Richard Piffer Soares de Campos[1]
Inez Valeria Pagotto Yoshida[1]
José Alberto Fracassi da Silva[1,2]

[1]Instituto de Química, Universidade Estadual de Campinas, Campinas, SP, Brazil
[2]Instituto Nacional de Ciência e Tecnologia em Bioanalítica, INCTBio, Campinas, SP, Brazil

Received October 30, 2013
Revised March 25, 2014
Accepted March 25, 2014

Research Article

Surface modification of PDMS microchips with poly(ethylene glycol) derivatives for μTAS applications

In this work is presented a method for the modification of native PDMS surface in order to improve its applicability as a substrate for microfluidic devices, especially in the analysis of nonpolar analytes. Therefore, poly(ethylene glycol) divinyl ether modified PDMS substrate was obtained by surface modification of native PDMS. The modified substrate was characterized by attenuated total reflectance infrared spectroscopy, water contact angle measurements, and by evaluating the adsorption of rhodamine B and the magnitude of the EOF mobility. The reaction was confirmed by the spectroscopic evaluation. The formation of a well-spread water film over the surface immediately after the modification was an indicative of the modified surface hydrophilicity. This characteristic was maintained for approximately ten days, with a gradual return to a hydrophobic state. Fluorescence assays showed that the nonpolar adsorption property of PDMS was significantly decreased. The EOF mobility obtained was 3.6×10^{-4} cm^2 V^{-1}s^{-1}, higher than the typical values found for native PDMS. Due to the better wettability promoted by the modification, the filling of the microchannels with aqueous solutions was facilitated and trapping of air bubbles was not observed.

Keywords:
Microchip electrophoresis / PDMS / Surface modification
DOI 10.1002/elps.201300531

Additional supporting information may be found in the online version of this article at the publisher's web-site

1 Introduction

The development of μTAS has grown over the last two decades. From liquid handling at microscale dimensions to sample treatment and analysis, it is difficult to find an analytical protocol that cannot be implemented on microchip format [1–4]. Using microchip format, it is possible to integrate many analytical steps on a single substrate with the advantages of reduced analysis time, low power, reagents and sample consumption, low generation of residues, high-throughput analysis, among other features [5–8]. These characteristics are interesting for numerous bioapplications, such as DNA analysis [9, 10], immunoassays [11], protein analysis [12], pathogen detection [13], and especially for studies concerning cell heterogeneity and single-cell analysis [14–18]. In this context, the properties of the substrate of the microchips are of utmost importance to achieve their expected result.

Among the polymeric materials used in μTAS fabrication as substrates, PDMS is by far the most important polymer [19] due to its elastomeric properties, optical transparency, biocompatibility, easy molding, and low fabrication cost [20, 21]. However, its application for aqueous systems can be troublesome due the intrinsic hydrophobicity presented by the material. This hydrophobic characteristic can also cause nonpolar analytes to be adsorbed on PDMS surface or even be absorbed into its bulk [1]. To overcome this problem, several methods for channel surface or bulk modification of PDMS microchips were proposed in the literature, including layer-by-layer deposition techniques [22–24], wet modifications after plasma oxidation [25], chemical vapor deposition [26, 27], silanization [28], bulk modification [29], dynamic surface modification [30], among others. Although all of these

Correspondence: Dr. José Alberto Fracassi da Silva, Instituto de Química, Universidade Estadual de Campinas, P.O. Box 6154, Campinas, SP 13083-970, Brazil
E-mail: fracassi@iqm.unicamp.br
Fax: +55-19-3521-3023

Abbreviations: ATR, attenuated total reflectance infrared spectra; **DVE-PEG**, poly(ethylene glycol) divinyl ether; **His**, L-histidine; **WCA**, water contact angle

Colour Online: See the article online to view Figs. 1 and 3–6 in colour.

methods are viable options to overcome the drawbacks of PDMS, they usually require specific instrumentation to be applied. In this sense, we present a simple method for PDMS surface modification that can be performed using common laboratory equipment. The principle used here can also be extrapolated to other PDMS surface modifications using compounds containing available vinyl groups in order to get different hydrophilic and electroosmotic surface characteristics. Therefore, in this article we present a characterization study of PDMS modification by poly(ethylene glycol) divinyl ether (DVE-PEG) covalent bonding. The modification was followed by FTIR spectroscopy, water contact angle (WCA) measurements, EOF mobility, and fluorescence microscopy, and the results were compared to native PDMS substrates.

2 Materials and methods

2.1 Reagents and materials

The PDMS substrates were prepared using the Sylgard 184 kit from Dow Corning (Midland, MI, USA), with molar mass around 2×10^7 g/mol. Poly(methylhydrogen siloxane) fluid (PMHS), as the DC1107 product, and platinum(0)-1,3-divinyl-1,1,3,3-tetramethyldisiloxane complex solution (platinum catalyst, Q2-7368) were also purchased from Dow Corning. DVE-PEG, MES, and L-histidine (His) were obtained from Aldrich (St. Louis, MO, USA). Rhodamine B, sodium borate, sulfuric acid, $FeCl_3$, KCl, NaCl, and LiCl were obtained from Synth (Diadema, SP, Brazil). Bis(2-methoxyethyl) ether solvent was purchased from Acros Organics (New Jersey, USA).

2.2 Instrumentation

Equilibrium WCA measurements for the native and functionalized PDMS were performed using an optical goniometer model DSA100 Standard (Krüss, Germany). All measurements were done using the sessile drop method of 10 μL deionized water (Direct Q-3 ultrapure purification system, EMD Millipore, MA, USA), where a drop is deposited by an automatic pipette. The shape of the drop was optically analyzed using Drop Shape Analysis software. Each WCA value is an average of five measurements in random locations on the substrate surface.

Attenuated total reflectance infrared spectra (ATR-FTIR) were obtained using a spectrometer model MB Series B-102 (Bomem, USA). All spectra were recorded at 45° of incidence for 256 scans with a resolution of 4 cm^{-1} in the range of 650–4000 cm^{-1}.

The setup for microchip electrophoresis consisted of a laboratory-made C^4D system [31], a signal generator (GV-2002, ICEL, Brazil) operating at 500 kHz and 1.1 V (peak voltage) amplitude, a high-voltage power supply (EMCO High Voltage, C Series, model C40), and a vacuum generator to assist the microchannel filling and cleaning. Data acquisition was made through a desktop computer, an NI USB-6009 interface module, and software written in LabView® (both from National Instruments, SP, Brazil).

EOF measurements were performed using the C^4D detector. Briefly, this method consists in monitoring the C^4D signal variation caused by the injection zone, which travels with the EOF velocity. When the injection plug reaches the C^4D detector, a negative peak is registered due to the lower conductivity of this zone and the associated migration time is used to calculate the EOF mobility. The injected plug was a solution containing 500 μmol/L of Li^+, Na^+, and K^+ and the background electrolyte used for the separation was the MES/His 20 mmol/L buffer, pH 6.0.

2.3 Substrates and device fabrication

2.3.1 PDMS preparation prior to modification

The PDMS substrate was obtained using the conventional method of fabrication, mixing the prepolymer and cross-linker, in the mass ratio of 10:1, respectively. For the ATR-FTIR spectra and WCA evaluation, the PDMS was placed in a polystyrene Petri dish and cured under a flat surface. The substrate obtained this way was submitted to the modification route and the properties were studied without the need of sealing against other substrate.

The PDMS substrate for the microchips with cross-shaped microchannels was obtained by rapid prototyping method, mixing the PDMS precursors (10:1 ratio, as above), and depositing it over a prefabricated SU-8 mold. The SU-8 mold was fabricated on a silicon wafer by conventional photolithography, in the Brazilian Nanotechnology National Laboratory (LNNano). The produced microchannels had 65 μm width × 15 μm height. The lengths of the injection and separation channels were 1.0 cm and 4.6 cm, respectively. The substrate containing the cross-shaped microchannels was punched at the end of each channel using a biopsy puncher (Ted Pella, Harris Uni-core, USA) to produce the inlet and outlet reservoirs (3 mm in diameter). The substrate containing microchannels and reservoirs was submitted to the modification route. In sequence, the modified substrate was irreversibly sealed against a printed circuit board containing copper electrodes and covered with a native PDMS thin film, using the O_2 plasma oxidation method.

2.3.2 Fabrication of electrodes for C^4D detection

To manufacture the electrodes, the copper present in the printed circuit board was removed by the controlled corrosion of the board using a 20% $FeCl_3$ solution. The electrode area was protected using a toner mask. The toner mask was initially drawn in graphical software (*CorelDraw*®), printed on a waxed paper using a laser printer (HP Laserjet 1300, Hewlett Packard, SP, Brazil), and transferred to the board using a laminator model BW-P320 (Bowey, Brazil). The copper was etched under stirring for 20 min. The toner was

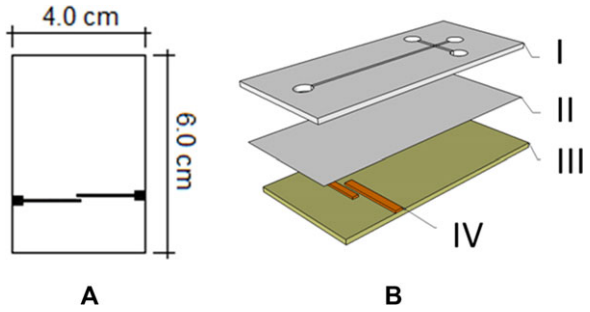

Figure 1. Microchip schemes. (A) Used design for the manufacturing of C^4D electrodes in a printed circuit board. Dimensions: 4.0 × 6.0 cm. Electrode width: 1 mm. Gap between electrodes: 0.5 mm. (B) Three-layer device for contactless conductivity detection where (I) is the substrate with cross-shaped microchannels, (II) is the thin polymer layer, (III) the printed circuit board, and (IV) the copper electrodes.

then removed using acetonitrile, obtaining the board with two electrodes. The shape and dimensions of the electrodes are shown in Fig. 1A.

The electrodes were spin-coated (model WS-650S-6NPP, Laurell Technologies, USA) with a thin layer of PDMS. This deposition was performed using the same 10:1 ratio mixture of PDMS precursors under the following spin procedure: 30 s at 300 rpm, 30 s at 600 rpm, 15 s at 900 rpm, and 30 s at 1000 rpm. This procedure resulted in a thin PDMS layer of approximately 60 μm. The final device obtained was the combination of the substrate containing the cross-shaped microchannels sealed against the board containing the C^4D electrodes (Fig. 1B).

2.4 Modification route

The modification route was adapted from the work of Chen et al. [32]. In this route, the already cured PDMS substrate must have Si-H bonds introduced to its surface in order to react with the DVE-PEG. This activation can be done through a displacement reaction. To do it, the PDMS substrate was immersed in a solution prepared with 30 mL of bis(2-methoxyethyl) ether, 10 mL of DC1107, and 0.5 mL of concentrated H_2SO_4 under stirring for 30 min. After that, the sample was rinsed with methanol and ethanol, kept at 80°C for 10 min in an oven, and left cool down to room temperature in a desiccator for 1 h under vacuum.

The Si-H activated substrate was placed into a second solution containing 25 mL of bis(2-methoxyethyl) ether, 5 mL of DVE-PEG, and two drops of the platinum catalyst. In this step, the hydrosilylation reaction between the Si-H activated surface and vinyl group of DVE-PEG takes place. The second solution was kept under constant stirring for 2 h. After that, the polymer was washed with bis(2-methoxyethyl) ether and left dry under vacuum for 10 min in a desiccator, and finally heated in an oven at 120°C for 4 h. A flowchart of the modification procedure can be seen in the Supporting Information 1. The products after the first and second reaction were characterized by ATR-FTIR.

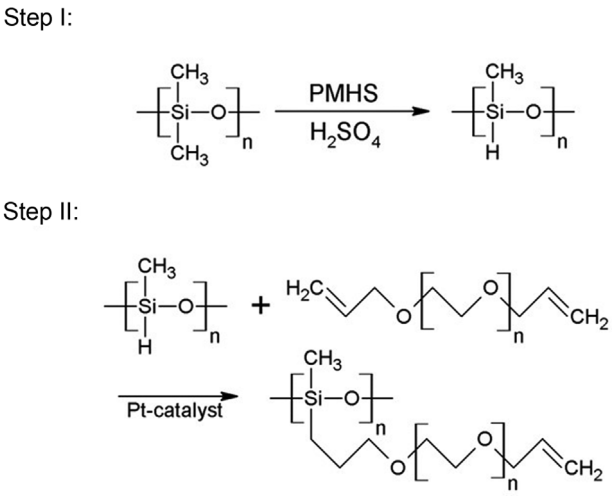

Figure 2. Reaction mechanism for DVE-PEG addition to PDMS surface. Step I: PDMS surface activation with Si-H bonds. Step II: DVE-PEG anchoring to the PDMS chain. Adapted from Chen et al. [32].

2.5 Adsorption studies

Adsorption studies were performed using flat substrates of native PDMS and DVE-PEG-modified PDMS. To achieve it, a droplet of 20 μL of a 100 μmol/L rhodamine B in 10 mmol/L sodium borate solution was pipetted over the substrates surfaces. This solution was already used in similar adsorption studies presented in the literature [33].

The droplet was left static over the flat substrates for 1, 5, and 10 min. The substrate was then dried using absorbent paper to remove the droplet by the capillarity. This was simply performed by touching the solution droplet with the absorbent paper. The substrate was then dried again using N_2 to ensure that no solution was left over the substrates. The region where the droplets were deposited was analyzed using a TCS SP5 confocal fluorescence microscopy (Leica Microsystems, Germany). The optimal image parameters were maintained constant for all image acquisition. The laser power was 675 μW, with a pinhole of 78.04 μm. The excitation wavelength used was 543 nm of an HeNe laser and the acquisition of the images was performed in the range of 560–590 nm.

3 Results and discussion

3.1 Surface characterization

The reaction mechanism for the modification of PDMS substrate with DVE-PEG is described in Fig. 2. In Step I of the proposed reaction, the Si-H groups are introduced through a

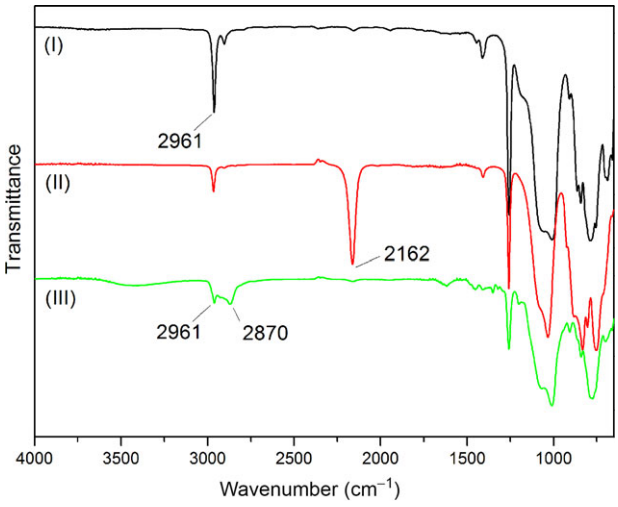

Figure 3. ATR-FTIR spectra for the three stages of PDMS modification: (I) native PDMS; (II) Si-H surface activated PDMS, and (III) DVE-PEG-modified PDMS.

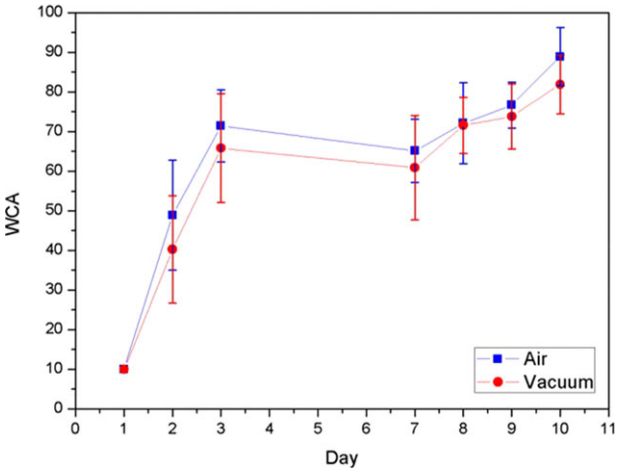

Figure 4. WCA values for the DVE-PEG-modified PDMS as function of the days after its preparation. Squares (-■-) indicate the air-stored polymer and circles (-●-) indicate the vacuum stored polymer.

displacement reaction catalyzed by sulfuric acid. In Step II, the DVE-PEG chains are anchored on the surface of the substrate due to the hydrosilylation reaction between the Si-H groups and $CH{=}CH_2$ group present in the PEG derivative. These two steps were monitored by ATR-FTIR, and the spectra for the native PDMS, for the Si-H surface activated PDMS and for the final modified product, are shown in Fig. 3.

By analyzing the Fig. 3, it is possible to conclude that the proposed reaction was successfully achieved. The presence of $–HSi(CH_3)$- groups in the PDMS-activated surface (step I) is indicated by an absorption band at 2162 cm^{-1} in spectrum II, characteristic of the Si-H bond stretching. The 2961 and 2870 cm^{-1} bands are associated with the presence of $\text{-}CH_2$ and $\text{-}CH_3$ in the polymer surface. The $\text{-}CH_3$ is a typical group for native PDMS, but the $\text{-}CH_2$ absorption is associated to the hydrosilylation reaction product, with the addition of the DVE-PEG on the PDMS surface by a $\text{Si-}CH_2\text{-}CH_2\text{-PEG}$ covalent bond. Thus, the proposed modification route proves to be suitable for insertion of DVE-PEG to the PDMS surface through covalent bonding.

3.2 WCA behavior

Figure 4 shows the WCA values for the DVE-PEG-modified PDMS stored in air and vacuum several days after its fabrication. In the first day after the substrate modification, the drop of deionized water formed a thin water film over the PDMS surface and the WCA obtained was so small that the drop analysis software was unable to calculate its value. The value was then graphically estimated and this behavior demonstrates the high hydrophilic characteristic of the surface at this time. Figure 4 also shows that the WCA values gradually increase and after ten days from the modification the WCA reaches values that are similar to those obtained for the native PDMS (about 101.5°). In fact, the obtained WCA values after ten days do not differ significantly from values found for the native PDMS reference, showing the restoration of the PDMS hydrophobic characteristic after this given time. The storage under air or vacuum did not reflect in particular differences in the WCA values, indicating that the restoration of the hydrophobic characteristic was not due to reactions between the substrate and atmosphere compounds.

Still, the DVE-PEG-modified PDMS retained the hydrophilic characteristic for a longer period compared to the plasma-oxidized PDMS stored under the same conditions. After three days of its modification, the WCA values reached 70° for the DVE-PEG-modified PDMS while the WCA for native PDMS treated with plasma and stored in air presented angle variation from 40 to 80° in the first 30 min after the oxidation, as reported by Zhao et al. [34].

3.3 EOF mobility

Figure 5 shows electropherograms obtained with the same DVE-PEG-modified PDMS device and measured seven and nine days after the device fabrication. The sample used was a mixture of 500 μmol/L K^+, Li^+, and Na^+ prepared from the respective chlorides dissolved in deionized water. The BGE was a 20 mmol/L MES/His solution. The sample was injected by loading the cross region of the microchip by applying 1 kV between sample and sample waste reservoirs for 20 s (traditional cross-form injection, [35]). The separation was driven by applying 1 kV between buffer and buffer waste reservoirs. Migration time of the injection plug does not vary too much for the measurements made between the seventh and ninth days (RSD = 4.8%) and the separation was obtained in 40 s. The EOF mobility obtained in both seventh and ninth day were $(3.7 \pm 0.1) \times 10^{-4}$ cm^{2}V^{-1}s^{-1} ($n = 7$) and $(3.6 \pm 0.2) \times 10^{-4}$ cm^{2}V^{-1}s^{-1} ($n = 9$), respectively. In fact, those values do not show significant difference at a 95%

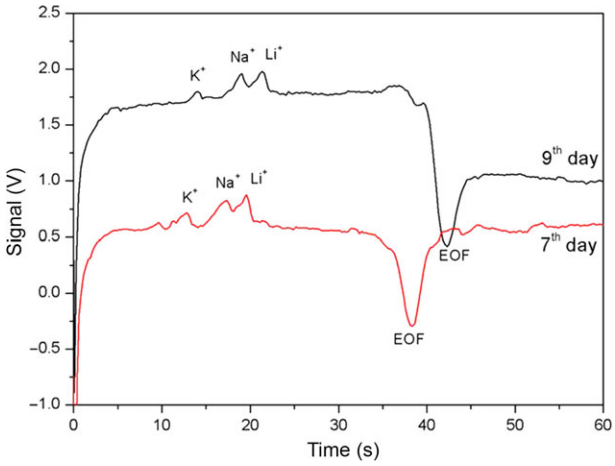

Figure 5. Electropherograms for the monitoring of the EOF mobility in the DVE-PEG-modified PDMS device. BGE: MES/His 20 mmol/L, pH 6.0; Separation voltage: 1 kV.

confidence level. Moreover, the practical aspect of the DVE-PEG-modified microchip is that the microchannels were more easily filled and washed than those made exclusively of native PDMS. The formation and trapping of bubbles inside the microchannel during the filling process was rarely observed. In comparison, the EOF mobility obtained for the native PDMS microchip was $(2.5 \pm 0.1) \times 10^{-4}$ $cm^2V^{-1}s^{-1}$ ($n = 6$).

Compared to other PDMS surface modifications found in the literature, we have found that the DVE-PEG-modified PDMS microchip presented one of the higher EOF mobility values, with magnitude comparable to O_2 plasma treated and multilayer polyelectrolyte modified PDMS [36–42]. The EOF mobility for a series of PDMS surface modifications can be found in the Supporting Information 2.

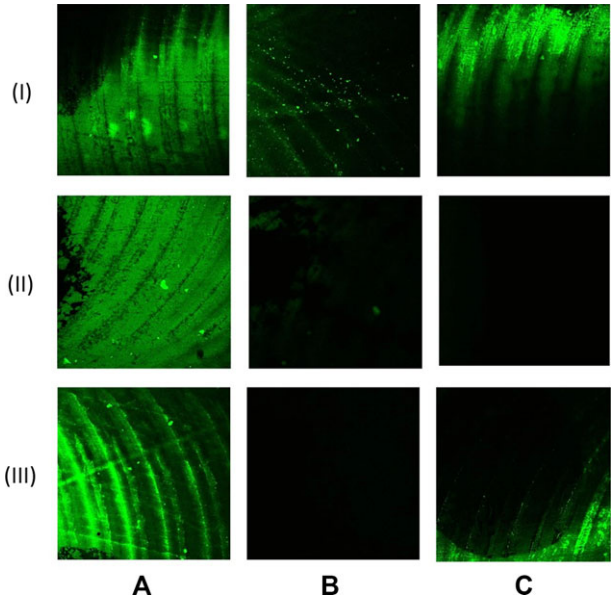

Figure 6. Adsorption measurements in (A) native PDMS and (B-C) DVE-PEG-modified PDMS substrates after the exposition to the rhodamine B solution for (I) 1 min, (II) 5 min and (III) 10 min. Laser power 675 μW, pinhole 78.04 μm, excitation laser 543 nm and image acquisition between 560 and 590 nm.

3.4 Adsorption studies

Comparative adsorption studies between the native PDMS and DVE-PEG-modified PDMS substrate were performed at the same experimental conditions and the regions where the solution of rhodamine B was deposited were analyzed by fluorescence confocal microscope. The results are shown in Fig. 6.

The acquired images suggest that the DVE-PEG-modified PDMS substrate is less prone to adsorption of the rhodamine B solution since the observed fluorescence, represented as the green color, is due exclusively to the amount of rhodamine B adsorbed in the polymer surface. It is possible to observe that the modified substrates also present some regions with fluorescence signal as high as those seen in the native PDMS. In fact, these images were chosen to elucidate the fact that the modification reaction may not be homogeneous in some points in the substrate, causing the sites where the rhodamine B adsorption was higher. This becomes clear when comparing the images in Fig. 6 (III-B) and (III-C). Both samples exhibit a wide area where the adsorption was not significant.

To quantify the adsorption of rhodamine B, the images were treated using ImageJ® software. We obtained histograms for the pixel counting with color intensity varying between black (0) and green (255). The histogram showed in Fig. 7A indicates a mean pixel value centered in 109.47 intensity for the 5 min exposition with rhodamine B to the native PDMS. For the same conditions, the DVE-PEG-modified PDMS substrate showed values of 6.86 and 3.77 (Fig. 7B and C). Table 1 summarizes the image treatment results for all fluorescence images presented in this work. Thus, it is possible to conclude that the DVE-PEG modification reaction could improve the PDMS surface properties, leading to a substrate presenting less adsorption of nonpolar analytes and therefore been more suitable to microfluidic applications that involve the handling of this type of analyte, such as in microchip electrophoresis of amino acids and peptides.

4 Concluding remarks

This work has shown a viable and simple route for addition of DVE-PEG in PDMS surfaces. Other PEG derivatives containing a $CH{=}CH_2$ group available for the hydrosilylation reaction can also be attached to the PDMS surface in order to obtain different surface properties (data not shown). This demonstrates the versatility of the method proposed by Chen et al. [32] and here adapted and applied to the development of devices for microfluidic applications.

Using the DVE-PEG, the modification route produced substrates with highly hydrophilic characteristics immediately after modification. The EOF measurements showed

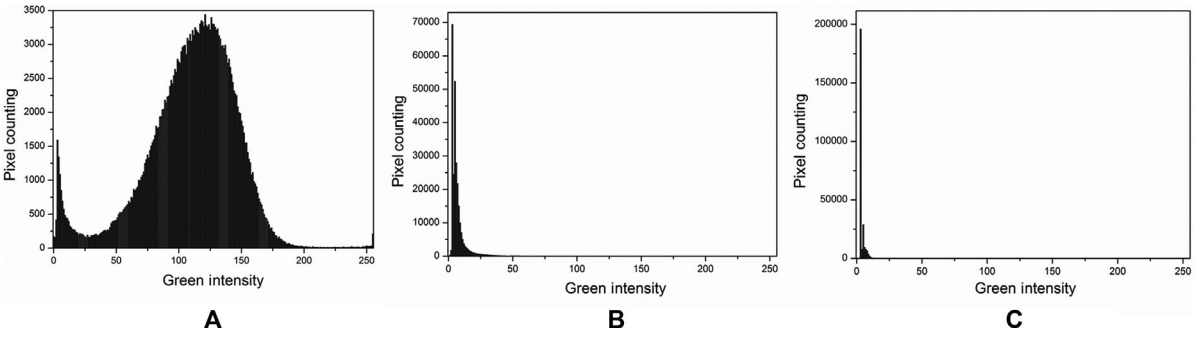

Figure 7. Histograms for pixel counting in the black to green scale for (A) native PDMS and (B and C) DVE-PEG-modified PDMS substrates after the 5 min exposure to the rhodamine solution.

Table 1. Mean pixel intensity for the samples of native and DVE-PEG-modified PDMS after exposition to the rhodamine B solution, referents to the images showed in Fig. 6

Exposition time	Substrate	Histogram mean intensity
1 min	Native PDMS (I-a)	74.82
	DVE-PEG-modified PDMS (I-b)	23.2
	DVE-PEG-modified PDMS (I-c)	60.54
5 min	Native PDMS (II-a)	109.47
	DVE-PEG-modified PDMS (II-b)	6.86
	DVE-PEG-modified PDMS (II-c)	3.77
10 min	Native PDMS (III-a)	90.85
	DVE-PEG-modified PDMS (III-b)	3.86
	DVE-PEG-modified PDMS (III-c)	25.08

constant electroosmotic mobility at the seventh and ninth days after the modification, with the value of approximately 3.6×10^{-4} $cm^2V^{-1}s^{-1}$. This value is higher than the value for the native PDMS for the same BGE conditions. In addition, the DVE-PEG-modified PDMS substrate showed less adsorption when in contact with a nonpolar analyte, which can be of great advantage for several applications.

In practical terms, the device could be easily handled, filled, and cleaned. In addition to the simplicity of the modification process, this is an indicative of the potential for the use of those devices in routine assays. The limitations of the modification are that the sealing process must be done irreversibly through plasma oxidation in order to ensure a more robust and longstanding device and that the modified PDMS surface recover its hydrophobicity over time. Although the DVE-PEG surface addition could not be reversed in a used device, the microchips were able to be used for nine days with no significant changes in the EOF correlated to the hydrophilicity changes. The lifetime of the device was still linked to the inherent disposable characteristic of PDMS and thus a study aiming stability of this substrate would be of great support in the further stability improvement of the obtained hydrophilicity. Additionally, the separation of a series of nonpolar compounds and its optimization should be done in future work.

We would like to thank the founding agencies FAPESP, CNPq, CAPES for the financial support, The National Institute of Science and Technology on Bioanalysis (INCTBio), Brazil, for the scholarship, and Brazilian Nanotechnology National Laboratory (LNNano) for the use of the microfabrication facility.

The authors have declared no conflict of interest.

5 References

[1] Arora, A., Simone, G., Salieb-Beugelaar, G. B., Kim, J. T., Manz, A., *Anal. Chem.* 2010, *82*, 4830–4847.

[2] West, J., Becker, M., Tombrink, S., Manz, A., *Anal. Chem.* 2008, *80*, 4403–4419.

[3] Dittrich, P. S., Tachikawa, K., Manz, A., *Anal. Chem.* 2006, *78*, 3887–3907.

[4] Nandi, P., Scott, D. E., Desai, D., Lunte, S. M., *Electrophoresis* 2013, *34*, 895–902.

[5] Vilkner, T., Janasek, D., Manz, A., *Anal. Chem.* 2004, *76*, 3373–3385.

[6] Auroux, P. A., Iossifidis, D., Reyes, D. R., Manz, A., *Anal. Chem.* 2002, *74*, 2637–2652.

[7] Reyes, D. R., Iossifidis, D., Auroux, P. A., Manz, A., *Anal. Chem.* 2002, *74*, 2623–2636.

[8] de Campos, R. P. S., Yoshida, I. V. P., Breitkreitz, M. C., Poppi, R. J., da Silva, J. A. F., *Spectrochim. Acta, Part A* 2013, *100*, 67–71.

[9] Wang, J., Aki, M., Onoshima, D., Arinaga, K., Kaji, N., Tokeshi, M., Fujita, S., Yokoyama, N., Baba, Y., *Biosens. Bioelectron.* 2014, *51*, 280–285.

[10] Tseng, Q., Lomonosov, A. M., Furlong, E. E. M., Merten, C. A., *Lab Chip* 2012, *12*, 4677–4682.

[11] Adel Ahmed, H., Azzazy, H. M. E., *Biosens. Bioelectron.* 2013, *49*, 478–484.

[12] Yang, M., Chao, T. C., Nelson, R., Ros, A., *Anal. Bioanal. Chem.* 2012, *404*, 1681–1689.

[13] Zuo, P., Li, X., Dominguez, D. C., Ye, B.-C., *Lab Chip* 2013, *13*, 3921–3928.

[14] Chen, Q., Wu, J., Zhang, Y., Lin, Z., Lin, J. M., *Lab Chip* 2012, *12*, 5180–5185.

[15] Choi, J., Jung, Y. G., Kim, J., Kim, S., Jung, Y., Na, H., Kwon, S., *Lab Chip* 2013, *13*, 280–287.

[16] Zare, R. N., Kim, S., *Annu. Rev. Biomed. Eng.* 2010, *12*, 187–201.

[17] Price, A. K., Culbertson, C. T., *Anal. Chem.* 2007, *79*, 2614–2621.

[18] Metto, E. C., Evans, K., Barney, P., Culbertson, A. H., Gunasekara, D. B., Caruso, G., Hulvey, M. K., da Silva, J. A. F., Lunte, S. M., Culbertson, C. T., *Anal. Chem.* 2013, *85*, 10188–10195.

[19] Coltro, W. K. T., Piccin, E., Carrilho, E., de Jesus, D. P., da Silva, J. A. F., da Silva, H. D. T., do Lago, C. L., *Quim. Nova* 2007, *30*, 1986–2000.

[20] Qiu, J., Hu, P., Liang, R., *Anal. Sci.* 2007, *23*, 1409–1414.

[21] Duffy, D. C., McDonald, J. C., Schueller, O. J. A., Whitesides, G. M., *Anal. Chem.* 1998, *70*, 4974–4984.

[22] Schmolke, H., Demming, S., Edlich, A., Magdanz, V., Buettgenbach, S., Franco-Lara, E., Krull, R., Klages, C.-P., *Biomicrofluidics* 2010, *4*, 044113 (1–12).

[23] Boonsong, K., Caulum, M. M., Dressen, B. M., Chailapakul, O., Cropek, D. M., Henry, C. S., *Electrophoresis* 2008, *29*, 3128–3134.

[24] Zhou, J., Khodakov, D. A., Ellis, A. V., Voelcker, N. H., *Electrophoresis* 2012, *33*, 89–104.

[25] Zhang, Z., Feng, X., Xu, F., Hu, X., Li, P., Liu, B.-F., *Anal. Methods* 2013, *5*, 4694–4700.

[26] Chen, H.-Y., McClelland, A. A., Chen, Z., Lahann, J., *Anal. Chem.* 2008, *80*, 4119–4124.

[27] Xu, J., Gleason, K. K., *Chem. Mater.* 2010, *22*, 1732–1738.

[28] Sui, G., Wang, J., Lee, C.-C., Lu, W., Lee, S. P., Leyton, J. V., Wu, A. M., Tseng, H.-R., *Anal. Chem.* 2006, *78*, 5543–5551.

[29] Deepak, K. L. N., Rao, S. V., Rao, D. N., *Pramana J. Phys.* 2010, *75*, 1221–1232.

[30] Garcia, C. D., Dressen, B. M., Henderson, A., Henry, C. S., *Electrophoresis* 2005, *26*, 703–709.

[31] da Silva, J. A. F., Guzman, N., do Lago, C. L., *J. Chromatogr. A* 2002, *942*, 249–258.

[32] Chen, H., Brook, M. A., Sheardown, H. D., Chen, Y., Klenkler, B., *Bioconjugate Chem.* 2006, *17*, 21–28.

[33] Roman, G. T., Culbertson, C. T., *Langmuir* 2006, *22*, 4445–4451.

[34] Zhao, L. H., Lee, J., Sen, P. N., *Sens. Actuators, A* 2012, *181*, 33–42.

[35] Tsai, C.-H., Yang, R.-J., Tai, C.-H., Fu, L.-M., *Electrophoresis* 2005, *26*, 674–686.

[36] Xiao, Y., Wang, K., Yu, X.-D., Xu, J.-J., Chen, H.-Y., *Talanta* 2007, *72*, 1316–1321.

[37] Wu, D., Luo, Y., Zhou, X., Dai, Z., Lin, B., *Electrophoresis* 2005, *26*, 211–218.

[38] Fan, D.-H., Yuan, S.-W., Shen, Y.-M., *Colloids Surf. B* 2010, *75*, 608–611.

[39] Li, M., Quan, H., Xu, G., Kim, D.-P., *Microchem. J.* 2013, *110*, 753–757.

[40] Hu, S., Ren, X., Bachman, M., Sims, C. E., Li, G. P., Allbritton, N., *Electrophoresis* 2003, *24*, 3679–3688.

[41] Wang, A.-J., Xu, J.-J., Zhang, Q., Chen, H.-Y., *Talanta* 2006, *69*, 210–215.

[42] Zhang, Q. L., Xu, J. J., Lian, H. Z., Li, X. Y., Chen, H. Y., *Anal. Bioanal. Chem.* 2007, *387*, 2699–2704.

