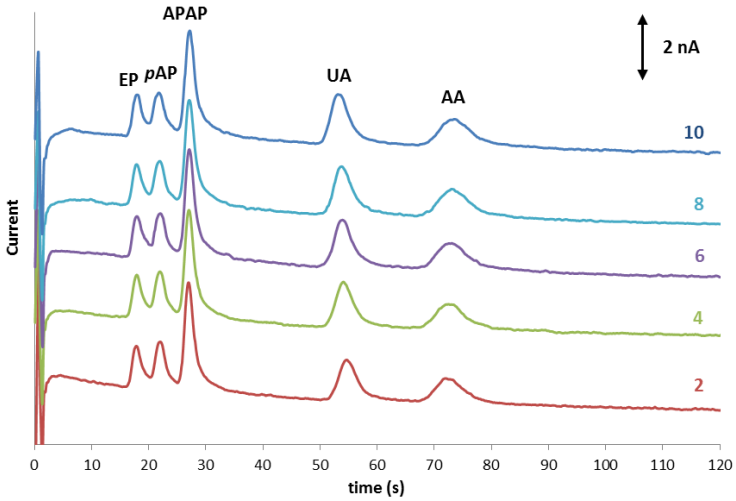

Figure 4*. Successive electropherograms for a mixture of EP (50 µM), pAP (50 µM), APAP (100 µM), UA (125 µM) and AA (250 µM) in a SU8/Pyrex microchip with platinum thin-film electrode. Conditions: 20mM MES, pH = 6.0;* V_{inj} *= +750 V,* t_{inj} *= 3 s,* V_{sep} *= +1000 V,* E_d *= +0.8 V.*

Analysis of real samples: Standard Additions Protocol

Uric acid in urine sample was determinated by using the addition standard method. The protocol of standard additions used in the microfluidic platform is shown in ***Figure 5***.

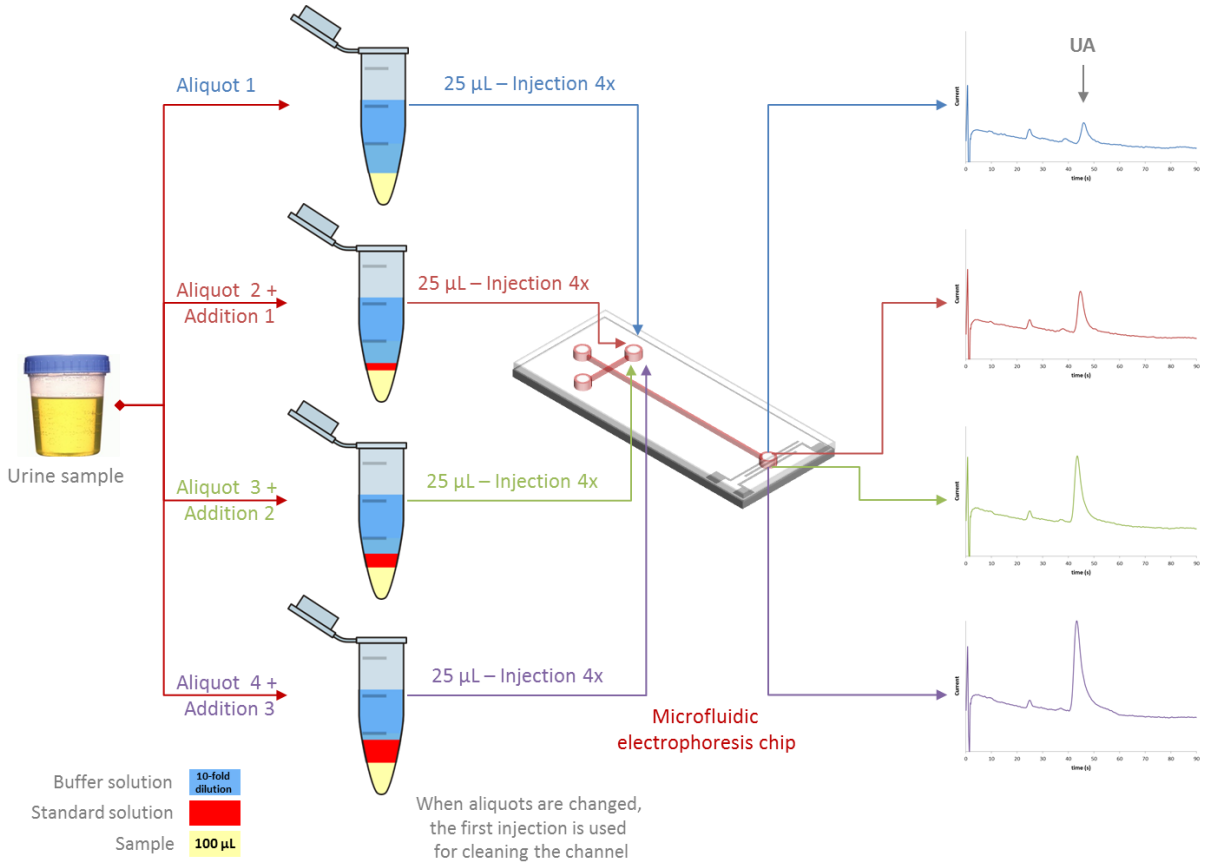

***Figure 5**. Scheme of the standard additions protocol used in the microfluidic platform*

Urine analysis

The analytical signal of UA obtained for a 10-fold dilute urine sample from a volunteer with antecedents of gout was about three times higher than it was in a healthy volunteer. Thus, in this case, higher amount of UA was spiked in order to get a significant increase of the analytical signal. ***Figure 6*** shows the electropherograms of the urine and spiked urine sample of an individual with antecedents of gout.

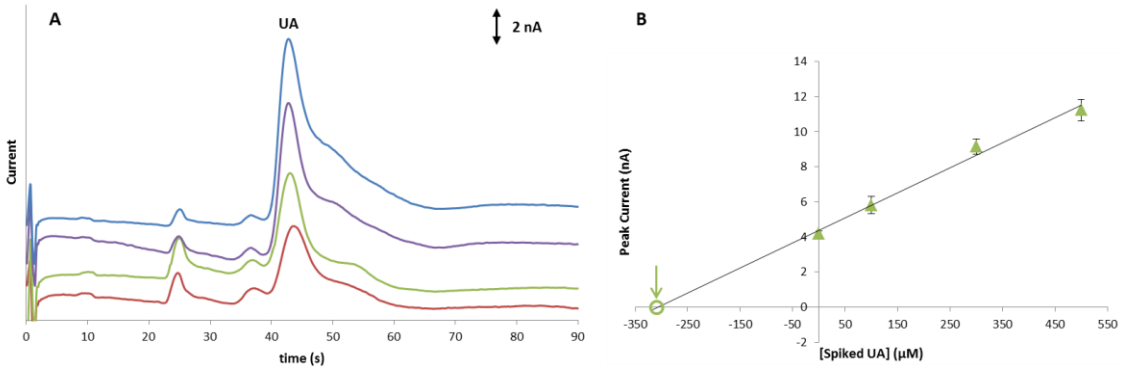

Figure 6. (A) *Electropherograms for a 10-fold dilute urine sample from a volunteer with antecedents of gout with successive additions of UA: 0 (a); 100 (b); 300 (c) and 500µM (d).* ***(B)*** *Standard additions calibration plot. Conditions: 20mM MES, pH = 6.0;* V_{inj} *= +750 V,* t_{inj} *= 3 s,* V_{sep} *= +1000 V,* E_d *= +0.8 V.*

The higher spike UA (500 µM) is out of the linear range for the UA in the analysis conditions. Thus, it was more useful and more accurate results are obtained if only the additions of 100 and 300 µM are employed in the determination of UA. In this sense, an extra dilution could be also performed in order to get results that are more accurate. When the peak current of the urine sample without additions is upper than 1 nA, further dilution of the sample has to be performed (e.g. from 10-fold to 15- or 20-fold), thus, the additions will be in the linear range and a better precision is achieved.

In a qualitative mode, it would be very easy to recognize a healthy from an unhealthy individual with the first analytical signal of the dilute urine sample.