Electronic Supplementary Information (ESI)

Fibre-based Electrofluidics on Low Cost Versatile 3D Printed Platforms for Sample Delivery, Separations and Diagnostics

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1. Platform fabrication

A base of 12 cm × 8 cm × 0.5 cm (width × depth × height) PMMA was made using a commercial hobby Laser System (Full Spectrum, Las Vegas, NV, USA) equipped with a CO_2 laser with an output of an invisible infrared beam at a wavelength of 10.6 µm and a maximum output of 40 W was used to cut the cast PMMA sheets. 7 × 11 holes of 3 mm squared were also cut uniformly around the platform allowing up to 77 reservoir positions. This base was designed using the free CAD software, Draftsight (SolidWorks Corp., Dassault Systemes, France) and sent to the Retina Engraver software (FullSpectrum, Las Vegas, NV, USA) as a print job to drive the laser system for the ablation process. Experimental parameters of laser power, scanning speed, and number of laser beam passes were set by using the Retina engraver software. Buffer reservoirs were designed using CAD software, SolidWorks (SolidWorks Corp., Dassault Systemes, France). Reservoirs designs were printed using Eden 260VS (Stratasys, MN, USA) with VeroClear SUP70 and water soluble support. Support material was removed by agitation in water for 4-6 hours followed by 2 hour in NaOH 2%. Finally, buffer reservoirs were rinse and subsequently soaked for 1 day Milli-Q water.

2. Theory

The apparent mobility of a compound (μ_{ap}) is the sum of the electrophoretic (μ_{ep}) and electroosmotic flow (μ_{EOF}) mobilities and can be expressed by means of equation (1S).

$$\mu_{ap} = \mu_{EOF} + \mu_{EP} \quad (1S)$$

The velocity of the analyte (v) is simply a function of the μ_{ap} and the electric field (E, voltage applied per fibre length) as described in equation (2S).

$$v_{ap} = \mu_{ap} E \quad (2S)$$

Any of these mobilities can be calculated following equation (3S) from the fibre length to the detector (L_D), the electric field applied (E), the observed migration time of the compound (t_m).

$$\mu = \frac{L_D}{E t_m} \tag{3S}$$

When a number of fibres (n) with different diameter are connected, the system can be seen as an electric circuit with n resistors in series. Therefore, effective electric field for a particular fibre (i) depend on the resistance (R) and the current of the system (I) per unit length of fibre (L). Thus

$$E_i = \frac{R_i I}{L} \tag{4S}$$

Assuming electrophoresis system as a continuous flow microfluidic device, the number of moles (N) traveling per unit of time (t) can be defined as

$$f(t,N) = \frac{\partial N}{\partial t} \tag{5S}$$

and considering fibres as a completely cylindrical pathway,

$$\frac{\partial N}{\partial t} = C_{AN} V \tag{6S}$$

where C_{AN} is the analyte concentration and V the cylindrical volume in a defined period of time. Volume can be express as $dV = \pi r^2 dL$, where r is the fibre radium and L is length, and $dL = v_{ap} dt$. Thus, equation (6S) and (2S) can be transformed and integrated to equation (7S) and (8S).

$$N = \int_0^t \pi C_{AN} r^2 \mu_{ap} E_i dt \tag{7S}$$

$$N = \pi C_{AN} r^2 \mu_{ap} E_i \, (t - t_0) \tag{8S}$$

3. Experimental:

Reagents: Tris-(hydroxylmethyl)amino-methane (Tris), 2-(cyclohexylamino)-ethanesulfonic acid (CHES), Tris-Borate-EDTA buffer (TBE), r-phycoerythrin, rhodamine B and fluorescein sodium salt of analytical reagent grade were obtained from Sigma-Aldrich (New South Wales, Australia). R-phycoerythrin was dissolved in 25 mM TBE at pH 8.5. Solutions were prepared in water from a Milli-Q water plus system from Millipore (Bedford, MA, USA), with a resistivity of 18.2 MΩ cm.

Fibres: 100% nylon with 803 ± 53 µm in diameter (woolly nylon stretch overlocking thread, QA thread, China), 100% silk with 573 ± 39 µm (stranded silk, 8.4 yd, Cascade House, Creswick, VIC, Australia) and 100% cotton with 397 ± 19 µm (mercerised twice, 8.7 yd, mouliné stranded, DMC, France) were used for fibre-based microfluidics. Diameters of the wetted fibres with 2.5 mM Tris/CHES pH 8.5 were measured in 10 different samples using an objective-type inverted microscope (Nikon Eclipse TE2000). In order to clean and eliminate impurities, fibres were soaked in Milli-Q water and sonicated during 10 minutes 3 times. Subsequently, fibres were water cleaned again and prepared for the separation system.

Electrodriven analysis: Voltage was applied using in-house built four channel high voltage power supply (CSL electronic workshop, University of Tasmania, Hobart, Australia). Fluorescence images were taken with a USB microscope AM4113T-GFBW (Dino-Lite Premier, Clarkson, WA, Australia) fitted with a blue light-emitting diode for excitation and a 510 nm emission filter. Fluorescent intensities were taken from images and videos using Image J.

3D printed buffer reservoirs were placed on the 7 × 11 positions platform accordingly to the desired structure. Unless state in the text, 500uL of 2.5 mM Tris/CHES buffer solution was dropped into the chambers and fibres were automatically wetted. Before applying voltage, the system was left for few minutes in order to achieve equilibration and avoid syphonage flow during the analysis. Experiments were carried out in the cathodic mode, where anode is in the inlet and cathode is in the outlet, following the electroosmotic flow. Electric field applied was -200 V cm-1 unless state differently elsewhere. USB Dino-Lite microscope was placed on top of the fibre platform. Experiments were performed at room temperature. Electrolysis of the bioactives are minimal since the current is only few tens of µA.

Current was controlled all the time. For Ohm's Law relationship, current was monitored (1 Hz) during one minute starting form the first minute of electrophoresis. This procedure was repeated 3 times every 50 V $cm^{-1}$from 0 to 500 for nylon, silk and cotton using a new fibre each time. The average results together with their correspondent standard deviation are represented in Figure 1S. Temperature raise due to Joule heating effect was also controlled with a FLIR E40 MSX infrared camera (Notting Hill, VIC, Australia).

In order to calculate the apparent mobility of the analyte signal was monitored at the 3, 4 and 5 cm from the injection point using equation (3). Average values are summarized in Table 1S shows the apparent mobility of the analytes at each fibre.

Table 1S: Apparent mobilities of the analytes in the fibres.

	Mobility (10^{-8} m^2 V^{-1} s^{-1})		
Analyte	**Nylon**	**Silk**	**Cotton**
Rhodamine B	3.3 ± 0.2	0.0	0.0
Fluorescein	2.6 ± 0.4	1.9 ± 0.1	0.0

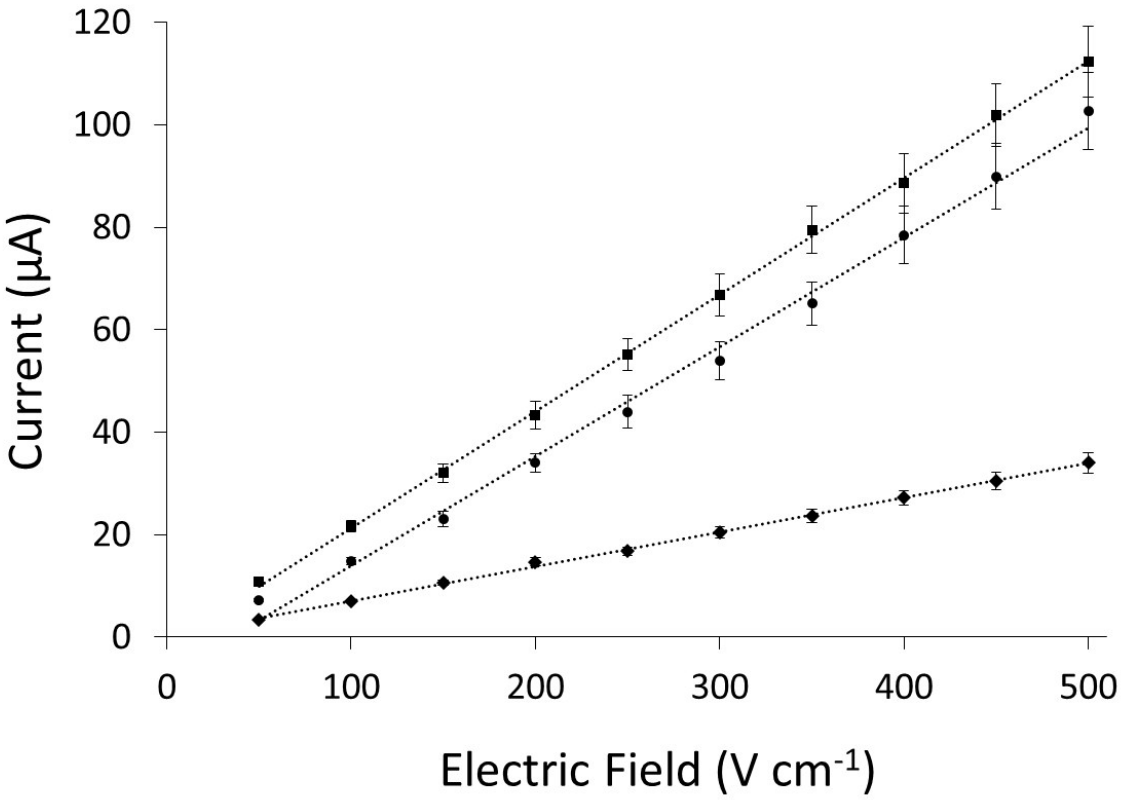

Figure 1S: Relationship between current and electric field for cotton (◆), silk (●), nylon (■) fibres using 2.5 mM Tris/CHES buffer at pH 8.5 obtaining slopes of 0.0671, 0.2139, and 0.2278, and correlations (R^2) of 0.9987, 0.9948, and 0.9995, respectively. Linearity demonstrated the Ohm's law dependence of the solution conductivity with the ionic concentration for the different fibre materials. Errors bars show the variation associated at the experimental values. Data was collected by triplicate using each time a new fibre.

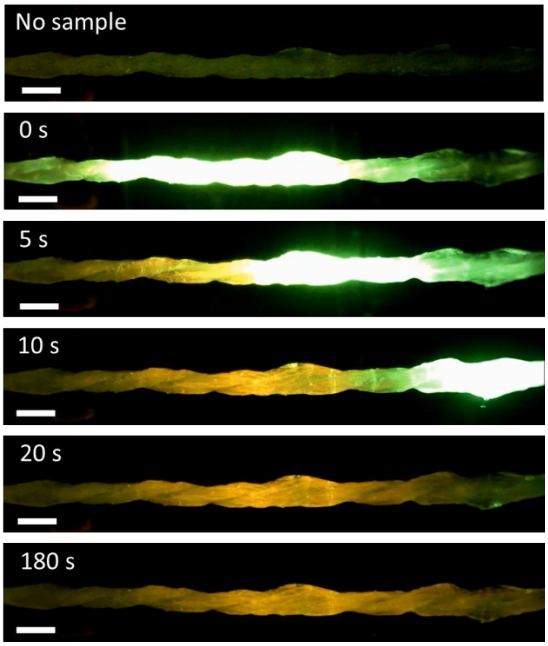

Figure 2S: Exploration of physical and chemical properties of silk. Separation due to the interaction of 30 µM rhodamine B (orange) and 2 µM fluorescein (green) mixture in silk fibre. After 3 minutes, rhodamine B has barely moved. Buffer: 2.5mM Tris/CHES at pH 8.5. Injection was performed by dropping 1 µL of sample mixture. Voltage applied in cathode: -200V/cm.