**Thiago Pinotti Segato**[1,2]
**Wendell Karlos Tomazelli Coltro**[2,3]
**André Luiz de Jesus Almeida**[4]
**Maria Helena de Oliveira Piazetta**[4]
**Angelo Luiz Gobbi**[4]
**Luiz Henrique Mazo**[1]
**Emanuel Carrilho**[1,2]

[1]Instituto de Química de São Carlos, Universidade de São Paulo, São Paulo, Brazil
[2]Instituto Nacional de Ciência e Tecnologia de Bioanalítica, Campinas, São Paulo, Brazil
[3]Instituto de Química, Universidade Federal de Goiás, Goiás, Brazil
[4]Laboratório de Microfabricação, Laboratório Nacional de Luz Síncrotron, Campinas, São Paulo, Brazil

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Research Article

# A rapid and reliable bonding process for microchip electrophoresis fabricated in glass substrates

In this report, we describe a rapid and reliable process to bond channels fabricated in glass substrates. Glass channels were fabricated by photolithography and wet chemical etching. The resulting channels were bonded against another glass plate containing a 50-μm thick PDMS layer. This same PDMS layer was also used to provide the electrical insulation of planar electrodes to carry out capacitively coupled contactless conductivity detection . The analytical performance of the proposed device was shown by using both LIF and capacitively coupled contactless conductivity detection systems. Efficiency around 47 000 plates/m was achieved with good chip-to-chip repeatability and satisfactory long-term stability of EOF. The RSD for the EOF measured in three different devices was *ca*. 7%. For a chip-to-chip comparison, the RSD values for migration time, electrophoretic current and peak area were below 10%. With the proposed approach, a single chip can be fabricated in less than 30 min including patterning, etching and sealing steps. This fabrication process is faster and easier than the thermal bonding process. Besides, the proposed method does not require high temperatures and provides excellent day-to-day and device-to-device repeatability.

**Keywords:**
Contactless conductivity detection / Microchip electrophoresis / PDMS / Wet chemical etching
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## 1 Introduction

Miniaturized electrophoresis devices have become a powerful tool in modern analytical chemistry [1–4]. In the chip-based electrophoresis systems, the reduced size and the low-power requirements are able to improve portability and bring together high levels of integration, yet maintaining low cost *per* device [5]. Furthermore, the miniaturized systems offer additional advantages over conventionally sized systems, including small consumption of sample and buffer, as well as short analysis time [6, 7].

Electrophoresis microdevices have been fabricated in a wide variety of substrate materials using both standard photolithographic procedures and newer microfabrication methods [8, 9]. In the last 10 years, the interest for polymeric substrates has increased significantly due to their low cost and easy of manufacturing [9]. PDMS is the most used polymer in microdevices fabrication, and this is attributed to its properties like biocompatibility, and easy of manufacturing. There are also a variety of components like mixers, valves and filters that are well integrated in PDMS chips [10, 11]. Since the first reports, however [12–14], glass substrates are still the most popular platform for microfluidics due to its similarity in the chemistry of fused silica capillary and silica particles [13–15]. Glass channels have shown higher separation efficiency [9], lower channel wall adsorption [9] and higher EOF [9, 16] than polymeric substrates. In addition, glass substrates also offer good mechanical and optical properties, high electrical insulation, low chemical reactivity and more effective heating dissipation [6, 17].

Glass microchips are often fabricated using photolithographic approaches combined to wet chemical etching [7]. The conventional fabrication processes of channels on glass substrates are laborious and cleanroom facilities are quite often required. The critical point on the manu facturing of glass channels is the sealing step, in which the channel needs to be assembled allowing fluids to flow through the device. The most employed method to bond glass channels is the thermal process, which it is carried out above 600°C. This approach, however, is

**Correspondence:** Professor Emanuel Carrilho, Grupo de Bioanalítica, Microfabricação e Separações, Instituto de Química de São Carlos, Universidade de São Paulo, Avenida Trabalhador São-carlense 400, P. O. Box 780, 13560-970 São Carlos, São Paulo, Brazil
**E-mail:** emanuel@iqsc.usp.br
**Fax:** +55-16-3373-9975

**Abbreviations: C$^4$D,** capacitively coupled contactless conductivity detection; **FL,** fluorescein; **HF,** hydrofluoric acid; **PR,** photoresist; **PT,** polyester-toner

not as straightforward as described in the literature. The thermal bonding method requires a careful cleaning of the glass substrates with acid solutions (*e.g.* piranha solution). Besides the cleaning step, thermal bonding is also a time-consuming process and, sometimes, the elevated temperature can damage other on-chip-integrated analytical features, such as electrodes or immobilized biomolecules [18, 19]. In addition to these critical points, thermal bonding is often irreproducible, thus resulting in glass microchips that are expensive for disposable devices [17]. Another process that is used to bond glass channels is the anodic bonding, which is carried out under temperature of 300–450°C and voltage of 400–1200 V. This method, however, requires one wafer to be a conductive material such as metal or silicon [5].

These disadvantages related to bonding glass channels have led scientists to investigate alternative sealing processes. Huang *et al*. [20] reported an attractive method to bond glass channels based on the use of a UV-curable glue of low viscosity at room temperatures. Allen and Chiu [21] described a calcium-assisted glass-to-glass bonding process, which is carried out during 1–2 h at 115°C. Alternative processes such as bonding with hydrofluoric acid (HF) [22, 23], bonding with sodium silicate [24], SU-8 [25], chemically treated polymeric membranes [26, 27] or another intermediate adhesive layers [6, 7, 28–30] have been found in the literature. Other bonding processes involving PDMS or PDMS/glass devices are described in the literature as well [30, 31]. In addition, many research groups have reported the bonding of glass microchips at room temperature without the requirements of cleanroom facilities [16, 32–36]. Such reported processes are strongly dependents of some factors as (i) multiple washing steps [32, 33, 35], (ii) need of an accurate holder to apply an equalized pressure between two glass plates [34] and (iii) still requiring instrumentation for sequential plasma activation of the glass surface [36]. However, all room-temperature bonding processes demand high level of cleanliness and flatness of the glass surfaces [32–36]. Furthermore, the integration of other analytical tools on chip, such as electrodes for electrochemical detection, is not compatible with most of the described bonding techniques at room temperature.

In this study, a cheap, fast and reliable way to seal glass microchannels based on the use of a thin PDMS membrane is reported. Electrophoresis microchips coupled either to LIF or to capacitively coupled contactless conductivity detection ($C^4D$) was used to show the analytical feasibility of the proposed approach. For $C^4D$ experiments, the PDMS membrane was also used to provide an electrical insulation between a glass wafer containing integrated electrodes and another glass wafer with the etched channels. The proposed method to bond glass channels has offered simplicity and high chip-to-chip repeatability. Furthermore, the proposed method is faster than other processes and it can be applied also in any chemistry laboratory without highly sophisticated instrumentation.

## 2 Materials and methods

### 2.1 Materials, reagents and samples

The following materials and chemicals were used as supplied. Soda-lime glass wafers ($26 \times 76 \times 1$ mm) were purchased from Glass Técnica (São Paulo, Brazil). Sylgard 184 and AZ4330 photoresist (PR) were obtained from Dow Corning (Midland, MI, USA) and Clariant (Sommerville, NJ, USA), respectively. HF was acquired from Synth (Diadema, São Paulo, Brazil) while sodium hydroxide, boric acid, sodium tetraborate, MES, L-histidine (His) as well as sodium, lithium and potassium chloride salts were purchased from Sigma-Aldrich (St. Louis, MO, USA). Fluorescein (FL) sodium salt and FITC were obtained from Sigma-Aldrich.

Run buffers (background electrolytes) were prepared weekly in ultrapure water (resistivity 18 MΩ cm). For LIF and $C^4D$, the run buffer solutions were 20 mmol/L boric acid/sodium tetraborate (pH 9.0) and 20 mmol/L MES/20 mmol/L His (pH 6.1), respectively. Stock solutions of every analyte (10 mmol/L each) were prepared daily in water, except FITC, which was prepared in acetone. Prior to LIF experiments, FL and FITC samples were diluted in run buffer. For EOF measurements, phosphate buffer solutions (15 and 20 mmol/L) were prepared at different pHs from potassium dihydrogen phosphate and dipotassium hydrogen phosphate (Mallinckrodt, Xalostoc, Mexico).

### 2.2 Microchip fabrication

The device layout was drawn using Corel Draw software version 11.0 (Corel) and printed on a high-resolution transparency film in a local graphic service (Journal Primeira Página, São Carlos, SP, Brazil), which was used as mask in the photolithographic step. The electrophoresis microchip design consisted of a double T-type format (gap = 200 μm), as shown in Fig. 1. Injection and separation channels were 15 and 60 mm long, respectively.

Our approach to produce glass electrophoresis microchips is shown in Fig. 2 and follows two parallel steps: one for the channels and other for the bonding plate. The printed transparency mask was placed on the top of a glass

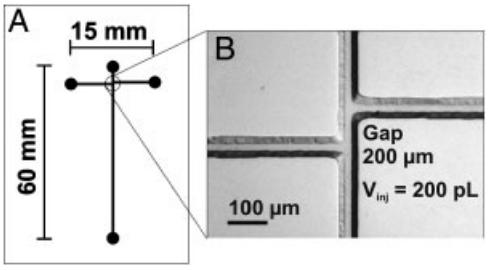


**Figure 1.** Presentation of the layout of the device (A), and its expanded view of the double-T injection system (B). The gap in the channel intersection was 200 μm.

 

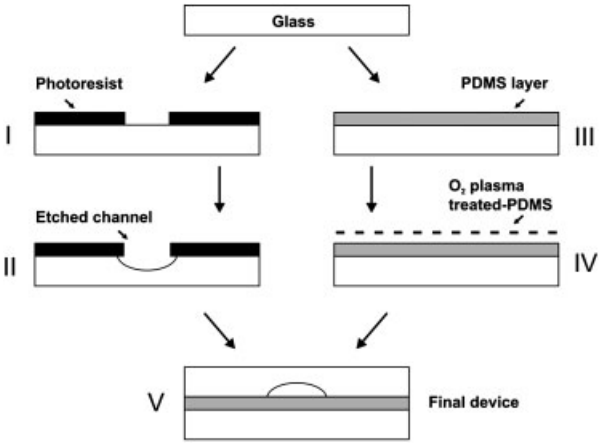


**Figure 2.** Microfabrication process of glass chip with sealing based on the use of a thin PDMS layer. I, patterning of PR step; II, wet chemical etching of glass with HF solution; III, spin-coating of a PDMS layer over flat glass surface at 3000 rpm during 10 s and baking at 100°C during 5 min; IV, plasma-oxidized PDMS during 1 min; V, final device after bonding step.

wafer previously coated with a 5 μm-layer of AZ4330 PR. The substrate was exposed to UV radiation for 15 s and developed in AZ 400 K developer solution for 2 min (Fig. 2I). Glass channels were etched with an etching solution consisted of 20% HF for 4 min under continuous stirring (Fig. 2II). The etching rate was $8 \pm 1$ μm/min. Following the etching step, substrates were rinsed with deionized water and the PR was removed with acetone. To access the microfluidic network, holes were drilled on glass-etched channels with a Dremel tool (MultiPro 395JU model, USA) using 1 mm diamond drill bits.

For bonding of the chip, another glass plate was spin-coated with a thin PDMS layer at 3000 rpm during 10 s (Fig. 2III). PDMS was prepared by a 10:1 mixture of Sylgard 184 monomer and curing agent. The thickness of this layer was *ca.* 50 μm. Before sealing, PDMS layer was cured at 100°C during 5 min in a hot plate. Glass channels and PDMS-coated glass substrates were placed in an oxygen plasma cleaner (Plasma Technology PLAB SE80 plasma cleaner) and oxidized for 1 min (Fig. 2IV). The two pieces were brought into contact immediately after removal from the plasma, obtaining a strong irreversible seal (Fig. 2V). As described in this section, the final device can be obtained in less than 30 min.

The resulting microchannels were characterized by SEM using a LEO 440 (Zeiss-Leica) scanning electron microscope, applying an accelerating voltage of 18 kV. The samples were sputtered with a 90 Å-thick gold layer prior to the SEM analysis.

In order to evaluate the bonding force, the resistance of the final device under different pressure values was investigated by using a HPLC pump (Shimadzu, model LC-20AT). For these experiments, HPLC connectors (Waters, stainless steel union size 1/16 in.) were glued to the solution reservoirs microchip using epoxy resin and then connected to a HPLC pump. The flow rate ranged from 10 to 500 μL/min.

Planar electrodes for $C^4D$ were fabricated on the glass surface and integrated to the microfluidic network using a PDMS membrane to provide the electrical insulation between electrodes and channel. The glass substrate was submitted to photolithographic procedures to define the electrode geometry. The electrode material, a 20 nm adhesion layer of titanium followed by 150 nm of gold, was sputtered over a glass plate substrate (Fig. 3I). The substrate with Ti/Au thin film deposited was spin-coated with a thin layer (*ca.* 5 μm) of AZ4330 PR at 3000 rpm for 30 s. After a prebake step (90°C/5 min), the electrode layout was patterned onto the PR layer using a high-resolution transparency photomask (Fig. 3II). The exposure time to the UV lamp was 15 s under a power of 10 mW/cm$^2$. Subsequently, the PR (patterned layout) was developed for 2 min using AZ 400 K developer, washed with deionized water and dried with $N_2$. The pattern of the electrode material was accomplished by soaking the substrate in Au-etch and 1% HF solutions to remove the Au and Ti films, respectively (Fig. 3III). The electrode material remained anchored to the glass plate only under the PR-protected patterns (Fig. 3IV). The remaining PR was removed with acetone and the glass plate washed with deionized water and dried with $N_2$. After finishing the electrode fabrication process, a thin PDMS layer was deposited over the surface to provide an electrical insulation as well as to allow an effective sealing as described in the previous section.

## 2.3 Electrophoresis procedures

### 2.3.1 Microchannel preconditioning

Before starting the experiments, the microchip was treated with 0.1 M NaOH, ultrapure water and buffer solutions for 30 min each. The rinsing steps were carried out by applying vacuum in the buffer waste reservoir. After preconditioning the channels, the run buffer in the sample reservoir was replaced with the sample solution. All reservoirs were filled

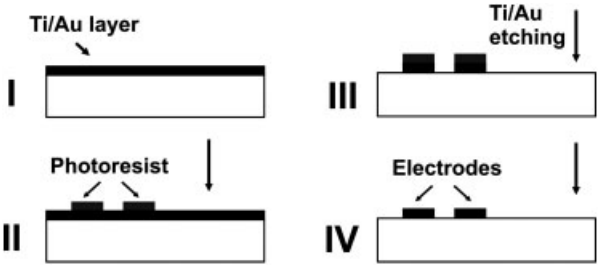


**Figure 3.** Fabrication process of gold electrodes on glass wafers. I, sputtering of a 20 nm titanium layer followed by 150 nm of gold; II, spin-coating of the PR layer; III, patterning of the PR step; IV, etch of the exposed Ti/Au followed by PR removing and washing steps.

 

with the same volume (50 μL), and the electrophoresis experiments were performed at room temperature.

#### 2.3.2 Measurements of EOF

EOF measurements were performed by the current-monitoring method [37] using alternated runs at two different concentrations of phosphate buffer (15 and 20 mM) under an electric field of 125 V/cm. EOF values were monitored over a wide pH range, varying from 2 to 12. A 30-day experiment was carried out in a single chip to evaluate the EOF behavior, as well as the stability at pH 7. The magnitude of the EOF over the entire pH range was compared before and after 30 days on the same microchip. EOF measurements were also carried out in three different chips to evaluate the chip-to-chip repeatability.

#### 2.3.3 Electrokinetic transport

The electrokinetic transport of the flow into microfluidic channels was accomplished by a bipolar single-channel high-voltage power supply (CZE 1000R, Spellman, Hauppauge, NY, USA) controlled by a computer equipped with a National Instruments (NI) interface (USB-6009 model). Electrokinetic injections were performed using an unpinched injection procedure [38]. For the present experiments, the injections were performed by applying a desired potential (+1 kV) for 10 s to the sample reservoir with the sample waste reservoir grounded, and all other reservoirs floating. Switching the high-voltage contacts and applying the corresponding separation voltages to the running buffer reservoir while maintaining the detection reservoir grounded and all other reservoirs floating performed the separations.

### 2.4 LIF and contactless conductivity detection

LIF detection was performed employing the original technique describing fluorescence microscopy detection by Hernandez *et al.* using a modified custom-made confocal system [39, 40]. To carry out these experiments, a compact system (IS BIOTECH, Porto Alegre, RS, Brazil) equipped with a 488 nm argon ion laser beam (1–50 mW optical output power) (LaserPhysics, Salt Lake City, UT, USA) was used. The resulting fluorescence signal was sent to the NI interface and monitored in real time using a program written in *LabVIEW*.

$C^4D$ experiments were carried out using a home-made system described previously [38]. A function generator (CFG-250, Tektronix) was used to generate an excitation signal for the conductivity detector. The data acquisition was also performed in a program written in *LabVIEW*. This software was also used to control the potential, and the time of the injection/separation steps. The electropherograms were recorded using a time resolution of 50 ms without any software filtering. To reduce electrical noise pickup, all measurements were carried out in a Faraday cage.

## 3 Results and discussion

### 3.1 Characterization of the microdevice

The proposed microdevice was characterized by SEM images, profilometry measurements, bonding force, EOF magnitude, stability and repeatability. Figure 4 shows a SEM image of the transversal section of a sealed device presenting a parabolic shape as a result of the isotropic etching with HF solution. A little underetching occurred but did not influence the analytical performance of the chip (Section 3.2). The resulting channel exhibited a smooth surface (RMS deviation below 5 nm, according to profilometric measurements) and 135 μm-wide at the opening of the channel, 85 μm-wide at the bottom of the channel and 35 μm-deep. The channel depth was controlled under an etching rate of $8 \pm 1$ μm/min.

The glass plate with channels was sealed against another glass plate, which was previously coated with a thin layer of PDMS using a spinner. By using a fixed spinning time of 10 s, the thickness of the PDMS layer was evaluated in function of the rotation speed. This parameter was measured by profilometry and the results are shown in Fig. 5. As expected, we observed that the greater the rotation speed, the thinner the PDMS layer. In order to obtain a strong, effective and reproducible sealing, experiments with different thickness (ranging from 20 to 120 μm) were carried out.

For thinner layers (below 40 μm), we experienced problems with the uniformity of the surface, and also problems with the generation of air bubbles, which did not allow an effective bonding. On the other hand, for thicker layers, a noticeable deformation at the edge of the PDMS-covered plate was observed. This effect is attributed to the high viscosity of the PDMS, which avoids the full contact between both plates. In addition, the use of thick layers can also block the channels during the bonding process. For this reason, the best results were obtained by using a PDMS layer with 50-μm thick. A chip-to-chip repeatability of 100% was routinely achieved with these optimized conditions ($n = 12$) and could be applied to mass production.

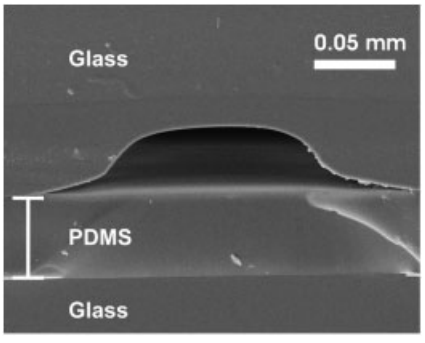


**Figure 4.** Scanning electron micrograph of the transversal section of the sealed glass channel with 135 μm-wide at the opening of the channel, 85-μm-wide at the bottom of the channel, and 35 μm-deep the height of the channel.

 

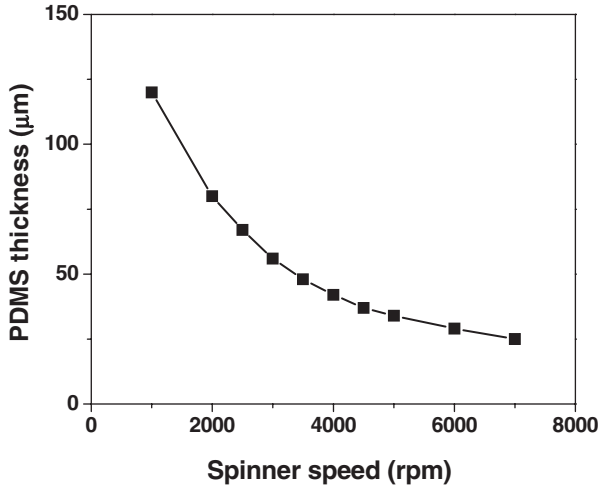


**Figure 5.** Thickness of the PDMS membrane in function of the spinner speed. The spinning time was 10 s.

The bonding force of the resulting device (or resistance of the bonding to pressure) was studied using a HPLC pump. The pressure generated inside microchannels was measured and the values ranged linearly from 1 to 40 kgf cm$^{-2}$ when adjusting the flow rate from 0.01 to 0.50 mL/min. The microchips did not show any leakage under this flow rate and pressure range.

The EOF is one of the most important parameters evaluated during the development of new platforms for electrophoresis. The EOF behavior is directly correlated to the channel wall composition, and is strongly affected by temperature, buffer pH and ionic strength [41]. The value of the EOF can provide information on the nature of the channel surface, and also can affect separation performance. Cathodic EOF, *i.e.* EOF that moves from anode to cathode, is an indicative of a surface with excess of negative charges, and the magnitude of the EOF is determined by the amount of surface charge [41]. Due to the importance of the EOF magnitude and stability for a successful separation on chip, its determination and optimization need to be studied [42, 43].

The magnitude of the electroosmotic mobility, $\mu_{eo}$, was evaluated with background electrolytes with pH values ranging from 2 to 12. As expected, a cathodic EOF was observed for all pH values. The $\mu_{eo}$ magnitude over this pH range was evaluated in a single chip during 30 consecutive days. Figure 6A shows the results obtained at the first day and also after 30 days. Each point in the graph is an average value of six determinations. It is important to note that the composition of the microfluidic channel consisted of three sides of glass (two side-walls and the bottom of the etched channel) and one side of PDMS (the flat bonding cover). Since the EOF magnitude did not alter significantly over 30 days for the pH range, we can infer that this profile is mainly dominated by the glass surface. It is well described that for PDMS devices (native and plasma-oxidized PDMS), the EOF changes quickly affecting the run-to-run repeatability [42, 44]. On the other hand, glass chips and fused silica capillaries provide highest and stable EOF due to their composition of the internal walls, which present greatest density of silanol groups [41, 45].

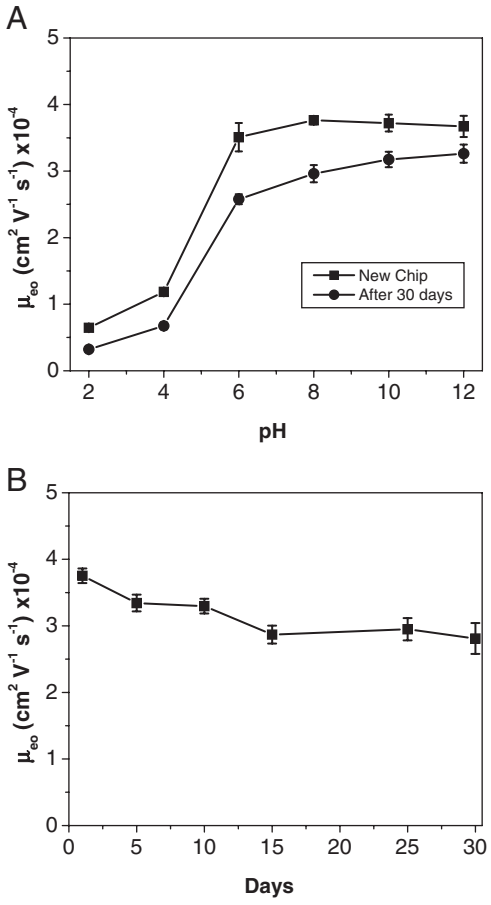


**Figure 6.** (A) Effect of the pH of the background electrolyte on the EOF magnitude measured by the current monitoring method for a new chip (■) and the same chip after 30 days (●). Each point in the graph is an average of six determinations; (B) EOF stability at pH 7 over the 30-day period.

The values of $\mu_{eo}$ at pH 7 for ten consecutive measurements (same day) was $3.6 \pm 0.2 \times 10^{-4}$ cm$^2$ s$^{-1}$ V$^{-1}$ showing a stable run-to-run performance. Figure 6B displays the $\mu_{eo}$ values determined over 30 days at pH 7. As shown in Fig. 6B, the data presented a slight decrease of *ca.* 20% over the period. This behavior was observed, probably, because the PDMS surface can be returned to its original state, *i.e.* the oxidized PDMS surface ($SiO_2$) could have been converted in its native form containing Si–$CH_3$ groups [44]. This problem can be minimized by adding an additional step for removal of low molecular weight oligomers, which allows the formation of a stable $SiO_2$ surface on PDMS for long periods [44]. The magnitude and the stability of the EOF generated in our device are in good agreement to other glass and quartz chips reported in the literature [9, 42, 45–48]. In addition, the EOF magnitude of the proposed device is higher than the one published for hybrid PDMS/

 

glass chips [9], which contained three surfaces of PDMS to one of glass.

### 3.2 Analytical performance

The analytical performance of the electrophoresis device was evaluated by using both LIF and $C^4D$ detection systems. For both detection systems, the injection volume was 200 pL. Figure 7 shows three injections of a mixture containing FL and FITC (50 and 150 μM, respectively). For the electropherograms shown in Fig. 6, the migration times to FITC and FL were $78.5 \pm 0.3$ and $93.4 \pm 0.3$ s, respectively for which the RSD values were below 0.4% ($n = 3$). The separation efficiency obtained for both analytes with the proposed device was around 25 000 plates/m. In addition, no noticeable tailing was observed for the proposed device. The high plate counts confirms that the electrophoretic profile is dominated mainly by the glass surface and little or no adsorption was detected on the PDMS layer, even for hydrophobic analytes. This is in agreement to the EOF results presented in the previous section, in which the silica inner wall of the glass is responsible for generating the stable and reproducible EOF.

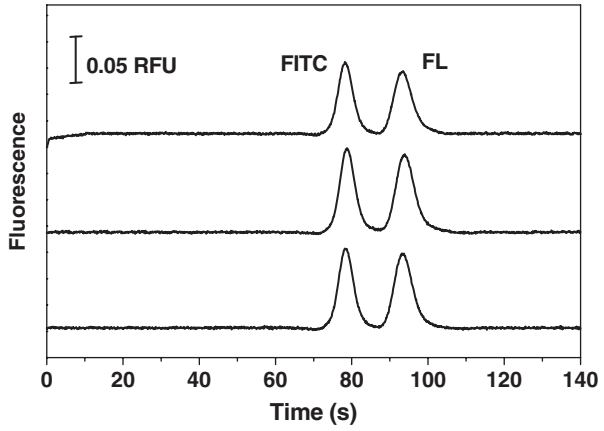


**Figure 7.** Electropherograms showing the repeatability on the separation of FL (50 μM) and FITC (150 μM) in a single chip. A 25 mM borate/boric acid was used as running buffer at pH 9. Injection and separation steps were performed by applying 1 kV/10 s and 2 kV, respectively. Detection was carried at 40 mm from the injection channel.

Using LIF detection, three different chips were evaluated and the results of electrophoretic current, EOF magnitude, migration time, peak area and separation efficiency are summarized in Table 1. All experiments were carried out using a mixture containing FL and FITC, however, for a simple comparison just the FL parameters are presented. According to the results summarized in Table 1, it can be inferred that the proposed approach to bond glass electrophoresis channels provided similar results when compared with other methods reported. For a quantitative intra-chip comparison, the RSD values found for electrophoretic current, migration time and EOF magnitude were below 6.5% each. The greatest RSD values for peak area (9.2%) and efficiency (13.6%) can be attributed to the differences in the EOF magnitude or to the channel dimensions (width and depth) from chip-to-chip. We believe, however, that these discrepancies are not representative for chemical analysis on microchips. Alternatively, the results summarized in Table 1 confirm that the proposed method is reliable, reproducible, fast and, most importantly, it does not require (i) high temperature and (ii) additional chemicals to bond the glass plates.

A homemade $C^4D$ system [38] was also used to evaluate the analytical performance of the proposed electrophoresis device. For this detection system, planar electrodes were integrated to the glass surface and were insulated from the physical contact to the solution in the glass channel by the PDMS layer. Figure 8 shows three electropherograms obtained from three consecutive injections of a mixture containing high-mobility cations – $Li^+$, $Na^+$ and $K^+$ (100 μM each). The detection was carried out by applying a sinusoidal waveform of 120-kHz frequency and 2-$V_{peak\text{-}to\text{-}peak}$ amplitude.

For the $C^4D$ experiments, the separation efficiency ranged from 27 000 to 47 000 plates/m. The separation efficiency obtained in our device is greater than that one reported to polyester-toner (PT) devices using this same detection system [33]. The LODs found for these experiments were around 25 μM for $K^+$ and $Na^+$, and 50 μM for $Li^+$(signal-to-noise ratio = 3). The LOD values found here are slightly higher than those reported previously on PT devices, but they were on the same order of magnitude. The lower LOD levels found with PT chips, however, were obtained with sample stacking which was not applied here [33]. As shown in Fig. 8, all electropherograms were recorded with baseline resolution.

**Table 1.** Comparison of electrophoretic parameters obtained for three different chips[a)]

| Chip # | Current in channel (μA) | EOF ($10^4$ cm$^2$ V$^{-1}$ s$^{-1}$) | Migration time (s) | Peak area (au) | Efficiency (plates/m) |
|---|---|---|---|---|---|
| 1 | $3.4 \pm 0.1$ | $3.3 \pm 0.3$ | $74 \pm 1$ | $6.1 \pm 0.4$ | $21\,400 \pm 100$ |
| 2 | $3.4 \pm 0.1$ | $3.0 \pm 0.1$ | $77 \pm 1$ | $6.5 \pm 1.3$ | $27\,900 \pm 800$ |
| 3 | $3.2 \pm 0.1$ | $3.4 \pm 0.2$ | $73 \pm 1$ | $5.4 \pm 0.3$ | $26\,700 \pm 300$ |
| Chip-to-chip RSD (%) | 4.5 | 6.6 | 2.2 | 9.2 | 13.7 |

a) Values of current and EOF are related to the channel itself, whereas migration time, peak area and efficiency are related to the injection of analyte (FL). Each value is an average of five measurements ±1 SD.

au, area units.

 

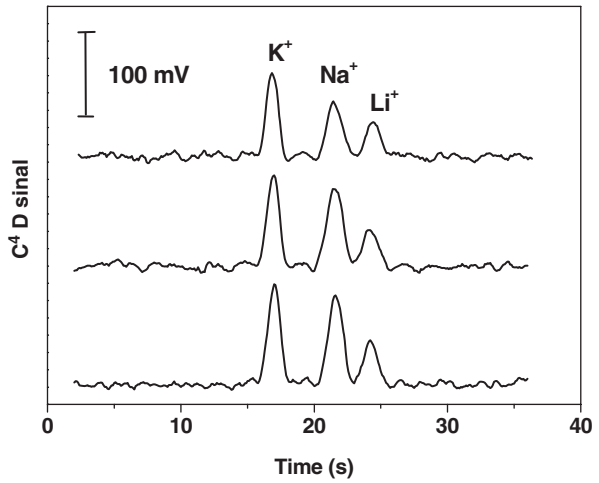


**Figure 8.** Electropherograms showing three consecutive separations of $Li^+$, $Na^+$ and $K^+$ (100 μM each) in a single chip using 20 mM MES/His as running buffer at pH 6.1. Injection: 1 kV/10 s and separation at 1 kV. Detection was performed applying sinusoidal wave with frequency of 120 kHz and amplitude of 2 $V_{pp}$.

## 4 Concluding remarks

The process we proposed here for sealing glass channels was fast and reliable. This sealing process provides similar analytical results in different microchips (with low RSD for analytical parameters). As published by distinct research groups [9, 48, 49], glass is the best platform for microfluidic applications. However, the high cost and the poor repeatability of chip-to-chip thermal bonding have directed the focus of some researches to the development of newer materials like PDMS [9, 50] with rapid sealing time. Alternatively, techniques to irreversibly bond polymeric materials, *e.g.* PDMS, are based on the use of silanes and other surface activators that can make the process very expensive in the long run, and unrepeatable, depending on the quality of these reagents [26]. The fabrication procedure presented here dispenses complex manipulation of the substrates and, due to the employment of the polymeric membrane, stimulates other instrumentation advances such as the integration of valves for the fluidic control on integrated lab-on-a-chip [51]. The use of the thin PDMS membrane has also shown versatility allowing the on-chip integration of microfabricated electrodes for $C^4D$ measurements.

A very similar process has been recently described and divulgated by Han and co-workers in the Chips & Tips section of the *Lab on a Chip* Journal (http://www.rsc.org/Publishing/Journals/lc/Chips_and_Tips/reversible_bonding.asp). The authors used a thin PDMS layer (1 μm thick) to provide the bonding after curing the PDMS. The limitation of thin PDMS layers is that high pressure should be avoided once this could lead to squeezing of PDMS into the channels and clogging of the chip, therefore, influencing the repeatability in the fabrication process. When compared to the process reported by Han and co-workers, our bonding technique offers a high reliability, robustness and repeatability for the fabrication of several devices in different days by any investigator. It is important to note that all these remarkable parameters – in terms of analytical repeatability – were found only for membranes with thickness around 50 μm. Furthermore, we can fabricate glass chips in less than 30 min including the cleaning, patterning, etching and sealing steps. These advantages can contribute to solve some problems related to bonding of glass channels, and also related to adsorption of analytes to the channel wall, commonly found in polymeric materials [9].

Another limitation is the compatibility of PDMS with organic solvents. As reported by Lee *et al.* [52], PDMS can suffer a swelling in contact with nonaqueous media. This effect has many implications and can provide changes the cross-sectional area of the channel and, therefore, the rate and profile of the flow. Changes in channel dimensions due to swelling can affect integration of the channel with components such as membranes, detectors, mixers or electrodes. The hydrophobic profile of the PDMS structure is the main drawback that avoids some applications to be performed on this kind of microfluidic platform. This property is not related just for PDMS devices. Other elastomeric substrate materials, like poly(urethane), have also shown problems due to the swelling [50]. Although the PDMS surface can become hydrophilic after oxidation with oxygen plasma, this hydrophilic surface is not stable over time and can be reverted to its original hydrophobic – form sometimes within hours. The extraction of unreacted oligomers from the bulk PDMS was shown to improve the stability of oxidized hydrophilic surface from hours to days [44, 52]. Furthermore, adsorption of biomolecules such as proteins, on channel walls or also on membrane surface is also a common problem for microfluidic devices fabricated in PDMS. As the membrane has a thickness of tens of micrometers, proteins can bind nonspecifically to its surface. To solve this problem and obtain results similar to glass surface, it would be necessary to generate PDMS membrane with stable hydrophilic surface, as described by Vickers *et al.* [44].

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