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An AC electroosmotic micropump for circular chromatographic applications

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Flow rates of up to 50 μm s^{-1} have been successfully achieved in a closed-loop channel using an AC electroosmotic pump. The AC electroosmotic pump is made of an interdigitated array of unequal width electrodes located at the bottom of a channel, with an AC voltage applied between the small and the large electrodes. The flow rate was found to increase linearly with the applied voltage and to decrease linearly with the applied frequency. The pump is expected to be suitable for circular chromatography for the following reasons: the driving forces are distributed over the channel length and the pumping direction is set by the direction of the interdigitated electrodes. Pumping in a closed-loop channel can be achieved by arranging the electrode pattern in a circle. In addition the inherent working principle of AC electroosmotic pumping enables the independent optimisation of the channel height or the flow velocity.

1 Introduction

Over the last few decades chromatographic separation techniques have become indispensable in the pharmaceutical, agricultural and chemical industries. Now quality control regulations are continually being tightened so that there is a permanent need to improve separation performance. Thanks to the advent of microseparation techniques such as capillary HPLC, capillary electrophoresis, capillary electrochromatography and capillary supercritical fluid chromatography, separation speed and power have greatly been increased. However, these latter techniques face performance limitations inherent in their working principle: for HPLC it is the pressure drop that limits the separation performance,[1] whereas for electroseparation methods, the applied voltage limits the separation performance. Since the potential for innovation and improvement of these conventional techniques have declined, the current challenge consists of innovation at a more fundamental or conceptual level.

The recently proposed concept of on-chip circular chromatography[2] is a good representation of this sort of innovation which may in turn break through the performance barriers. Circular chromatography (CC), like cyclic separation methods,[3] results from the desire to recycle the analytes coming out of a separation column in order to increase the analysis time, while reducing the physical length of a column. However circular chromatography differs from cyclic separation methods in that there is no discontinuity along the column, that is, a seamless flow (re-entrant flow) is generated along a single closed-loop channel. Analysis time can be varied at will. Since the plate number is proportional to analysis time, extremely high plate numbers could be generated for difficult separations but low plate numbers when separations were simple, with the benefit of a reduced analysis time.

In order to develop a circular chromatographic system, the first step consists of finding a suitable method for pumping in closed-loop channels. So far only magnetohydrodynamic (MHD) pumping has experimentally been investigated for closed-loop channels.[4] A maximal flow velocity of 40 μm s^{-1} was reported for a 30 μm height channel.[4] MHD pumping systems turned out to be intrinsically limited because of the channel height-dependence of the flow velocity,[4] hence alternative pumping methods were investigated.

In the following work, AC electroosmotic pumping (ACEOP) was chosen for the following reasons: firstly this method was theoretically described and experimentally demonstrated in linear channels.[5–7] It is suitable for closed-loop channels and generates flow rates that are sufficiently high to perform open channel chromatography (a velocity of up to 450 μm s^{-1} , close to the electrode surface, has been reported).[7] Secondly an advantage of AC electroosmotic flow (ACEOF) stems from the fact that the flow velocity is independent of, the channel height h so that h can theoretically be minimized in favour of the separation quality without encountering pumping power limitations. Another advantage of ACEOF stems from the low power required for pumping (an AC voltage of typically 1 V_{RMS} and 5 kHz) and consequently the experimental system requires minimum instrumentation.

This paper shows that ACEOP is suitable for fluidic pumping in a closed-loop channel and it discusses the potential of circular chromatography using this pumping method.

2 Theory

a. AC electroosmotic pumping principle

As shown in Fig. 1, an AC electroosmotic device is made of an interdigitated array of unequal width electrodes located at the bottom of a channel, with an AC voltage applied between the small and the large electrodes. The driving forces of ACEOF are the coulombic forces exerted on the double layer above the electrodes due to the tangential component of the electric field.[8] The forces generated are always directed away from the small space between the electrodes and parallel to the electrode surface, regardless of the

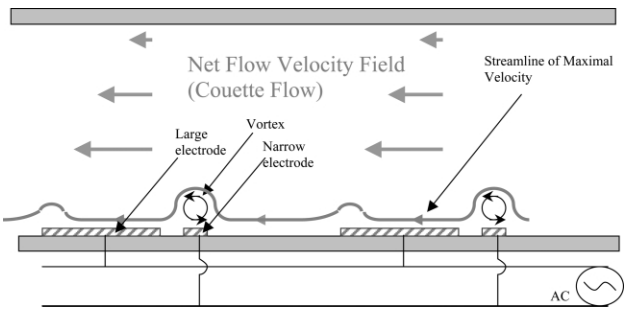

Fig. 1 Schematic representation of an ACEOF profile.

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polarity of the electrodes, *i.e.* independent of the phase of the AC field applied. Directional pumping of the whole fluid then results from the geometric asymmetry of the electrodes,[6] and can be explained by the fact that the fluid shear force penetrates further into the solution above the larger electrodes.

As depicted in Fig. 1, small vortices form above the narrow electrodes.[6] According to the models of Ajdari[5] and of Brown *et al.*,[6] these vortices scale with the electrode size and are of the order of the narrow electrode width. Although no study has yet shown the influence of the channel height on the net flow velocity, the presence of these vortices can be speculated to cause a dramatic drop of the net flow for channel heights approaching the order of the narrow electrode width. In addition these vortices may increase the longitudinal diffusion with respect to the flow direction by mixing the analyte plug and thus be considered as an intrinsic phenomenon that reduces the separation resolution. So far theoretical studies and experimental demonstrations agree on the behaviour of ACEOF with respect to the applied frequency and voltage. It has been shown that velocity profile for AC electroosmosis is zero at high and low frequencies and maximum at an intermediate characteristic frequency f_0 expressed as eqn. (1):

$$f_0 = \frac{\lambda_D \sigma}{\varepsilon \pi^2 x} \tag{1}$$

where x is a characteristic length for the electrode array (4.3 micrometer for the array used), λ_D is the thickness of the double layer, ε and σ are respectively the permittivity and the conductivity of the solution.[6] It was also shown that the flow velocity linearly increases with the square of applied voltage, the upper limit of which is restricted by electrolysis at the electrodes. Thus f_0 and $V_{0,max}$ will characterise the pumping limits of an ACEOF device.

b. Couette flow as a first order model for the velocity field

In order to quantify the potential of ACEOP for circular chromatography, a first approach consists in considering a simple theoretical model for the velocity field. As quoted in the previous section, the driving forces of the ACEOP take place at the bottom of the channel, within the double layer. The net flow then is formed by the effect of viscous drag in the channel solution. Neglecting the flow profile in the double layer and the influence of the small vortices, the flow can be considered equivalent to Couette flow (see Fig. 1). This flow profile also occurs in shear driven chromatography as demonstrated by Desmet *et al.*,[9] who pointed out the potential for separation power improvement that could result from simply switching one driving force to another. Thus assuming that the pressure drop is negligible and that the diameter of the vortices is negligible compared to the channel height, it is reasonable to adopt the Couette flow model for theoretically describing the flow velocity field that is generated by ACEOP. Therefore the mean velocity u_m can be expressed as in eqn. (2):

$$u_m = \frac{1}{2} u_{max} \tag{2}$$

where u_m is the velocity, averaged over the channel length, of the fastest streamline flowing at the bottom of the channel. Eqn. (2) shows that the fluid velocity is independent of the channel height. It should be noted that the potential of ACEOP in terms of separation power comes from this absence of correlation between h and u_m. Indeed h can be minimized at will in order to improve separation speed and resolution, as shown in the following section. It is interesting to note that ACEOP is thus well suited for miniaturization. In practice, however, the independence of h with respect to u_m is only valid if the narrow electrode width can be neglected compared to the channel height.

c. Circular chromatography

In conventional linear LC, the separation efficiency (plate number) is limited by the channel length due to high pressure drops, which explains why the plate height plays a key role. In a circular chromatograph, the analysis is stopped when sufficient analyte separation is obtained. Consequently the plate generation rate, representing the rate at which separation is obtained, is the crucial parameter describing the quality of the chromatographic device. Chromatographic theory for a rectangular channel of infinite width gives the plate height HETP of an analyte with a capacity factor of one and with a triangular flow profile as in eqn. (3):[9]

$$\mathrm{HETP} = 2\frac{D_m}{u_m} + \left(\frac{2}{5}\frac{h^2}{D_m} + \frac{1}{6}\frac{\delta^2}{D_s}\right) u_m \tag{3}$$

where h is the channel height, u_m is the solution mean velocity (m s^{-1}), δ is the thickness of the stationary phase layer (m), and D_m and D_s respectively represent the analyte diffusion coefficient in the mobile and stationary phase (m^2 s^{-1}). Assuming typical values of $\delta/h = 0.25$, $D_m = 10^{-9}$ m^2 s^{-1}, and $D_s = 0.5D_m$, the plate height expression becomes as given in eqn. (4):

$$\mathrm{HETP} = 2\frac{D_m}{u_m} + 0.421\frac{h^2}{D_m} u_m \tag{4}$$

As it is known that the plate number $N = u_m t/\mathrm{HETP}$, we can express the plate generation rate as:

$$\frac{N}{t} = \left(\frac{2D_m}{u_m^2} + 0.421\frac{h^2}{D_m}\right)^{-1} \tag{5}$$

Eqn. (5) shows that for a sufficiently small channel height, the plate generation rate scales with the square of the solution velocity. Because h and u_m are independent when ACEOP is used, the standard approach for optimising an ACEOF driven circular chromatograph consists of decreasing h as much as possible, combined with maximizing the solution velocity. For ACEOF, the practical limits are the minimum channel height, which is determined by the vortices forming above the narrow electrodes, and the maximum velocity, which is limited by electrolysis occurring at too high a voltage. On the basis of the experimental results given later, the maximal plate generation rate of ACEOF-driven circular chromatograph can be estimated using eqn. (5).

3 Experimental

a Devices

A two or three layer approach was chosen to produce the devices, resulting in a PDMS/Glass chip (Fig. 2, top) and a Glass/Laminate/Glass chip (see Fig. 2, bottom). The channel and microelectrode patterns are shown in Fig. 3.

In both cases, the bottom plate was fabricated using a 1 mm thick glass substrate, which was ultrasonically cleaned and a 5 nm layer of chromium was thermally evaporated onto the surface followed by a 70 nm gold layer. A circular array of asymmetric electrodes was then formed in the gold–chromium layer using standard photolithography in a class 10 000 cleanroom. Each electrode pair has the following dimensions: 4 μm wide electrode, 5 μm wide gap, 26 μm wide electrode, 16 μm wide gap. This 50 μm wide unit is repeated 564 times along a circle with a 4.49 mm radius, a section of which is shown in Fig. 3.

In order to fabricate the top plate of the glass/laminate/glass chip, a 30 μm layer of dry film laminate resist (DFLR) (Laminar 5038, Shipley, UK) was laminated onto a 0.5 mm thick pre-cut, pre-drilled glass slide. Access holes for fluidic and electrical connections were micro-milled in the glass slide. 100 μm channels were then excimer laser machined into the DFLR using an Exitech 8000 Series Microfabrication Workstation, incorporating a Lambda Physik Compex 110 excimer laser, operating at 248 nm (KrF). This system allows sub-micron positioning and accurate machining of the workpiece, all under computer control.

The laser machined micro-channels were then aligned with the circular electrode array using a mask aligner (Tamarack MAS-12). As the DFLR remains slightly adhesive after being coated onto the glass, the whole device could be transferred to a thermal press whilst maintaining alignment. The device was heated to 130 °C under an initial pressure of 0.5 MPa, held at 130 °C for 10 minutes, and then allowed to cool naturally to room temperature, under pressure. This sealed the laminate to the electrode structure, forming the channel structure (Fig. 2 and 4). Finally solution reservoirs were glued to the chip, fabricated from Luer lock fittings of syringe needles.

An alternative lid structure was also investigated, which consisted of a PDMS cast lid complete with the channels and ports. This consisted of the closed-loop separation channel, the filling channel and the injection channel (Fig. 4). The master used for molding the PDMS patterns was formed by using standard photolithography in a 100 μm thick SU8 photo resist layer spin-coated on a glass plate. Then the PDMS cover lid was drilled using a pre-filed syringe needle in order to obtain the access holes for fluidic and electrical connections.

After cleaning ultrasonically the two parts of the chip, the PDMS cover lid proved to be sufficiently adhesive to ensure a water-tight sealing for the experiment. Finally, additional pieces of PDMS were used to form fluidic reservoirs and placed on top of the PDMS lid, around the ports.

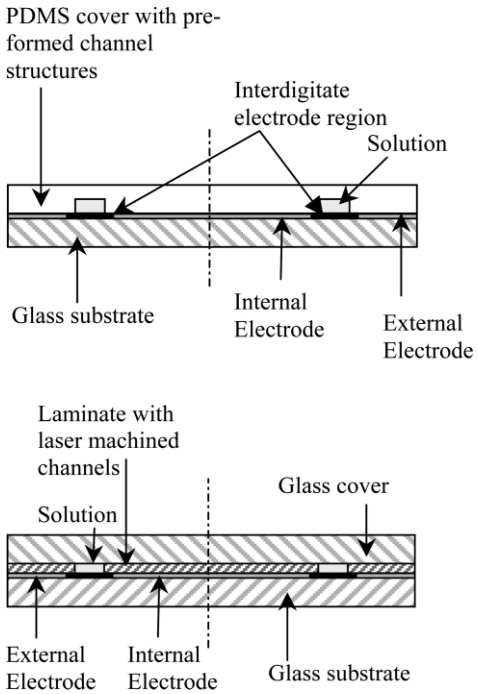

Fig. 2 Top: structure of the PDMS/glass chip (section X–X of Fig. 4). Bottom: structure of the glass/laminate/glass chips (section X–X of Fig. 4)

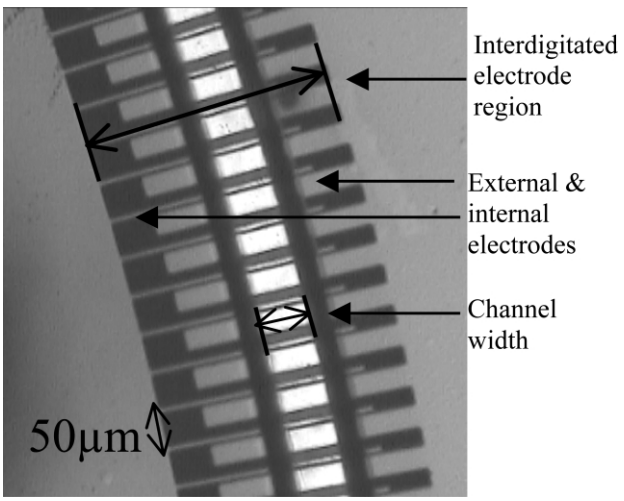

Fig. 3 Optical micrograph showing a section (See ZOOM in Fig. 4) of a laminate bonded device. The lighter region, where the laminate has been removed, is the channel. The circular, asymmetric electrode array can be seen to extend either side of the channel. The internal electrode has small fingers and the external one large ones.

b AC electroosmotic pumping setup

The internal and external electrodes (see Fig. 2) were respectively connected to the 0° and 180° phase terminals of a custom made four-phase sine wave generator (University of Glasgow, UK). From a computer coupled to the sine wave generator, the amplitude and the frequency of the desired signal could be set using a PASCAL program. The velocity measurements were carried out by optical microscopy using CCD camera and a standard VCR.

c Measurements

The measuring solution was a dispersion of polystyrene spheres in a 10^{-4} M solution of KNO_3. To visualize several flow streamlines in the channel cross-section, three types of spheres of different diameters (0.2 μm, 0.5 μm and 3 μm (Molecular Probes, Leiden, The Netherlands)) were injected in the solution and dispersed at 0.2%. Prior to filling the device, the electrolyte solution was deaerated by passing helium through it for 15 minutes. Helium was provided from a gas bottle (BOC, Guildford, UK), using a syringe needle to introduce the gas into the solution vessel. In the case of the glass/laminate chip, the filling could be carried out by using capillary forces due the hydrophilic property of the internal channel walls. Due to hydrophobicity of the PDMS, an alternative method was developed to fill the PDMS/glass chips, which proved faster and more simple. After filling one of the reservoirs with the de-aerated solution, a vacuum pump was connected to each empty reservoir and the electrolyte solution aspired.

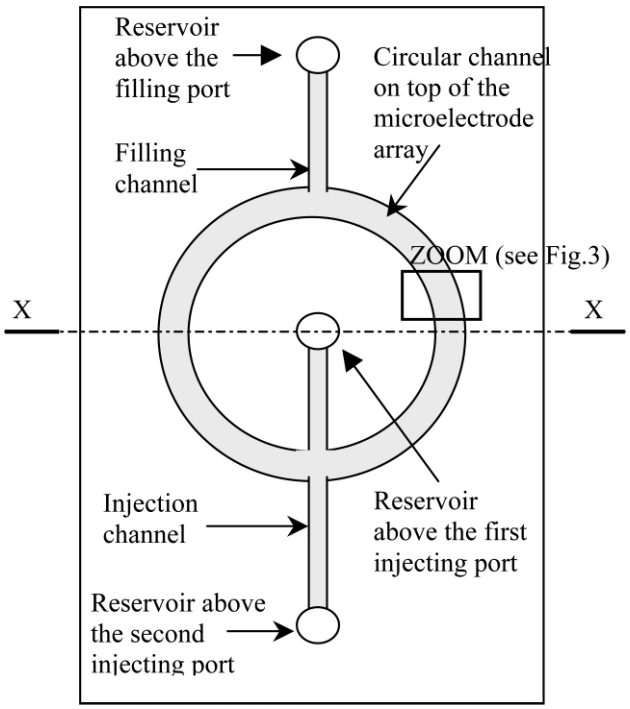

Fig. 4 Schematic of the channel structure with fluidic inlet and outlet ports

4 Results and discussion

a Pumping

When an AC voltage was applied to the electrodes, the spheres were observed to move inside the closed-loop channel away from the small electrode, across the narrow gap, towards the large electrode. The spheres were observed to follow the circular pattern of the channel and therefore it was shown that circular ACEOF electrode arrays can be used for pumping in a closed-loop channel.

b Frequency dependence

In order to characterize the ACEOF-driven circular chromatograph, it was first undertaken to measure the frequency dependence of the solution velocity. Using the glass/laminate/glass device with a channel height of 35 μm, an AC electric field was applied to the electrodes with the voltage set to 1 V_{RMS} and the frequency decreasingly from 20 kHz to 5 kHz. Frequencies lower than 5 kHz were not applied due to bubble formation by electrolysis of water. The pumping velocity was found to be approximately inversely proportional to the applied frequency between 5 kHz and 20 kHz (Fig. 5). Considering the measurement error (discussed fully in section 4d), a more precise shape of the curve could not be established. Using eqn. (1), and taking $\lambda_D = 30 \times 10^{-9}$ m, $\sigma = 0.00145\ \Omega^{-1}\ m^{-1}$ and $\varepsilon = 7.08 \times 10^{-10}$ F m^{-1} we obtain for a 10^{-4} M solution of KNO_3, a driving frequency, $f_0 = 1.4$ kHz, which can be in accordance with the data obtained assuming that the maximum velocity is not yet reached.

c Voltage dependence

In these experiments a PDMS/glass device was used with a channel height of 100 μm. After filling the device with the deaerated 10^{-4} M solution of KNO_3 containing polystyrene beads, an AC field was applied to the electrodes where the frequency was set to 5 kHz and the voltage was increased from 0.3 to 1.8 V_{RMS}. The application of voltages any greater than 1.8 V_{RMS} led to electrolysis and bubble formation. An approximately linear relationship between applied voltage and measured velocity was found (Fig. 6) with a maximal velocity of about 50 μm s^{-1} at an applied voltage of 1.8 V_{RMS}. From the theory, the flow velocity is expected to vary linearly with the square of the applied voltage. The difference between the theory and the results shown in Fig. 6 is attributed to the uncertainty of measurements.

d Uncertainty of measurements

The importance of using beads of different diameters is manifest: it enabled visualisation of the flow velocity and the small vortices (Fig. 1). Indeed, immediately after applying the AC voltage, small beads (0.2 and 0.5 μm diameter) were trapped by the vortices and observed in a backward and forward motion, whereas the 3 μm diameter beads continued to flow if not adsorbed. The size-discriminatory behavior of vortices indicates that they are approximately 3 μm in size, which is in agreement with Ajdari[5] and Brown *et al.*[6] predicting the dimension of the vortices to be of the order of the narrow electrode size (4μm).

The flow was characterized using the velocity of the fastest observed streamline u_{max}. From u_{max} the flow mean velocity can be directly derived using eqn. (2). As shown in Fig. 1, AC electroosmotic pumping gives rise to a non-planar flow close to the electrodes, which makes it difficult to determine the velocity. Hence, to determine u_{max}, we assumed that the polystyrene beads moved with the fastest streamline, which is questionable. Indeed, immediately after applying the AC voltage to the electrodes, a large majority of tracer beads were trapped by vortices or adsorbed. Both the latter phenomena dramatically decreased the bead concentration so that only a few streamlines were visible. Therefore the measured maximal velocity u_{max} can be assumed to be the fastest velocity observed for a bead or group of beads and will probably be an underestimation of the true velocity.

e Electrolysis

Electrolysis and hydrolysis taking place at the gold electrodes must be considered as the main limiting factor for the functioning of the device. Its negative effect is threefold: firstly electrolysis enhances bubble formation obstructing visualisation of the channel. Secondly electrolysis leads to the deterioration of the electrodes until the chromatograph is ineffective. Thirdly, analytes, especially the electrochemically unstable ones, can be decomposed. Electrolysis will be smallest at low applied voltage amplitudes.

Since the electrolyte solution was deaerated prior to filling, it is assumed that bubble formation was only due to electrolysis. At 5 kHz, the first gas bubbles formed at applied voltage of 1.8 V_{RMS}. In order to estimate the gold electrodes lifetime, the operating time was noted for the PDMS/glass device at 5 kHz and with a voltage increasing from 1 to 2 V_{RMS}, until most of the electrodes had dissolved in the electrolyte. The electrode lifetime was estimated to be approximately 90 minutes, which is currently a limitation of AC electroosmotic pumping. Future improvement of the device should aim at reducing electrode erosion, for example using new electrode materials such as ITO.[7] However the tendency to reduce the size of the electrodes in order to increase the flow velocity will shorten the lifetime of the device due to the deterioration of the electrodes. One method to overcome this limitation could be to cover the electrodes by using a dielectric protection film. Such a protective layer would have the added advantage of hindering electrolysis. However one must be aware that the voltage drop across this dielectric layer would require the application of higher voltages.

5 Potential of circular chromatography

The plate generation rate of the glass/laminate/glass device can be calculated as 0.26 plates per second, and of the PDMS/glass devices

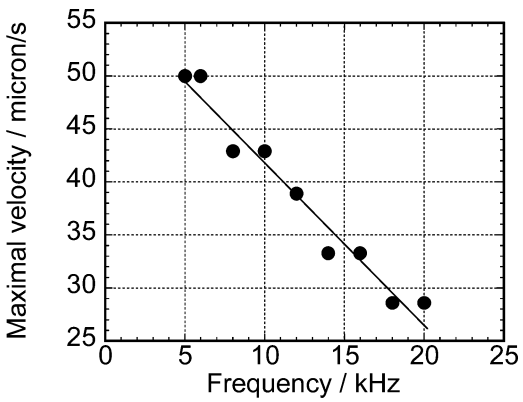

Fig. 5 Pumping velocity as a function of frequency (V_{RMS} = 1 V) in a 30 μm high channel (glass/laminate/glass device).

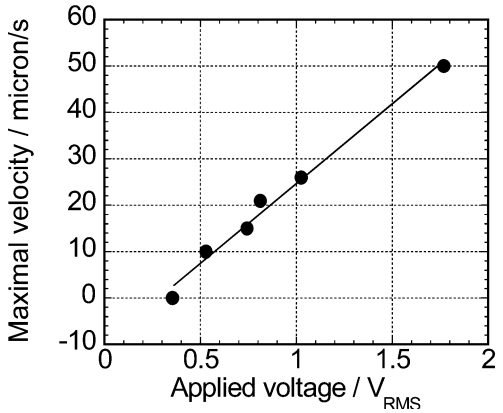

Fig. 6 Pumping velocity as a function of applied RMS voltage at 5 kHz in a 100 μm high channel (glass/PDMS device).

as 0.135 plates per second. These values are very low compared to those typically obtained using conventional HPLC columns (about 8 plates per second). Nevertheless much higher performances can be reached by increasing the flow velocity or optimizing the channel height, the great advantage of AC electroosmotic pumping being that various velocities and channel heights can be independently investigated.

As shown in the work of Mpholo *et al.*,[7] the perspective of development of AC electroosmotic pumping is promising. Indeed his work confirms that the flow velocity increases when decreasing the dimensions of the electrodes while keeping the same ratios between the electrode widths and the spaces in-between. It also shows that velocities of more than 450 $\mu m\ s^{-1}$ were obtained, close to the electrode surface. Therefore limits to the pumping performance are set only by the accuracy of the manufacturing processes used. This work also shows that, by placing two electrode arrays face to face, a plug flow can be obtained with velocities of up to 100 $\mu m\ s^{-1}$. It should be noted that in the case of a closed-loop channel, back pressure will vanish so that higher average velocities can be expected.

Limits to the channel height will primarily be due to the presence of vortices above the small electrodes. From the discriminatory behavior of the vortices with respect to the spheres of different diameter, it can be seen that the size of these vortices is of the order of magnitude of the width of the small electrodes. Assuming the influence of vortices to be negligible for the AC EOF, if the channel height is at least ten times the small electrode width and assuming that the resolution limit of the current manufacturing processes is 1 micron, the smallest channel height for an ACEOP possible using such techniques is 10 microns. In the case of a triangular flow profile eqn. (5) shows that for sufficiently high velocities, the plate generation rate is only determined by the second term and this is proportional to the square of the channel height. For a channel height equal to 10 μm, the plate generation rate will be 25 plates per second, which is a similar value to conventional HPLC columns. In the case of a plug flow obtained by placing two arrays of electrodes face to face, the low limit of the channel height will be imposed by the interference of the electric fields created by the top and the bottom electrodes.

6 Conclusions

Alternating current electroosmotic pumping (ACEOP) has been demonstrated in a device designed for circular chromatography. Solution velocities of up to 50 $\mu m^{-1}s$ were observed in 35 and 100 μm high channels. Electrode erosion has been identified as the main limiting factor in this work. The pumping methods specially adapted for circular chromatography will be those able to create a re-entrant flow and to increase at most the plate generation rate. ACEOP is one of them and is very promising. Compared to magnetohydrodynamic pumping, its potential originates from one additional degree of freedom which enables the independent optimisation of the channel height or the flow velocity. On the one hand, future improvements will be the natural consequence of miniaturisation trends since smaller electrodes lead to higher flow velocity. On the other hand the future of ACEOP depends on developing a suitable method of avoiding deterioration of the electrodes.

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