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Application of microfluidic chip with integrated optics for electrophoretic separations of proteins☆

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Abstract

This paper describes the fabrication, the characterization and the applications of a capillary electrophoresis microchip. This hybrid device (glass/PDMS) features channels and optical waveguides integrated in one common substrate. It can be used for electrophoretic separation and fluorimetric detection of molecules. The microfluidic performance of the device is demonstrated by capillary zone and gel electrophoresis of proteins.

Keywords: Lab-on-a-chip; Integrated optics; Microfluidic; Capillary electrophoresis

1. Introduction

For a number of years, lab-on-a-chip (LOC) technology has attracted growing interest, in particular in the domain of biomedical sciences [1–6], allowing high throughput operation and analysis of a minute amount of samples. Detection by means of laser-induced fluorescence is one of the most popular techniques associated with micro capillary electrophoresis. However, this conventional detection method relies on bulky detection systems (lenses, microscope objectives), and delicate light coupling components, leaving the portability of LOC system slightly controversial.

To overcome these limitations, various concepts of the integration of optical detection systems have been proposed [7–19]. In our previous paper [20] we described in details the fabrication method and the basic optical characteristics of a microsystem, featuring integrated microfluidic and optical detection system. As our microoptical components are highly temperature sensitive, classical thermal bonding methods had to be excluded from the fabrication process. This gave rise to the concept of hybrid glass/PDMS device has attracted our attention. PolyDiMethylSiloxane (PDMS) is simple to fabricate, it bonds easily at room temperature to the glass substrate in a reversible way (or in an irreversible way, if the bonding is carried out in oxygen plasma). Moreover, PDMS features high chemical resistance and optical transparency. Unfortunately, in the native form this polymer does not have ionisable groups on its surface and these groups are necessary to drive fluids by means of electroosmotic flow (EOF) [21]. According to Pallandre's review, different surface treatments have been applied to PDMS, in order to improve its surface properties [22] and to create a superficial electric charge, necessary for electroosmosis. In our device, microchannels are etched in glass substrates and a PDMS film is used as a cover. It appears from our experiments that in such structures the electroosmotic flow does exist indeed. The EOF characteristics were evaluated in this paper.

Microfluidic systems also hold great promise for realizing multidimensional separations in a simple integrated device. Exemplary devices were fabricated in full glass [23–26], polycarbonate [27], double layer PDMS [28], poly(methyl methacrylate) (PMMA) [29,30]. Although PDMS channels have been already described in PDMS/glass device [31–36], no glass channels have been reported in such hybrid device.

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The articles mentioned above explored the application of microfluidics to multidimensional peptide and protein separations [25,26,37,38]. Proteins have drawn our particular attention as our long term goal is to develop a diagnostic platform, capable of direct analysis of blood or saliva samples, in search for specific biological species—like cancer markers for instance.

Finally, in this paper, various separation modes like capillary zone electrophoresis (CZE), and capillary gel electrophoresis (CGE) protocols were examined. To demonstrate the separation potentialities of the device, CZE and CGE were tested with a mixture of streptavidin (MW: 60 kDa) and Cy3 (MW: 766 Da), but also with a mixture of well defined proteins, namely carbonic anhydrase II (pI = 5.9, MW = 29 kDa) and β-lactoglobulin A (pI = 5.1, MW = 18.28 kDa) [39]. Adequate protocols were developed and applied on chip for the separation of model proteins mixture.

2. Experimental

2.1. Chip fabrication

The fabrication of the optical waveguide in soda-lime glass plate was realized by the Na^+/K^+ ion exchange method resulting in the local change of the refractive index of glass [20,40]. Then, the microfluidic structure, comprising two intersecting channels, was fabricated in the same substrate by means of UV photolithography and wet etching methods. The substrate was covered with a layer of PDMS (Sylgard 184, Dow Corning) polymer, c.a. 1 mm thick and liquid reservoirs were made in this layer. This fabrication process was described in details in our previous work [20].

The depth and the width of the microchannel were 20 μm and 80 μm, respectively, with 5 cm long separation channel.

2.2. Measurement set-up

The in-depth characteristics of the experimental setup have been demonstrated previously [20]. Briefly, the programmable high voltage power supply (Labsmith HVS448-3000D, Livermore, CA) was used to apply electrical potentials between −1.5 kV and +1.5 kV of each of the four reservoirs. A 532 nm fibered laser (DPGL-2050, Suwtech, Shanghai, China) was aligned with the optical waveguide (10 μm wide) to excite labelled proteins. At the detection area, the fluorescence emission was collected by the microscope BXFM (Olympus, Rungis, France) perpendicularly to the chip surface, and detected by a silicon photodiode based detector (2151M, New Focus, San Jose, CA). The microfluidic analyses were recorded by a chromatography data system called Azur (Datalys, Saint-Martin d'Hères, France).

2.3. Chemicals

Sodium hydroxide (NaOH 1 M), hydrochloric acid (1 M), ortho-phosphoric acid (85%), glycerol (99.5%), sodium dodecyl sulphate (SDS, 99%), sodium tetraborate decahydrate (borax, 99%), boric acid (99%), phosphate buffer at pH 7.2 and rhodamine 6G were supplied by Sigma–Aldrich (Saint-Quentin Fallaviers, France), Cy3 antibody labelling kit and streptavidin-Cy3 were purchased from GE Healthcare (Saclay, France). β-Lactoglobulin A (Lac A, pI = 5.1, MW = 18.28 kDa), carbonic anhydrase II (CA II, pI = 5.9, MW = 29 kDa) and eCAP SDS-200 kit, were obtained from Beckman Coulter (Villepinte, France).

The analyses performed for comparison were carried out using a P/ACE 2100 capillary electrophoresis system (Beckman-Coulter, Fullerton, CA, USA) equipped with a UV detector. Acquisitions were performed by means of P/ACE 2000 software version 2.0 (Beckman-Coulter). The samples were systematically injected in hydrodynamic mode (injection pressure 0.5 psi, i.e. 3.4 kPa) and their analysis was carried out using fused silica capillaries of 27 cm (20 cm effective length) × 50 μm i.d. × 375 μm o.d. purchased from Thermo Electron (Thermo Electron France, Les Ulis, France). The analyses were carried out at 25 °C and the pH of running electrolytes was measured before utilization, at this temperature, with a model IQ 240 pH meter (I.Q. Scientific Instruments Inc., San Diego, CA, USA). The electrolytes were systematically degassed by sonication by means of an Ultrasonic Cleaner model 2510 (Branson Ultrasonic, Danbury, USA). The detection was performed by photon absorption at 254 nm.

2.4. Conditions

Before each experiment, the substrate of the microchip was conditioned by incubation for 10 min in 1 M NaOH, rinsed in double distilled water, top covered with the PDMS layer and flushed with degassed running buffer. In capillary gel electrophoresis (CGE), the lab-on-a-chip device was also flushed with 1 M hydrochloric acid, rinsed with water before filling with a sieving matrix.

2.4.1. EOF measurement

To avoid any disturbances from the channel intersection [41], all the EOF measurements where carried out in a single channel. In order to measure the electroosmotic flow, we used two previously published independent methods: current monitoring [42] and indirect fluorescence method [43,44]. In the first case, we monitored changes of the intensity of the current flowing through the channel when a diluted electrolyte replaced progressively a more concentrated buffer. The electrical current was monitored, indirectly by measuring the voltage drop on the resistor of known value (1 MΩ), connected in series with the microfluidic channel. When an electrical field was applied along the channel, the current decreased until the second solution completely replaced the previous electrolyte. The electroosmotic mobility was calculated taking into account either the duration of this transient step or the slope of the current–time characteristics. EOF was also evaluated by indirect fluorescence method. In this case, the channel was filled up first with rhodamine 6G (excitation wavelength: 532 nm; collection wavelength: 560 nm) diluted in buffer as a background electrolyte. Then, a neutral marker (H_2O/ethanol (V/V)) was electrokinetically injected. An inversed fluorescent peak was detected, when the neutral marker attained the detection region at the end of the channel. The measured migra-

tion time allowed for the calculation of the electroosmotic flow.

2.4.2. Capillary zone electrophoresis (CZE)

The channels were filled with 10^{-2} M borate buffer, pH 9.2. Two different mixtures of molecules were separated by CZE in this paper. In all of the cases, the separation potentialities were estimated by resolutions (Rs) and efficiency (N) calculated at the half peak height of the peak [45]. For comparison between different devices, both of those parameters were calculated in function of the separation length (Rs/cm and efficiency/m) as it was described in literature [46].

First, a mixture of 10^{-6} M of streptavidin tagged with Cydye3 (Cy3) and 10^{-5} M pure Cy3 was separated as a proof of CZE concept of our device. Second, a mixture of proteins: carbonic anhydrase II and β-lactoglobulin A labelled with Cy3 was separated by CZE. The Cy3 labelling was performed as follows: 1 mg of total protein was prepared in 1 mL of 5×10^{-2} M of phosphate buffer to react with a molar excess of Cy3 in 30 min at room temperature. The majority of non-conjugated dye was removed by gel filtration.

A three-step separation process, already described in literature [47–49] was elaborated on our device. Electrokinetic injection, performed at 100 V/cm, was used to inject analytes from the sample reservoir to the waste reservoir of the injection channel. The size of the sample plug was defined at the channel intersection with an electrokinetically driven pinching step.

Then, the plug was injected during 5 s into the separation channel at 244 V/cm. A pullback voltage was applied at the end of the injection channel to limit sample leakage [49]. The length of the separation channel was 5 cm and the separation electric field was 344 V/cm. The separation distance was 2.7 cm.

This separation was also performed with the use of a commercial full-glass microchip (X3530, Micronit, Enschede, The Netherlands) as a reference. The same chemicals were used, but the length of the separation channel was only 4 cm in this case, with the electric field of 400 V/cm. The detection area was chosen to obtain a separation distance of 3 cm. The size of the plug (100 μm) was defined during injection by the geometry of the double T junction, without any pinching step. The fluorescence was excited by a mercury lamp at 532 nm.

For the separation of proteins in CZE, the same arrangement of electrical potentials was used during the injection and plug forming step. However, the separation field strength was reduced to 300 V/cm. The separation length was 5 cm and a borax buffer of 10^{-2} M was used.

2.4.3. Capillary gel electrophoresis (CGE)

For the experiments with CGE, the experimental protocol which is already described in the literature [46,50] was followed. After conditioning, all the channels were coated with the eCAP SDS-200 gel (Beckman-Coulter) by filling the injection reservoirs and the separation channel input with this polymer, and then, by applying vacuum at the output of the separation channel. Special care was taken in order to avoid the generation of air bubbles and this was controlled with an optical microscope. Then, the gel was replaced by the diluted SDS sample buffer (Tris–HCl buffer with 0.1% of SDS, pH 6.6) in the reservoirs of the injection channel. Before the injection, the sample mixture was prepared in SDS sample buffer and heated for 5 min at 90 °C. The channels were equilibrated during 5 min at 400 V/cm. The plug was formed by a floating step at 200 V/cm during 30 s and separation was accomplished electrokinetically at 300 V/cm.

3. Results

3.1. Microfluidic characterization

3.1.1. Electroosmotic mobility

As an electroosmotic flow was used to drive sample in our device, EOF was an important phenomenon to characterize. In the case of our hybrid device, around 75% of the surface of the microchannel walls consists in soda-lime glass, the rest being the native PDMS. Hence, we could expect the electrokinetic flow to be supported by the presence of the negative charge at the glass fraction of the channel.

Different methods have been described to characterize the electroosmotic mobility: periodic photobleaching [51], direct flow visualization method [52], indirect amperometric method [53], indirect fluorescence method [54], and current monitoring method [40]. Several reports have shown possibilities of employing current monitoring method for different materials like glass [21,55], PDMS [21,55,56], composite material [57–59] or hybrid devices (microchannels fabricated in PDMS) [33,35].

Before proceeding with EOF measurements, it is essential to define the type of flow, i.e. anodic or cathodic. By changing the electrical polarity across the channel (negative or positive), we found that the electroosmotic current has a cathodic character in our case.

To establish the value of the electroosmotic mobility, the current change induced by replacement of a 2×10^{-2} M borax buffer by a 1.5×10^{-2} M borax buffer was monitored. The modification of current in case of replacement of a 1.5×10^{-2} M borax buffer by a 2×10^{-2} M borax buffer was also recorded (Fig. 1).

As it is shown in the Fig. 1, in both cases, the current stabilized after approximately the same time. Thus, no influence of the order of the sequence of buffers injection (1.5×10^{-2} M replaced by 2×10^{-2} M or the reverse) was observed for this single channel device. Two methods of calculation were used to estimate the electroosmotic mobility. In the first approach, the electroosmotic mobility was calculated according to $\mu_{EOF} = L/(t \times E)$, where μ_{EOF} is the electroosmotic mobility, L the channel length (m), t the time (s) necessary to reach a constant current and E the electric field (V/m) [33]. The results are presented in Table 1. A mean electroosmotic mobility of 4.6×10^{-4} cm^2 V^{-1} s^{-1} was measured when 1.5×10^{-2} M of borax buffer was replaced by 2×10^{-2} M. The deviation of the current plateau of 6.5% was observed after six measurements. As shown in Table 1, the mean μ_{EOF} measured after the replacement of 2×10^{-2} M by 1.5×10^{-2} M was 4.35×10^{-4} cm^2 V^{-1} s^{-1}. However, taking into account the standard deviation of 6.5%, one can say that no influence of the order of replacement was

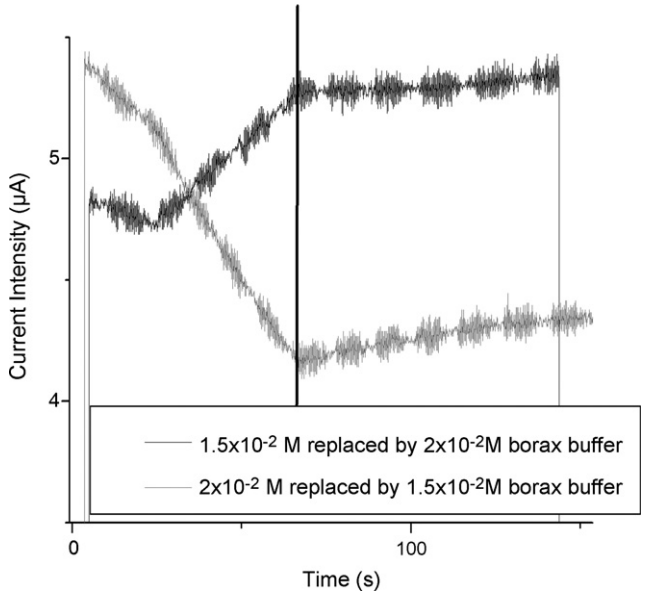

Fig. 1. Illustration of the results obtained with the current monitoring method when 1.5×10^{-2} M was replaced by 2×10^{-2} M of buffer (black curve) and the opposite (grey curve).

observed. When the device was reconditioned with 0.1 M NaOH between experiments, the reduction of the standard deviation from 6.5% to 2.5% was found.

Taking into account these results, a second calculation method for electroosmotic mobility, adapted from Ren et al. [42], was employed for comparison. In this case, the average electroosmotic mobility was calculated from the slope of the current–time relationship:

$$\text{slope} = \frac{\Delta I}{\Delta t} = \bar{u}_{\text{mean}} \frac{E \times A(\lambda_2 - \lambda_1)}{L} = \bar{u}_{\text{mean}} E \left(\frac{1}{R_2} - \frac{1}{R_1} \right) \quad (1)$$

where, $\bar{u}_{\text{mean}}$ is the average electroosmotic velocity (cm/s), A the cross-section area of the channel (cm^2), E the electric field (V/cm), $(\lambda_2 - \lambda_1)$ and $(1/R_2 - 1/R_1)$ are the conductivity difference and the difference between the resistances of the high-concentration solution and the low-concentration buffers, respectively. The experimental values of the resistance of each buffer were calculated from the slope of the Ohm's law. As calculated, results of the electroosmotic mobility are also presented in Table 1. The electroosmotic mobility around $4.7 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$ was estimated with this calculation method, without any influence of the order of the replacement protocol. It is interesting to note that both of these methods gave comparable results, albeit the slope measurement method demonstrated better accuracy.

Table 1
Electroosmotic mobility measured by different methods

	Electroosmotic mobility $\times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$	
	End point method	Slope method
1.5×10^{-2} M replaced by 2×10^{-2} M	4.6 ± 0.3	4.7 ± 0.2
2×10^{-2} M replaced by 1.5×10^{-2} M	4.4 ± 0.1	4.7 ± 0.1
Indirect fluorescence method	4.73 ± 0.05	

Relative standard deviation estimated from six experiments.

To confirm the above results, an independent approach was also tested. In this case, the electroosmotic mobility was calculated from the migration time of a neutral marker (H_2O/ethanol) in a fluorescent background constituted of rhodamine 6G in the running buffer, according to the well-known Eq. (2). Here, L_{eff} corresponds to the effective length of separation between the intersection of the two channels and the detection area. L is the full length of the separation channel and V the voltage applied.

$$\mu_{\text{EOF}} = \frac{L \times L_{\text{eff}}}{V \times t} \quad (2)$$

The results obtained by indirect fluorescence method are also summarized in Table 1. So, with this method, the average electroosmotic mobility was found to be around $4.70 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$ with good reproducibility (standard deviation of $0.05 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$).

In comparison with literature, our hybrid device had an intermediate EOF between full-glass chip ($5.45 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$) [55] and native PDMS device ($3.28 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$) [56]. Compared with another hybrid device ($\mu_{\text{EOF}} = 3.7\text{–}4.0 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$) [32,33], our chips featured higher EOF mobility, which is promising to drive sample in separation experiment. PDMS oxidized chip had a μ_{EOF} measured between $4.89 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$ [57] and $5.7 \times 10^{-4}\ \text{cm}^2\ \text{V}^{-1}\ \text{s}^{-1}$ [55]. Although the oxidized PDMS had a higher density of surface charge and a hydrophilic surface [60], we preferred to keep PDMS in its native form because the oxygen plasma treated sample had a short effective lifetime [21,61], resulting in fast modification of the electroosmotic mobility as a function of time.

3.1.2. *Influence of different additives on the electroosmotic mobility*

Improvement of the resolution in capillary zone electrophoresis can be achieved by reducing, enhancing or reversing the electroosmotic mobility. In this aim, permanent coating or dynamic coating can be envisaged [62]. The use of coating with liquid sieving gel, or solid crosslinked gel is interesting but difficult to integrate on microchip. For example, on one hand, it is difficult to obtain a uniform wall coating and, on the other hand, liquid gel could diffuse into interconnected area [63,64]. Moreover, air bubbles are frequently generated when a microchannel is filled with a sieving matrix.

In our work, the modification of the electroosmotic flow by addition of SDS surfactant or glycerol in the buffer was tested. Since the native PDMS layer was used as a top cover, a hydrophobic inner surface of the channel was expected after curing, which could induce bubble formation [61]. Addition of 2% of pure SDS to the solution of borax buffer (V/V) dynamically coated the walls and increased the cationic μ_{EOF} by almost 40% (Fig. 2) in comparison with the untreated chan-

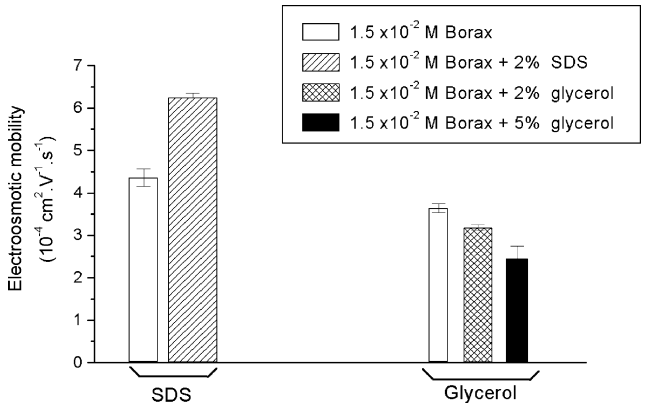

Fig. 2. Influence of additives on the electroosmotic mobility. The relative standard deviations were calculated on six measurements for glycerol and SDS.

nel. The μ_{EOF} was increased from 4.4×10^{-4} cm^2 V^{-1} s^{-1} ($\pm$0.1) up to 6.2×10^{-4} cm^2 V^{-1} s^{-1} ($\pm$0.1) in six reproduced experiments. These results confirm data published recently by Mourzina et al. where they observed a more hydrophilic surface of PDMS after treatment with SDS [35]. Here, we presumed that SDS at a concentration above its critical micellar concentration reduces the surface tension of aqueous solution by hydrophobic–hydrophobic interaction [65,66]. In fact, the hydrocarbon tail of the surfactant forms an adsorbed layer on PDMS [61]. Consequently, polar head group of the surfactant is exposed to the aqueous solution in the channel and creates a hydrophilic layer of increased charge density in the microfluidic channel [35,67].

In all the experiments made, no clogging of the channel was observed.

In another approach, glycerol was added to the running buffer in order to increase viscosity leading to a decrease of the electroosmotic flow. Furthermore, glycerol reduces protein adsorption on walls [66].

As the μ_{EOF} of any solution is inversely proportional to the viscosity, the amount of glycerol should be carefully controlled in order not to stop the EOF completely. In this study, we decided to add 2% (V/V) of glycerol which reduced the electroosmotic mobility by 13% (Fig. 2). For comparison, we also tested the addition of 5% of glycerol leading to the diminishing of 33% of the EOF. In the recent paper published by Busnel et al., they also studied the influence of glycerol on the electroosmotic mobility [68]. In comparison, our results showed higher efficiency of added glycerol. We consider that the difference arises from the PDMS layer we used, compared with fused silica capillary in their case. An additive effect between viscosity from glycerol and hydrophobicity from PDMS could explain the decrease of the electroosmotic mobility.

3.2. Separations of streptavidin/Cy3 mixture

In order to test the separation potentialities of our device, we eluted a mixture of Cy3 fluorescent dye and a commercial Cy3 tagged-streptavidin. During the separation experiments, sample concentrations of roughly 10^{-6} M to 10^{-5} M were used.

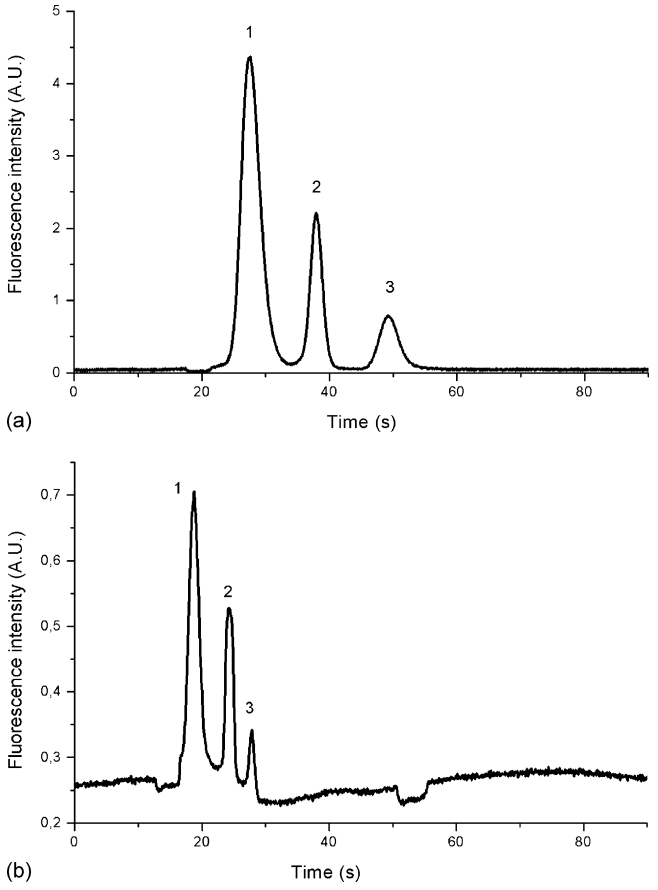

Fig. 3. (a) Electrophoretic separation of 10^{-5} M of free Cy3 and 10^{-6} M of Cy3 tagged streptavidin in 10^{-3} M borax buffer at pH 9.2 on PDMS/glass lab-on-a-chip. Field strength: 344 V/cm. The injection step duration was 5 s, the length of the separation channel was 5 cm and the separation distance was 2.7 cm. (b) The same mixture separated on Micronit full glass chip. Here, the length of the separation channel was 4 cm, the separation distance was 3 cm and the field strength was 400 V/cm. In both cases, the peak 1 was identified as Cy3-streptavidin, the peak 2 as native Cy3 and the peak 3 as hydrolyzed form of Cy3.

The analytical conditions (i.e. the size of the plug and the ionic strength of the electrolyte chosen) were optimized in order to achieve this separation by CZE on PDMS/glass chip. As Cy3 is pH independent, the separations were performed in alkaline borax buffer to obtain a significant electroosmotic flow. Before the separation, each component was tested individually both on our chip and with the use of an uncoated silica capillary by classic capillary electrophoresis system, in order to obtain reference profiles.

Fig. 3a, illustrates the separation profile of Cy3-streptavidin and Cy3 obtained in less than 1 min on the chip. The first peak was identified as streptavidin and the second as different forms of Cy3. For Cy3, the first peak was the native form of Cy3 and the second one was attributed to its hydrolyzed form [69–71]. The experiments were reproduced 6 times and we obtained a resolution on streptavidin/native Cy3 pair of Rs = 1.56 (Rs/cm: 0.58) with our hybrid PDMS/glass device. The efficiency for hybrid device varied from 16518 theoretical plates/meter for streptavidin to 78037 theoretical plates/meter for native Cy3. For comparison, the efficiency in Micronit chip was much higher

from 27500 theoretical plates/meter for streptavidin to 164800 theoretical plates/meter for hydrolyzed form of Cy3. As the Micronit lab-on-a-chip devices were fabricated in full glass, the generated power was better dissipated than in PDMS/glass device. So, we can explain the previous results as regards efficiency taking into account the electric field that we can applied on the chips dissipating the Joule heating. Indeed, the electric field applied is higher in Micronit chip (400 V/cm) compared with PDMS/glass chip (344 V/cm), consequently the efficiency is higher in full gall chip [72].

To show the stability of our microfluidic device, the separations were reproduced during 3 days, with the low time standard deviation of 2%. We believe that this deviation was mainly due to insufficient ambient temperature control.

It is important to note that we obtained the same separation profile (Fig. 3b) for both devices. The resolution measured on the streptavidin/native Cy3 pair on the full-glass device was Rs = 1.22 (Rs/cm: 0.41) with a time standard deviation of 2.6%. So, it means that the resolution obtained for this pair of solutes on PDMS/glass device was better than for the commercial chip.

Concerning the reproducibility, we believe that the better performance of our chip is mainly due to the integrated optics. In this case, the separation length is strictly defined as a distance between the channel intersection and the optical waveguide. On the contrary, when working with the Micronit chip, it was necessary to align the microscope-based detection system for each measurement independently. This might have introduced some variations of the separation length from one test to another.

3.3. Preliminary separation of protein mixture by CZE

Fig. 4 illustrates the separation of a protein mixture by CZE. β-Lactoglobulin A and carbonic anhydrase II which were injected into the device directly after modification of the mixture with Cy3. At this point, we would like to emphasize that, according to the literature data [39], labelling of a protein with Cy3 leads to a population of protein bearing one Cy3 or multilabelled protein resulting in various charge/mass ratio. This gives rise to multi-peaks for each component in UV absorption (Fig. 4b).

To confirm this hypothesis, different proteins were characterized by us before and after labelling with an uncoated capillary on a P/ACE 2100 system. And as we expected, after labelling a signal divided in two peaks was frequently observed. However, other explanations have been suggested in literature. For example CA II supplied by Sigma has been observed as two peaks in isoelectric focusing [64]. In the same way, an early article [73] described that Lac A can be expected as a dimer when the separation is carried out under non-reducing conditions as we performed our experiments. However, in our case, we think that the filtration system used to remove excess of Cy3 was not efficient enough; this is the reason why the first group of two peaks was assigned as unlinked Cy3 (peaks 1 and 2 in Fig. 4a and b). This hypothesis was confirmed with the apparent mobility of Cy3 when it was injected alone. According to their charge/mass ratio, CA II should appear before the Lac A on the electropherogram. This order was confirmed by previous work [39].

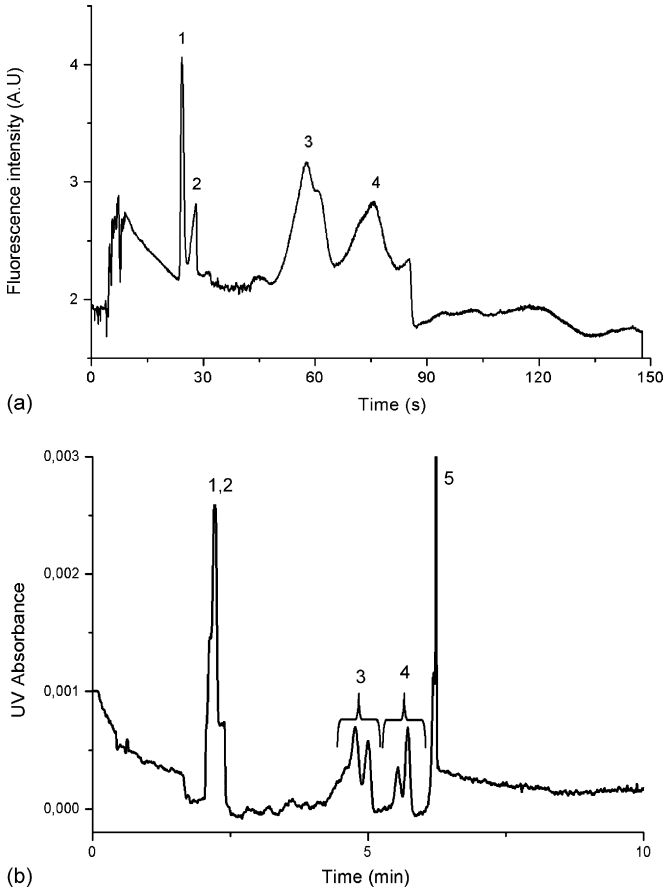

Fig. 4. (a) CZE separation of β-lactoglobulin A and carbonic anhydrase II on chip at 320 V/cm in 10^{-2} M borax buffer. The injection step duration was 5 s and the separation length was 2.7 cm. Proteins were detected by fluorescence. (b) Separation of the same mixture in 10^{-2} M Borax buffer. For comparison in this case, an uncoated capillary (75 μm i.d.) and a P/ACE 2100 system with UV detection was used. Hydrodynamic injection was performed at 0.5 psi during 3 s; separation length was 20 cm (full capillary length was 27 cm) and the separation voltage of 7500 V was used. In both cases, peaks 1 and 2 were identified as Cy3. Due to multi-labelling, carbonic anhydrase II and β-lactoglobulin A were interpreted as doubled peaks, annotated 3 and 4, respectively. The peak 5 detected with the fused silica capillary corresponds to an electrical spike.

Although, due to the insufficient resolution, we were not able to separate a protein with various amounts of Cy3, separation of the two proteins were achieved on our device with the resolution Rs = 1.07 (Rs/cm = 0.40). The efficiency measured on carbonic anhydrase II and β-lactoglobulin was about 30000 theoretical plates/meter. For comparison, by using classical capillary electrophoresis we obtained better efficiency (roughly twice higher than lab-on-a-chip device). This can be attributed to the higher adsorption of protein on our PDMS/glass device. On the other hand, the efficiency of the peak of Cy3 was much better for the lab-on-a-chip device than for the classical capillary electrophoresis. According to the 862510 theoretical plates by meter calculated of the peak 5, and the irreproducibility of this peak, it was attributed to an electrical spike.

When we compared the resolution per unit of separation distance the separation of β-lactoglobulin A and carbonic anhydrase II was better on chip. For instance, the resolution of proteins by using classical capillary electrophoresis Rs = 3.6

(Rs/cm = 0.18). The separation Cy3/carbonic anhydrase pair was around Rs = 9.6 (Rs/cm = 0.48) with the classic capillary electrophoresis compared with Rs = 3.4 (Rs/cm = 1.26) for the PDMS/glass device. According to these results, it is clear we can use our lab-on-a-chip device to separate a mixture of proteins. Further improvements of the experimental conditions have to be found in order to detect different ratio of Cy3 by proteins.

3.4. Separation of protein mixture by CGE

In order to avoid the problems related to multi-labelling, we tried to separate those proteins only by size, using capillary gel electrophoresis.

To perform this separation, the same protein mixture (CA II and Lac A tagged in Cy3) was injected into the microfluidic channel coated with the SDS 14–200 sieving gel. This matrix was useful, because of its optical properties which are compatible with our integrated optics. The separation was carried out at the electric field of 300 V/cm. The electropherogram is presented in Fig. 5, where the first peak corresponds to β-lactoglobulin A (18 kDa) and the second peak is the one of the carbonic anhydrase II (29 kDa). Although the separation is poor (Rs = 0.7), this electropherogram was repeated six times with a time deviation of 1.3%.

Two main reasons were emphasized to explain the lowest resolution obtained with CGE compared with CZE. At first, it was advised by the Beckman supplier to use the sieving matrix with a polyacrylamide coated neutral fused capillary (43 cm of separation length) to achieve good separation. For comparison with these advices, the hybrid device (2.7 cm of separation length) we fabricated was uncoated and the surface was heterogeneous, resulting in residual electroosmotic flow. At second, Yao et al. proved that a channel depth of 20 μm was too shallow for gel to pass correctly all along the channel [50]. They advised to work with a channel depth of 40 μm. Some new lab-on-a-chip devices and surface treatments were actually tested to improve the separation and to suppress the electroosmotic flow.

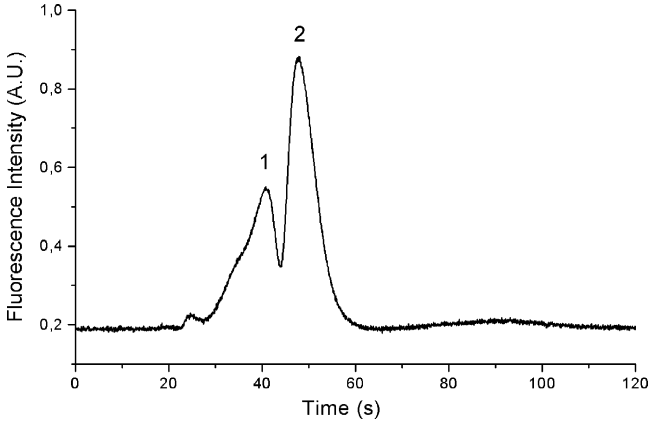

Fig. 5. CGE separation of two proteins mixture in a microfluidic channel coated with eCAP SDS 14–200 sieving matrix. The sample was prepared in SDS buffer and injected with a floating voltage step at 100 V/cm; the electric field during the separation was 300 V/cm. The peaks 1 and 2 were identified as β-lactoglobulin A and carbonic anhydrase II, respectively.

4. Conclusions

In our previous work [20], we demonstrated the fabrication technology and the optical performance of the integrated detection system in a PDMS/glass device. In this paper, we have reported the application of our concept, with the special attention on the separation of proteins mixtures.

First, the electroosmotic flow was measured using two different methods: current monitoring and indirect fluorescence. Both of these methods revealed the cathodic character of EOF, with the value of electroosmotic mobility intermediate between fully glass and PDMS chips.

Then, two different methods of EOF modification were verified. In the first approach, the addition of SDS increased the electroosmotic flow by 40%. In the second case, the addition of glycerol was employed to achieve the opposite effect. These results proved that we are able to tune the electrokinetic behaviour of the PDMS/glass device.

Electrophoretic separations were carried out on: the mixture of Cy3-Streptavidin with Cy3 and also on the mixture of proteins (β-lactoglobulin A and carbonic anhydrase II).

In the first case, the sample mixture was analyzed by CZE in less than a minute, with good time reproducibility and resolution.

This experiment was also carried out using a commercial microchip as a reference. The PDMS/glass device had better resolution than the tested commercial system.

In the second case, the protein sample was analyzed by CZE and CGE. In CZE, we were able to resolve each protein, although the separation of isoforms was not possible. Only broad zones were observed for each protein. In our opinion, it is the multi-labelling of proteins with Cy3 which is responsible for this disadvantageous effect. Nevertheless, the proteins separation was better with the chip device than with the classical capillary electrophoresis. Some labelling steps have been reported on chip as preparative step [74–76]. However, the excess of free dye can make further experiments, like trypsin digestion for instance [75,77,78] problematic. A possible option is to fabricate a device with a separation channel bifurcated at one end, in order to sort labelled protein in one channel and Cy3 in another one [79]. The realisation of this concept is fully feasible as the separation of proteins from Cy3 presented here, features good resolution.

Finally, in our effort to avoid the problem of multi-labelling, CGE approach was employed, in order to separate the proteins by their sizes only. We were capable of separating the sample with correct time repeatability, however, the resolution was relatively poor.

Thus, we believe that working with deeper microchannels and testing others sieving matrix could improve this resolution.

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