SDS CAPILLARY GEL ELECTROPHORESIS OF PROTEINS IN SU-8 MICROCHANNELS.

M. Agirregabiria, F. J. Blanco, J. Berganzo, K. Mayora and J. M. Ruano
MEMS/MST Department, IKERLAN S. Coop. Pº J.M. Arizmendiarrieta Nº 2,
Mondragón (Guipuzcoa), Spain.
Magirregabiria@ikerlan.es

The miniaturisation of capillary electrophoresis devices provides many advantages for the separation of different biomolecules [1]. This technique offers high efficiency, versatility, speed and economy of analysis since low consumption of reagents and analytes is required. Thus, numerous works have been reported during last years related with electrophoresis microchips.

Initially, the chips were fabricated on silica and glass. Nevertheless, the interest in using polymers has been growing due to their low costs. This work describes a SDS-capillary electrophoresis of proteins carried out in a microdevice made of SU-8. As far as we are concerned, this is the first time that this polymeric material is used for this purpose.

Firstly, an electrophoresis microchip is fabricated by an SU-8 multilayer technology described in [2]. It is based on successive bonding and releasing steps of photopatterned SU-8 layers. Thus, the inlets/outlets and the electrodes are left in contact with the outside world, simplifying the packaging method. Microchips, 3.5 cm long and 1.8 cm wide, with microchannels of 100x 20 μm in cross section are successfully fabricated and packaged. Figure 1 shows one of these devices and the cross section of the channel can be seen in Figure 2.

The device has been thoroughly characterized [3]. First of all, the generated current after applying voltage is measured to ensure that generated heat is well dissipated (see Graph1). After that, the microchannels are filled with Tris-Glycine-SDS buffer (pH=6.8) containing polystyrene beads and their velocity is measured after applying voltage. This velocity, shown in Graph 2, is composed of an electrophoretic mobility due to the presence of a charge in an electric field, and of an electrosmotic mobility, arising from the double layer effect. The two effects are taken into account to calculate the electrosmotic flow of our devices. Thus, an electrosmotic flow of 6.2×10^{-4} cm^2/Vs is calculated, proving an adequate and stable electrosmotic flow. Finally, the unspecific adsorption of proteins in microchannels is also characterized.

Once the microdevice is fabricated, packaged and characterized, an electrophoresis of proteins is achieved. The electrophoretic mobilities of trypsin (20KDa), glyceraldehyde (36KDa), BSA (66KDa) and phospholylase (97KDa) are measured in polyacrylamides of four different pore sizes (%T=6, 8, 10 and 12). Firstly, the protein samples are prepared labeling with a Cy5 fluorocrome. Then, they are diluted in a Laemli sample buffer to a final concentrations of 25, 14, 7.5 and 5.1μm, respectively. Subsequently, they are electrokinetically injected in the microchips to measure their electrophoretic mobilities. Ferguson plots and the calibration line of our device are obtained. They are shown in Graph 3 and 4. Finally, the separation of trypsin and phosphorylase is carried out. These proteins are successfully separated in only 20 seconds (see Figure 3).

In conclusion, it is proved that the SU-8 fabrication technology described in our previous work [2] is suitable for electrophoresis and for lab on a chip applications. Regarding fabrication, it is advantageous in comparison to the glass since its fabrication is less complicated, expensive and time consuming. Furthermore, the obtained electrophoresis results are comparable to the glass.

References:

[1] S. Yao, D. S. Anex, W. B. Caldwell, D. W. Arnold, K. B. Smith and P. G. Schultz, Proc. Natl. Acad. Sci., **96,** (1999), 5372

[2] **M. Agirregabiria**, F. J. Blanco, J. Berganzo, M. T. Arroyo, A. Fullaondo, K. Mayora and J. M. Ruano-López, Lab Chip, **5**, (2005),545.

[3] **Maria Agirregabiria**, F. J. Blanco, J. Berganzo, M. T. Arroyo, M. Tijero, J. García, I. Aramburu, K. Mayora and J. M. Ruano-López, Eurosensors XIX, (2005)

Figures:

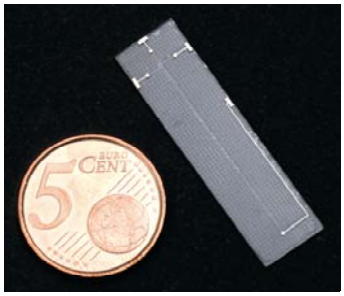


Figure 1: A photograph of a fabricated electrophoresis chip.

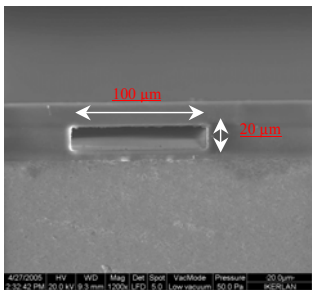
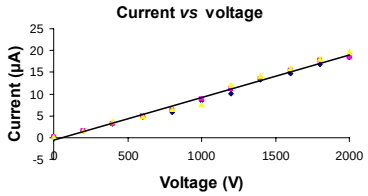
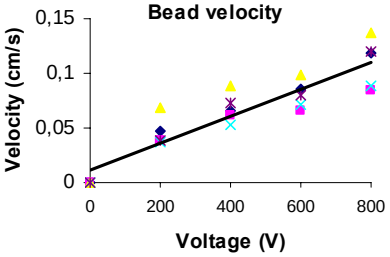


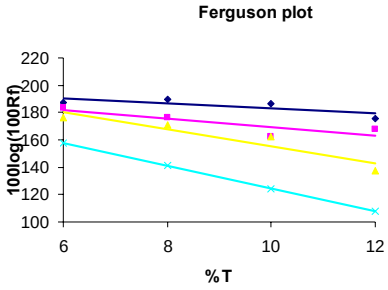
Figure 2: A SEM photograph of a fabricated microchannel in cross section (20µm height and 100µm width). There is no interface between SU8 layers. The sidewall profile is high-quality.



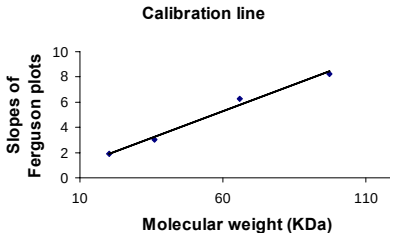
Graph 1: The generated current when voltage is applied. The heat is well dissipated because the plot is a line.



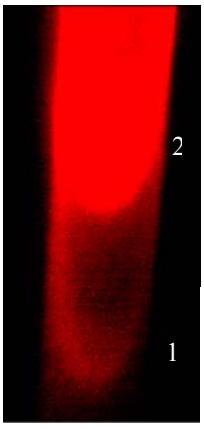
Graph 2: The speed of the beads under a electric field.



Graph 3: Ferguson plots for 4 proteins with different molecular weights determined at a constant electric field of 58V/cm, in SDS-Tris-Glycine buffer, at pH=6.8.



Graph 4: The calibration line of our device. Slope of the Ferguson plots vs molecular weight.



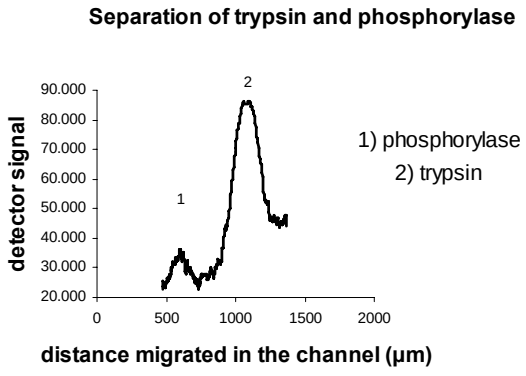


Figure 3: A photograph of the trypsin (25µM) and phosphorylase (2.5 µM) after being separated and their respective electropherogram.