Capillary electrophoresis characterization of a rapid prototyped PMMA chip for particle analysis

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Abstract—
A rapid and cheap method has been developed for the fabrication of a capillary electrophoresis chip for the preliminary characterization of particles. The microfluidic chips were fabricated using polymethyl methacrylate (PMMA) with integrated platinum electrodes without the need of using high technology microfabrication techniques. The chips were characterized using electroosmotic flow (EOF) with different channel treatments. The electrodes were characterised with impedance and conductivity measurements using both static and electrophoretic flow, respectively. Nine micron diameter particles were detected and their electrophoretic mobility determined using capillary electrophoresis and conductivity detection.

Index Terms— **Nanobiotechnology: Capillary electrophoresis (CE), Electroosmotic flow (EOF), polymethyl methacrylate (PMMA), rapid prototyping.**

I. INTRODUCTION

One of the first Lab-on-a-Chip devices was a gas chromatography system developed in 1975 by Terry and Angell [1]. They used silicon as the substrate material.

More recently, low cost polymer Lab-on-a-Chip devices have been developed. Depending on the method of fabrication used, it is important to take into account the properties of polymers. The EOF values for plastic materials are generally lower than for glass materials due to the lower density of charge on the surface resulting in a lower zeta potential. PDMS has been widely used in microfluidic applications, but it is less suitable than PMMA for capillary electrophoresis (CE). PMMA is a hydrophobic polymer and generate a stable EOF [2].

A number of research groups have studied the EOF behavior in PMMA[3] devices. Different examples of separation systems using CE have been developed using PMMA, testing the efficacy of dynamic coating of chips to prevent the non-specific interactions of analytes with the channel walls and also to suppress the EOF.

For these type of devices, there is a huge number of fabrication methods. It is common to use soft-lithography fabrication techniques which have to be performed inside a clean room. However, there are some cases that these installations are not available or have very high costs.

A number of groups have introduced a number of ideas of rapid prototyping of microfluidic devices. These include the fabrication of PMMA chips using a wire for imprinting a microfluidic channel [4], the development of thermo set polyester (TPE) channeled devices using standard lithography procedures[5], the rapid bonding techniques [6] and the fabrication of integrated electrodes using wires [7],[8]. If the optical properties of devices are important, such as laser induce fluorescence detection technique, it has been found that PMMA material does not have good optical properties[9].

CE is an attractive method for separation and analysis, as it offers faster analysis times than other separation methods, uses smaller sample volumes and it can be easily automated. CE is suitable for particle analysis, as it can provide both size and surface characteristics in a quick and simple way[10].

Characterization of particle size is normally done using the Laser Light Scattering technique, but this is a very difficult method. In order to characterise particles' surface, this can be done by Doppler Velocimetry Laser (LDV), that is another complex method and both of them high cost instrumentation.

Optical detection using fluorescence is the most popular method of detection for electrophoresis microchips[11. Although it is a very sensitive method, it has some limitation: including high cost instrumentation and the need to label non-fluorescent molecules. Other techniques have been developed such as amperometry, potentiometry and conductometry. Conductrimetry [12] is based on the conductivity properties of the solutes of interest whereby the signal arises from the conductivity differences from the bulk of the solution and not-specific reactions occurring on the electrode surface.

In this paper we have developed a quick and cheap method of fabricating capillary electrophoresis chips in a standard laboratory, with a simple one channel microfluidic device with integrated electrodes for the preliminary testing of particles. In order to study the characteristics of the particles and measure their electrophoretic mobility we have to reduce the EOF in the channel. This was carried out using a dynamic coating which is a blocking protein know as UltraBlock[13].

II. THEORY

The bulk main flow in capillary electrophoresis is generated by the EOF which is the bulk fluid flow of the background electrolyte (BGE) when an electric field is applied.

The EOF of the BGE is typically greater than the electrophoretic mobility of the negative solute ions so that all ions are carried in the same direction.

In normal operation, the direction of the electroosmotic flow is toward the negative charged cathode, which means the buffer flows from the source vial, through the capillary, through the detector, to the destination vial.

Charged solute molecules are separated due to differences in their electrophoretic mobilities. Negatively charged anions are attracted to the positively charged anode and vice versa

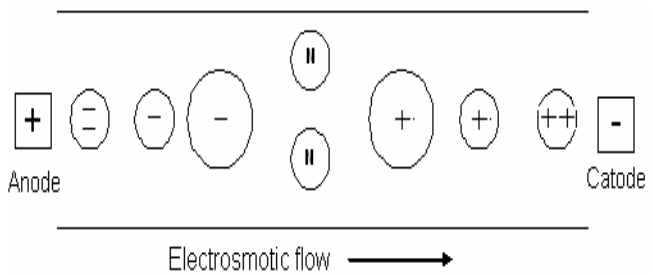

Fig1: *Ions flowing in order, according to their charge, toward the cathode as a result of electrosmotic flow*

Commonly fused silica capillaries are used in CE and the surface silanol groups (Si-OH) can be ionized to negatively charged silanote (Si-O-) groups at above pH 3. When the BGE is passed through the capillary the cations are attracted to the negatively charged anions. This inner layer of strongly held cations is known as the fixed layer. As these cations are not of sufficient density to neutralize all the anions a second layer of cations is formed known as the mobile layer[14]. In case of a PMMA surface it is ionized using the same process but PMMA does not have silanote groups.

When an electric field is applied the mobile layer is attracted to the cathode, and as the cations are solvated they carry the remaining BGE with it.

The velocity of the BGE under the electric field is definded by equation (1). Where μ_{EOF} is the mobility of the BGE by some intrinsic parameters of the solution [2].

The value of velocity of the electrosmotic flow, v_{EOF} (ms^{-1}) (1) and the electrophoresis mobility of the BGE, μ_{EOF} ($m^2 V^{-1}s^{-1}$) (2) can be calculated using the following equations respectively :

$$v_{EOF} = \mu_{EOF} \times E \qquad (1)$$

$$\mu_{EOF} = \frac{\delta e}{\eta} \qquad (2)$$

Where δ is the thickness of the double layer, e is the charge per unit surface area, η is the viscosity of the buffer and E is the applied electric field Vcm^{-1}.

Under the influence of an electric field, an electrically charge solute will migrate through the buffer. Separation is achieved because solutes migrate through the capillary because they have different electrophoretic mobilities. The solution around the molecule also imposes a frictional drag on the molecules as they move. The mobility (3) and velocity (4) of a molecule are represented by:

$$\mu_{EP} = \frac{q}{6\pi\eta r} \qquad (3)$$

$$v_{EP} = \mu_{EP} \times E \qquad (4)$$

where q is the net charge and r is the radius

III. EXPERIMENTAL

A. Chemicals

Phosphate buffered saline (PBS) was purchased from Sigma Aldrich. Sodium hydroxide pellets were purchased from Riedel-de Haën. Elisa ultrablock was purchased from AbD Serotec. Water was purified with a Mili-Q water purification system.

The 9.976 micrometers microspheres Fluoresbrite™ (Carboxy Yg i.e), purchased from Polyscience.

PBS was prepared by dissolving one tablet in 200 mL of Mili-Q water to make a 10mM phosphate buffer, and this solution was diluted to 1:10 and 1:100 with Mili-Q water.

Four grams of sodium hydroxide was dissolved in 100ml of deionised water.

Elisa UltraBlock was used as received.

Microspheres were prepared by dissolving 1drop in 5ml of PBS 10mM

B. Apparatus

For the capillary electrophoresis a PS468 power supply and 600 floated resistivity detector (FRD) (I-BIO Pte Ltd, Singapore) were used and for the impedance measurements an Agilent 4294A Precision Impedance Analyser was used. For the comparison measurements, we used a Corning conductivity meter 441 (Corning, USA).

C. Fabrication Materials

PMMA 500 µm thick sheets (Goodfellow, UK) were used for the fabrication of the microfluidic chips. Tungsten 125 µm wires (Advent, UK) were used for the fabrication of the microfluidic channels and 50 µm or 25 µm platinum wires (Advent, UK) were used for the electrodes. A metal clamp (size 25 by 80 mm) was used for bonding it the two PMMA

pieces. The clamp was placed inside an oven (LTE Scientific, UK) set at a temperature of 150ºC. Arraldite 2014 epoxy (Amidata, Spain) was used to connect plastic reservoirs to the channel inlet and outlet.

D. Fabrication

A rapid and cheap prototype method was developed for the fabrication of PMMA chip with integrated platinum electrodes [15].

A PMMA 500 µm sheet (Goodfellow, Huntingdon, UK) was cut into two 40 mm by 15 mm pieces. In one of the PMMA sheets, 1 mm holes were drilled with a driller (Dremel 389l, Amidata Spain) using drills of 1mm at either end to make the inlet and the outlet of the chip (shown in Figure2).

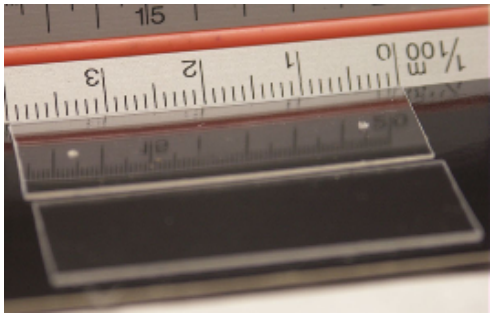

Fig 2. *Two PMMA pieces with one side drilled with 2 x 1 mm holes.*

Electrodes were placed on the same PMMA piece. To position the electrodes, two small slits were made, using a scalpel, on either side of the PMMA piece making sure that the slits were as close as possible to each other. Platinum wires, 50 µm (Advent, Oxford, UK) in diameter, were then placed across the PMMA sheet and one side of the wire was attached to the cut slit. A soldering iron was used to weld the wire to one side of the PMMA sheet holding it in place and allowing it to be pulled taught across the piece of PMMA and toward the cut slit on the other side. The platinum wire was welded to this side holding it firmly in place. Figure 3 shows the two electrodes after repeating the procedure above resulting in two electrodes spaced very close. To reduce the gap between the two electrodes a pair of tweezers with the help of an inverted microscope. Using this method we were able to reduce the gap between 350 µm and 14 µm.

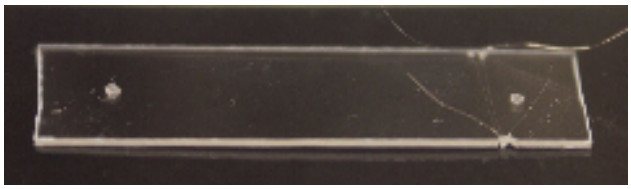

Fig 3. *50 µm platinum wires (shown by arrow) attached to the drilled piece of PMMA.holes.*

A 125 µm cut piece of tungsten wire was used to fabricate the channel (Advent, Oxford, UK) resulting in a straight channel, as shown in Figure 4.

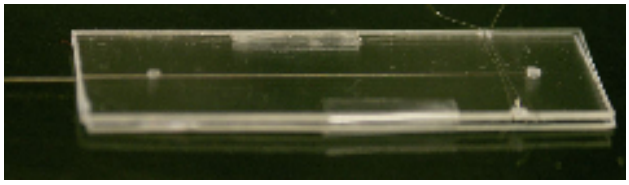

Fig 4. *Bonded chip with integrated platinum electrodes and tungsten wire (protruding from one side of the device)*

The following procedure was found to be the best way of positioning the tungsten wire in the correct position. First a clamp was placed in its side so that the PMMA piece, with integrated electrodes, could be easily placed on top of the lower clamp jaw. It is important that the dimensions of the clamps jaws are slightly larger than the dimension of the chip. The tungsten wire was then placed and positioned on top of the integrated electrode PMMA piece and the second blank PMMA piece, of the same size, was placed on top of this. Once this was done the tungsten wire could be easily positioned so that one end of the wire was in line with one of the drilled holes and the other end of the wire was protruding across the other drilled hole and extending outside of the microfluidic device, to enable removal of the wire once the two pieces were bonded together. The clamp was then firmly tightened and placed in a pre-heated oven at 150 °C. After 5 minutes the clamp was re-tightened and placed in the oven for a further 10 minutes. The clamp was then taken out of the oven and the tugsten wire stretched with tweezers and the bonded chip was allowed to cool. Figure 5 shows an image taken after the tungsten wire was removed from the PMMA device.

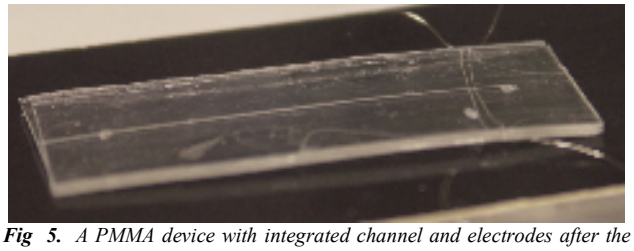

Fig 5. *A PMMA device with integrated channel and electrodes after the removal of the tungsten wire*

This resulted in a straight channel with integrated electrodes. It was found that the platinum electrodes were not disturbed by the removal of the tungsten wire. Micropipettes tips were used for the inlet and outlet reservoirs. Araldite 2014 epoxy adhesive (Amidata, Spain) was used to seal the end of the channel from where the tungsten wire had been removed and the heads micropipettes tips sealed to the inlet and the outlet holes. Figure 6 shows the finished device with external wires which were attached to the platinum electrode wires using either solder or silver epoxy.

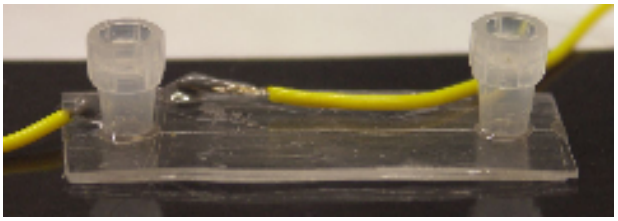

Fig 6. *Finished device connected with external wires and reservoirs*

A zoom (Figure. 7) shows a close up of the channel and the two integrated electrodes.

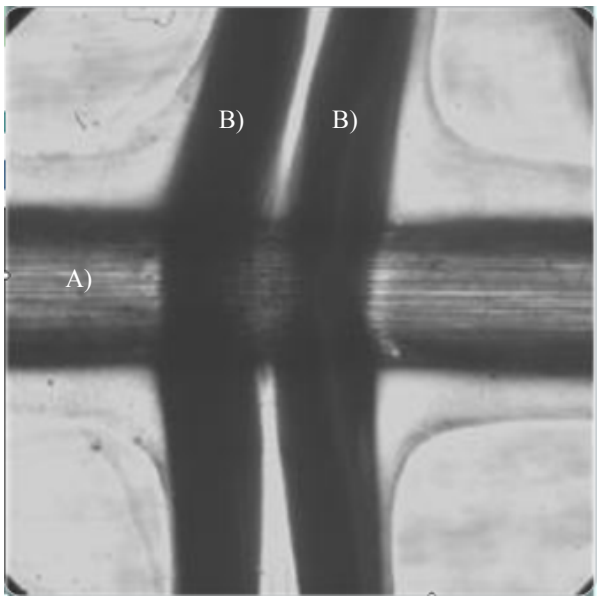

Fig 7. *A microscopic image of the channel, A), and two electrodes B), showing a distance between the electrodes to 23 µm*

IV. RESULTS AND DISCUSSION

IV.II. FABRICATION OF PMMA MICROFLUIDIC DEVICE

A quick and cheap fabrication method has been developed which is ideal for the preliminary testing of a microfluidic device.

The polymer PMMA was used in this study in preference to PDMS, although PDMS is an ideal material for the rapid prototyping of microfluidic chips, PDMS has some disadvantages as it is more permeable to oxygen and has an instable surface when used for electrophoresis applications [16]. PMMA is a commonly used polymer for CE experiments [13], as it is less hydrophobic than PDMS[17], because it has a high charge on its surface.

At first it was tested with a silver wire for the channel's manufacture, but it was found that the silver wire broke when it was withdrawn from the chip. The tungsten wire is perfect for the chips that have equal or less length than 6 cm and its strong enough to be taken out without breaking.

Also tests were made with two tungsten wire for chips with crossed channels and the results were satisfactory.

The most critical point was to establish the distance between the platinum electrodes with 25 µm and 50 µm of wire diameters, because of its low reproducibility. Although, in practice, chips with a separation between electrodes below 50 µm can be manufactured.

The manufacturing cost of one chip is about 4.00€ and the fabrication time is approximately 50min. In larger quantities, the cost would decrease.

If we compare it with the conventional procedures in a clean room, using lithography and deposition of the electrodes by evaporation, the cost of these procedures would be much higher and the manufacturing time would be around 2 days.

The described procedure could be a good solution for people that do not have access to micro-fabrication facilities and for those that would like to try out an idea.

This is a useaful method for doing a preliminary test or introducing students to microfluidics.

If the test is successful, the final device can be done with traditional lithography or soft-lithography [18].

IV.II. MEASUREMENTS OF EOF

PMMA's electroosmotic flow could be increased or decreased, using surface modification,[13] it is possible to increas its EOF with NaOH, or to reduce this charge with UltraBlock, The EOF can also be modified using other parameters such as the buffer's pH, concentration, ionic strength and its temperature.

The channel and reservoirs were filled with a 0.1 mM PBS solution, and, a 300 V voltage was applied to the inlet reservoir which was filled with a 1 mM solution.

A 300 V potential was used to reduce the effect of "joule heating". A higher voltage was not required as particles were not being separated in this study.

The value of velocity of the electrophoresis flow, v_{EOF} (ms^{-1}) (5) and electrophoresis mobility of BGE, μ_{EOF} ($m^2 V^{-1} s^{-1}$) (6) was calculated using the following equations respectively:

$$v_{EOF} = \frac{L}{t} \qquad (5)$$

$$\mu_{EOF} = \frac{v_{EOF} \times L}{V} \qquad (6)$$

Where t is time for the current to stabilise, L is the total length between two reservoirs and V is the applied voltage.

Channel and reservoir were cleaned with Milli-Q water between each run for 10 seconds in 5 runs.

A. Measurement of EOF in non treated PMMA channels

Figure 8 shows the measurement of an untreated channel.

When the more concentrated PBS fills the channel the current increases and it stabilises once the channel is completely filled. The time that the current takes to stabilise is used to calculate the electrophoresis velocity and the electrophoresis mobility using equations (5) and (6) respectively. This is the current monitoring method described in [12].

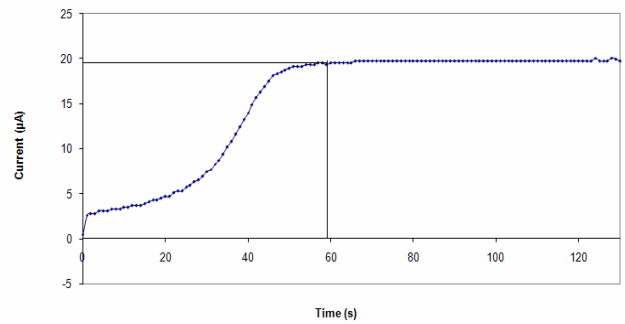

Fig 8. *The result of the injection of a 1 mM PBS solution into the microfluidic channel filled with a 0.1mM solution. The line indicates when the current is constant and the time that has been taken to calculate the* μ_{EOF} *and* v_{EOF} *.*

Three consecutive runs were made and the results of each one can be seen in the Table I. Table I shows an average of electrophoresis velocity of 0.0456cm/s, a EOF average of 0.00041 cm2/V·s and a relative standard deviation (RSD%) of 15.56%. RSD is similar to other published works [13].

Although the buffers and size channel used in other studies were different, it is possible to calculate the ionic strength, with equation (7), and make a comparison.

$$I = \frac{1}{2}\Sigma c_i \times z_i \qquad (7)$$

A higher ionic strength decreases the velocity of the EOF. In bibliography[13] the ionic strength was 549mM/149mM, and the EOF 1.4x10^{-4} cm^2/V·s. In this work the ionic strength is 32mM/3,2mM and the EOF 4.1 x10^{-4}(cm^2/V*s). With there values own results are comparable with previous studies.

TABLE I
SHOW THE RESULTS IN NON TREATED PMMA CHANNELS

Run	Velocity (cm/s)	EOF mobility(cm^2/V*s)
n=1	0.0529	0.00048
n=2	0.0450	0.00041
n=3	0.0388	0.00035
Average	0.0456	0.00041
RSD %	15.56	

B. Measurement of EOF in NaOH treated PMMA channels

The channel and reservoirs were filled with NaOH solution using positive pressure for 10 seconds. Then the NaOH solution was removed and Milli-Q water was injected for 2 seconds. The channels were then filled with 0.1mM PBS y and the inlet reservoir filled with 1 mM PBS. Once filled, 300V was applied for the EOF measurement.

In Figure 9, it can be seen that the time required to stabilize the current is lower, so it indicates that there is a v_{EOF} and a μ_{EOF} higher, as it can be seen in Table II.

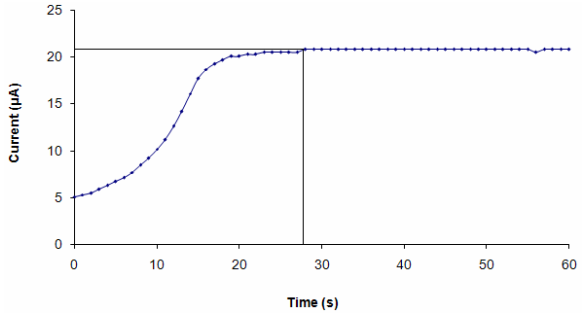

Fig 9. *The result of the injection of a 1 mM PBS solution into the microfluidic channel filled with a 0.1mM solution. This channel was treated with NaOH. The line indicates when the current is constant and the time that has been taken to calculate the* μ_{EOF} *y el* v_{EOF} *. Now the time is smallest*

TABLE II
SHOW THE RESULTS WITH NAOH TREATED CHANNEL

Round	Velocity (cm/s)	EOF mobility(cm2/V*s)
n=1	0.0978	0.00088
n=2	0.0818	0.00074
n=3	0.1	0.00090
Average	0.0932	0.00084
RSD %	10.65	

Treating the channel with NaOH introduce more negative charges on the channel walls, producing a thicker double layer. This leads to an increase of EOF, as it can be seen in the equation (2).

RSD with NaOH treated was better than non treated PMMA channel.

C. Measurement of EOF with UltraBlock treated PMMA channels

The process of treatment of the Ultra Block is the same as the one describe with the NaOH.

In Figure 10 it can be observed that the current stabilization takes longer than the other treatments.

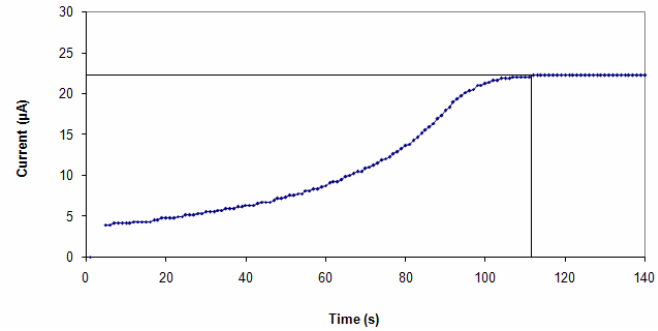

Fig 10*.With UltraBlock treated channel the time to stabilized is higher than non treated channels.*

TABLE III
SHOW THE RESULTS WITH ULTRABLOCK TREATED CHANNEL

Round	Velocity (cm/s)	EOF mobility(cm2/V*s)
n=1	0.0253	0.00023
n=2	0.0243	0.00022
n=3	0.0225	0.00020
Average	0.0240	0.00022
RSD %	5.88	

With the channel treated with UltraBlock there is a bigger improvement with the RSD%, as show in Table III.

The decrease of the v_{EOF} and μ_{EOF} is due to the treatment with UltraBlock, which reduces the capillary's wall charges, that reduces a δ thickness of the double layer, decreasing the electrophoretic velocity.

IV.III. CHARACTERISATION OF INTEGRATED PLATINUM ELECTRODES

The devices were tested with impedance and conductivity measurements using both static and electrophoretic flow, respectively. For the static flow experiments, impedance detection (Figure 11) was performed and for the electrophoretic experiments (Figure 12), conductivity data was recorded. Impedance measurements were taken at a range of frequencies to measure the sensitivity of the integrated platinum electrodes with three different solutions, each with a different ion concentration and conductivity. Figure 11A shows the recorded impedance phase and Figure 11B shows the impedance module responses (logarithmic scale) for two different solutions with the PBS 1mM water having the highest impedance and the 10 mM PBS solution having the lowest impedance. These results show a good sensitivity between the two platinum electrodes to detect different ion concentration solutions. Figure 11 also shows a good resistive behaviour for the 1 mM and 10 mM PBS buffers with impedance phases close to 0 degrees for a considerably large frequency range for both solutions.

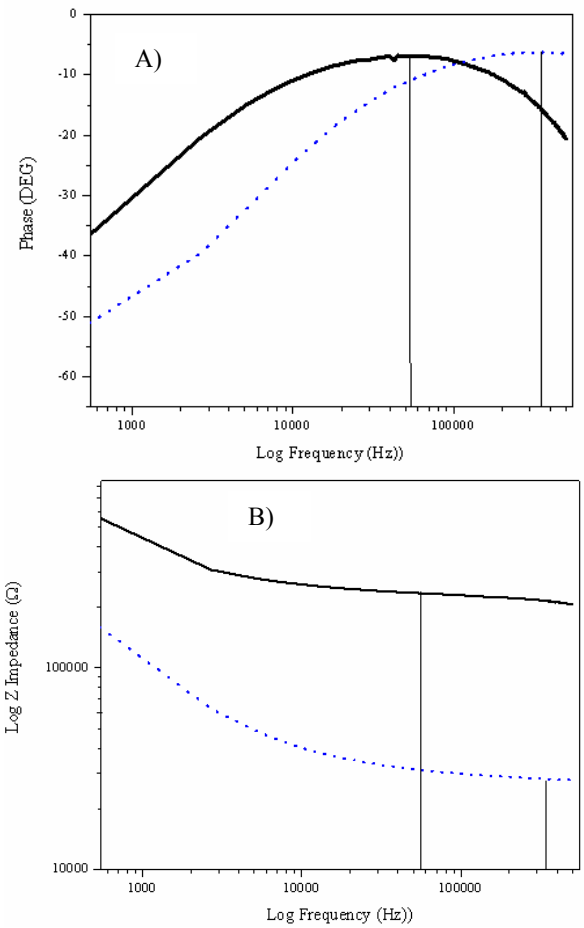

Fig 11. . *Dots line are solution to10mM PBS and solid line is 1mM PBS.The vertical line show the frequency that in phase is more near to 0ª, hence the zone Impedance is the resistivity and not have zone capacitive.*

Table IVIV shows a comparison for the 1 mM and 10 mM solutions between the impedance experimentally measured when the phase of the impedance between the two electrodes was close to zero and the calculated impedance values (R) using Equation (8)for the three different chips,

$$R = \rho \frac{l}{S} \qquad (8)$$

where ρ is the resistivity of the buffer, l is the distance between the two electrodes and *S* is the section area of the channel. Table IV shows good agreement between the impedance measurements and calculated values for the three different chips. The measurements correspond to three different chips with different electrode gaps of 962 µm, 447 µm and 364 µm.

TABLE IV

COMPARISON SHOWING GOOD AGREEMENT BETWEEN THE MEASURED AND CALCULATED IMPEDANCE FOR 3 FABRICATED CHIPS, EACH WITH DIFFERENT SEPARATION DISTANCE BETWEEN ELECTRODES

	Chip 1		Chip 2		Chip3	
	Electrode dist: 962 µm ChannelØ: 143 µm		Electrode dist: 447 µm ChannelØ: 135 µm		Electrode dist: 364 µm ChannelØ: 131 µm	
	Spectra/KΩ	Calc./KΩ	Spectra/KΩ	Calc./KΩ	Spectra/KΩ	Calc./KΩ
PBS 1mM	466	520	270	286	238	188
PBS 10mM	55	58	37	32	28	22

To test the device under electrophoretic conditions the channel and outlet reservoir were filled with a 0.1 mM PBS solution and the inlet reservoir was filled with a 1 mM solution. A 300 V voltage was then applied to the inlet reservoir and the outlet reservoir was held at ground. Figure 3 shows the resulting conductivity data, measured between the two integrated platinum electrodes.

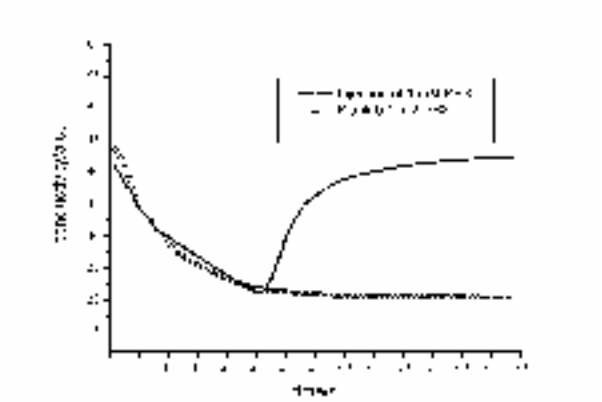

Fig 12. *Response of the integrated platinum electrodes under electrophoretic conditions (300 V) showing the electrophoretic injection of a 1 mM PBS solution into a channel filled with 0.1 mM PBS solution compared to a blank trace where a 0.1 mM solution was injected into a channel filled with a 0.1 mM PBS solution.*

Figure 12 shows the change in baseline compared to the blank signal as the front of the 1 mM solution reached the integrated platinum electrodes.

I.V.IV. TESTING OF SYSTEM WITH PARTICLES

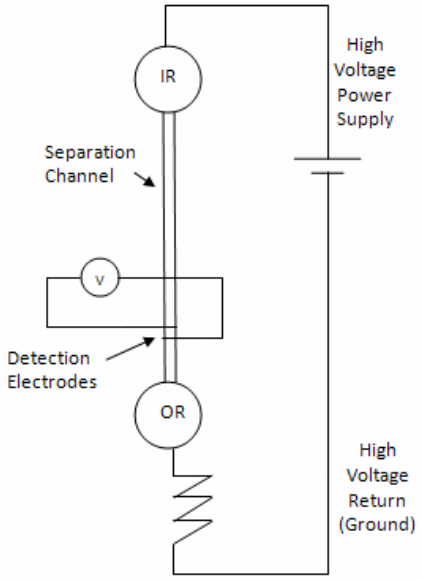

Fig 13. *Schematic diagram of the circuit of the FRD microfluidic system. Inlet reservoir, IR, outlet reservoir, OR.*

The detection of the particles is based on a passive detection system[12], At a fixed applied separation high voltage, the potential difference, V, is measured across the detection electrodes, as illustrated in Figure 13. The constant current, I, passing through the separation channel is measured at the high voltage return point (ground). With these two parameters, the resistance of the solution, R, passing through the detection electrodes can be simply determined by the floating resistivity detector (FRD) as expressed by Ohm's law (9)

$$R = \frac{V}{I} \quad (9)$$

At given length of the detection window, L and a fixed cross section area of the separation channel, A, the resistivity of the solution, ρ, can be obtained with the formula depicted in Equation (10).

$$R = \frac{L \times \rho}{A} \quad (10)$$

The channel is filled with buffer to generate a constant potential difference and obtain a baseline signal. When the particles of a different resistance enter between electrodes the potential difference change and the signal is detected. The channel was treated with UltraBlock during 10 seconds in order to decrease the EOF as has been showed earlier, cleaned with water for 2 seconds and filled with 1 mM PBS. An applied voltage of 300 V was used to introduce the particles into the channel from the injection reservoir. Due to the reduction of the EOF and the higher electrophoretic mobility of negatively charged particles the applied voltages were changed around so that the particles migrated from the

negative to the positive electrodes. With 300V applied and the channel filled with charged particles peaks were recorded as they passed over the platinum electrodes, as shown in Figure 13. Validation of the particle detection was by means of observing the particles as they passed over the electrodes using a Photron FastCam ccd camera mounted on an Olympus IX71 inverted optical microscope and the appearance of the peaks on the software recording the voltage. To confirm this, a more robust method of validation will be developed in further work.

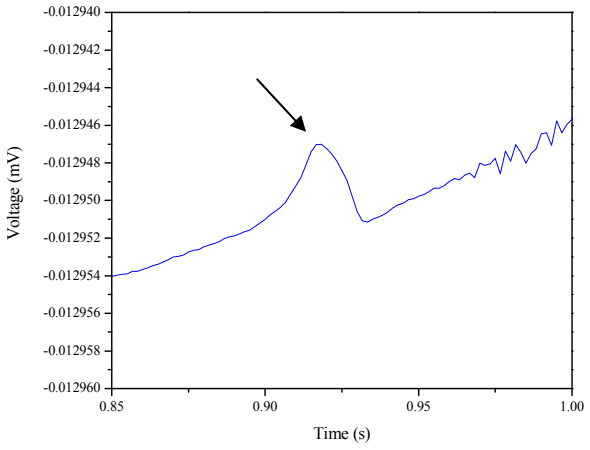

Fig 13. *A particle passing over the electrode array resulting in a broad peak indicated by the arrow.*

Table V shows the values of electrophoretic velocity and electrophoretic mobility for the latex micro particles. Measurements were determined using equations (5) and (6) respectively, but now, L for calculate velocity is the total distance of electrodes.

TABLE V

THE ELECTROPHORETIC VELOCITY AND MOBILITY OF 5 PARTICLES

Particles	Velocity (cm/s)	EOF mobility(cm2/V*s)
n = 5	0,0171	0,000156
RSD %		10,3275

Table 5. *The electrophoretic velocity and mobility of 5 particles.*

V. CONCLUSIONS

We have developed a method of fabrication that is quick and cheap and ideal for the preliminary testing of a microfluidic device with integrated electrodes. The fabrication of the PMMA chip was carried out in a standard laboratory without the requirement of any special equipment

This work shows that the treatment of wall channels is possible to control the electrosmotic flow using NaOH and UltraBlock and is comparable to previous publications.

Particles were detected using capillary electrophoresis integrated with a conductivity detector.

The objective for future work is to characterize particles and cells in smaller diameter channels fabricated using more conventional methods of lithography.

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REFERENCES

[1] A.Manz, N.Grabber, H.M. Widmer, Sensors and Actuators, 1979, 26, 1880-1886.
[2] G.Xu, J.Wang, Y. Chen, L. Zhang, D. Wanga and G. Chen, Lab Chip,2006,6, 145-148.
[3] Chen, H.Guo, Y. Shen, Y. Hu, Y. Sun, Sensor & Actuators B, 2006, 114, 1100-1107.
[4] L. Martynova, L. E.Locascio, M. Gaitana, G. W.Kramer, R. G.Christensen and W. A. MacCrehan, Anal. Chem., 1997, 69, 4783-4789
[5] G. S. Fiorini, G. D. M. Jeffries, D. S. W. Lim, C. L. Kuyper and D. T. Chiu *Lab Chip*, 2003, 3, 158–163
[6] E. Igarta., M. Arundell, H. Morgan, J. M. Cooper, *Lab Chip*, 2002, 2, 65–69
[7] C. D. García, Y. Liu, P. Anderson. and C. S. Henry, *Lab Chip*, 2003, 3, 324–328
[8] M. A. Schwarz, B. Galliker, K. Fluri, T. Kappes and P. C. Hauser, *Analyst*, 2001, 126, 147–151
[9] A.Piruska, I. Nikceveic, S.H. Lee, C. Ahn,W.R.Heineman, P.A.Limbach,C.J.Seliskar, Lab.Chip, 2005,5, 1348
[10] M. A.Rodriguez, D.W. Armstrong, 2004, 800, 75-25
[11] Rios,A.,Escarpa,A.,Gonzalez,M.C.,Crevilen, A.G.,Trends Anal. Cehm.2006,25, 467-479
[12] E. T. T. Tay, W. S. Law, S. P. C. Sim, H. Feng, J. H. Zhao and S. F. Y. Li, Electrophoresis, 2007, 28.4620-4628
[13] N. Naruishi, Y. Tanaka, T. Higashi and S. I. Wakida, Jour. Of Chromo. 1130 (2006) 169-174
[14] D.R. Bake. (1995) Capillary Electrophoresis(1st ed.)[Book]
[15] M.Arundell, A.Colom, O. Castillo, J.Samitier, Lab on a Chip, to be published
[16] G.S. Fiorini, G.D.M. Jeffries, D.S.W. Lim, C.L. Kuyper and D.T. Chiu, Lab Chip,2003,3, 158-163
[17] Dharma R., T. Bhushan and B. Bhushan, Trans. Nanotec, 2006, 5, 228-231
[18] T.Koerner, L. Brown, R. Xie, R. D. Oleschuk, Sens. Actuat, 2005, 197, 632-639