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Short communication

Versatile method for electroosmotic flow measurements in microchip electrophoresis

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ABSTRACT

A novel versatile method for the determination of low or high electroosmotic mobility values in microdevices of variable microchannel design is presented. The electroosmotic flow (EOF) calculation is based on the difference between the apparent and effective mobilities of a reference compound. The proposed method uses microchip frontal electrophoresis for the determination of these mobilities. This requires simple monochannel microchip design and demonstrates versatile and time-saving procedure when compared to conventional current monitoring method when measuring low EOF. It has been applied successfully to the characterization of different coating procedure in glass and poly(dimethylsiloxane) microchips.

1. Introduction

The development of microfluidic chip-based miniaturised technology, so-called Lab-on-Chip and μ-total analysis system (μTAS), has brought the performance of analytical chemistry to a new level. The requirements for minimal space used, portability, ease of manipulation, inexpensive manufacturing cost, minimal sample and reagent consumption have determined the attractive features of miniaturised analytical systems [1–4]. With the development of microfluidic technologies, increasingly diverse materials such as glass, poly(dimethylsiloxane) (PDMS) or cyclic alkene copolymer (COC) [5] are employed for a wide variety of applications. These materials present various surface properties. In particular, they present different hydrophobicities and/or zeta potential and thus lead to different electroosmotic mobility values when performing microchip electrophoresis [6]. Depending on the experiments performed, it can be necessary to modify the microchannel surface [7] in order to prevent analyte adsorption or to decrease electroosmotic flow (EOF).

To characterize these surface treatments, it is often necessary to evaluate EOF in microchip. Several methods have been proposed for this purpose [8,9]. Current monitoring method, which is widely used, was introduced by Huang et al. [10] and consists in monitoring the current level while a higher concentration buffer is being replaced by a lower concentration buffer in a microchannel. Pittman et al. [11] showed that current monitoring technique should be applied with caution in microfluidic systems as the measurement technique itself can alter EOF and the effect of the intersecting channels in the microchip design can mislead the measurements. Chen et al. reported a method to determine EOF by indirect laser-induced fluorescence detection [12]. Nevertheless, this method requires an optimisation of the injection and separation voltage program. Moreover, it can be expected that in case of low EOF determination the experiments should be very long. Another method to measure EOF in microchip is more widely used in traditional capillary electrophoresis and consists in adding a neutral compound to the investigated mixture as the EOF marker [13,14]. Nevertheless, the interaction of hydrophobic EOF marker and microchannel walls can limit this technique as the combination of reduced hydrophobic properties with the suitable properties for a specific detection technique is rather rare. Moreover, these methods are well adapted for high electroosmotic mobility values, but require long analysis times and can become less accurate when measuring low EOF. In capillary format, different papers have been published [15,16] especially the one of William and Vigh [17] who have developed a fast method to measure low EOF. However, as this method requires precise hydrodynamic injection of a neutral marker and employs pneumatically driven mobilization, it is not easily transposable to microchip format. Xu et al. have introduced the possibility to measure EOF by amperometric detection [18]. This method allows the detection of non-electroactive analytes based on amperometric response of dissolved oxygen in solution. This method required an electrochemical detection as well as a special design integrating carbon fiber disk as working electrode. Recently Wang et al. [19] have reported a fast method for low or zero EOF measurement in microchips. It is based on detecting the effective mobility of analyte in fast-electroosmosis

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microchip, while the apparent mobility of the analyte is obtained in the investigated microchip. The electroosmotic mobility is extracted from the difference of the apparent and effective mobility of analyte. To apply the method a microchip with cross-type channel combined with end-channel amperometric detector is required.

This paper reports a very simple and versatile method that can be used for the determination of very low or high EOF in microchip of any design. It consists in measuring the apparent mobility of a model compound by frontal electrophoresis, while its effective mobility is calculated using the same method with the addition of electroneutral EOF marker. As it is performed in a single channel, it is applicable to microchips with variable designs. In addition, the necessity of analyte injection is dismissed. This technique was applied to investigate EOF in glass and PDMS monochannel microchips to evaluate the ability of two coating procedures to decrease EOF in both chips.

2. Materials and methods

Unless otherwise stated all chemicals were of analytical grade and came from Sigma–Aldrich (Saint-Quentin Fallavier, France). Sylgard 184 (PDMS) was from Dow Corning (Midland, USA). Hydroxypropylcellulose (HPC, M_r 100,000) was from Scientific Polymer Products (Ontario, NY, USA). Water was produced with an Alpha Q Millipore system (Molsheim, France). A 20 mM borate buffer pH 9 was prepared with distilled water, the pH was adjusted using 0.1 M NaOH and the solution was filtered with 0.22 μm nylon filter. Fluorescein and rhodamine B were used as marker compound as the detection was fluorescence based. An alternative fluorescent compound could also be employed for this purpose.

Microchip analyses were monitored by an inverted fluorescence microscope system (IX-71, Olympus, France) equipped with a spectral filter 460–490 nm and a 100 W mercury lamp. A camera (XCD-X710, Sony, New York, NY, USA) was mounted on the microscope and NI Vision software (Alliance Vision, Montélimar, France) was used for camera control and image processing. A 6000 V high-voltage power supply (Micralyne, Edmonton, Canada) was used to apply electric fields to the microchannel through platinum electrodes placed in the reservoirs. All system operations were performed with Labview 7.1 (National Instruments, Austin, TX, USA) programmed through a personal computer. Both glass and PDMS microchip experiments in this work were performed in single channel microchips (length = 7.3 cm, width: 50 μm, depth: 20 μm). All experiments were performed at room temperature (23–25 °C). Glass microchips were purchased from Micronit Microfluidics (Enschede, The Netherlands). Polyether ether ketone (PEEK) Nanoport reservoirs from Upchurch (Oak Harbor, WA, USA) were bonded around the wells to increase the reservoir volume. PDMS microchips fabrication was done according to soft lithography technique [20–23]. Poly(dimethylacrylamide-co-allyl glycidyl ether) (PDMA-AGE) was synthesized in house according to the previously published procedure [24] A 0.1% (m/v) PDMA-AGE solution was introduced into the channel, which has been previously activated with 1 M NaOH for 15 min. The polymer solution was left in the channel for 30 min and then pneumatically removed. The coating procedure with HPC was adapted from previous work [25] where it has been used for bare silica capillaries. The microchannel was filled with 5% (m/v) HPC aqueous solution, this solution was then removed from the channel, leaving only a thin layer of HPC solution on the microchannel surface. The microchip was then heated at 140 °C for 30 min in a GC oven, and was merely rinsed with water.

For current monitoring experiments the channel and one of the reservoirs were filled with a 20 mM borate buffer pH 9, while the other reservoir was filled with 18 mM borate buffer pH 9. The concentrations of the two buffers have been chosen quite close to each other so that electroosmotic mobility values are quite close in these buffers. An electric field of 112 V cm^{-1} was applied and the current-time profile was recorded every second. Frontal electrophoresis experiments were carried out according to the following procedure. Borate buffer was introduced into the channel and one of the reservoirs pneumatically. The second reservoir was filled with the solution of marker dissolved in the identical buffer. The detector was placed at a given location in the channel. The electric field of 250 V cm^{-1} was applied. This value was chosen to decrease analysis time, but some experiments have been performed at 112 V cm^{-1} and did not lead to significant differences in mobility measurements. The polarity of the applied voltage was adjusted regarding the magnitude of EOF.

3. Results and discussion

A novel method to determine EOF in microchip is proposed in this paper. This method consists in a frontal electrokinetic injection of a solution of a charged fluorescent marker diluted in an alkaline buffer in a microchip previously filled with an identical buffer. As it requires only one channel to perform the experiment, this method can be applied to a number of microchip geometries. Fluorescein, which is negatively charged at alkaline pH [26], has been chosen as fluorescent marker. It has been preferred to a neutral molecule in order to decrease analysis time in case of low EOF measurements and to decrease marker adsorption onto the channel wall. Electroosmotic mobilities were obtained by subtracting fluorescein electrophoretic mobility to its apparent mobility, according to the following equation:

$$\mu_{eo} = \mu_{app} - \mu_{ep}. \quad (1)$$

However, fluorescein electrophoretic mobility was unknown. It has been determined thanks to the frontal injection of a fluorescein and rhodamine B mixture in a bare silica microchip. Rhodamine B has been used as EOF marker, because it is globally neutral at alkaline pH. A typical electropherogram obtained with this method is shown in Fig. 1. The first derivative of the fluorescence signal has been calculated to ensure accurate determination of migration times. Using this method, fluorescein electrophoretic mobility was determined to be $-32.7 \pm 0.5 \times 10^{-9}$ m^2 V^{-1} s^{-1} ($n = 5$). To validate these experi-

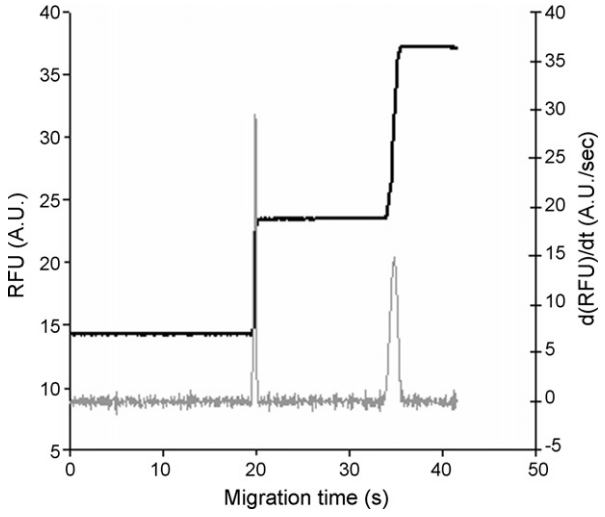

Fig. 1. Typical frontal electropherogram obtained in bare silica microchip for the determination of fluorescein effective electrophoretic mobility. Injection of rhodamine B (first peak) and fluorescein (second peak), at 0.1 mg/mL each, namely 0.2 and 0.3 μM, respectively. Electric field: +250 V cm^{-1}. Black line: electropherogram. Grey line: first derivative of the previous electropherogram.

ments, fluorescein electrophoretic mobility has also been obtained using capillary electrophoresis with UV detection and formamide as EOF marker. The values obtained in microsystems and in capillary were almost identical. Moreover, these experiments in capillary format showed that no Joule heating effect was observed with the electric fields used. As heat dissipation is known to be more efficient in microchip format, it can thus be considered that no temperature change due to Joule heating effect was observed in all the experiments performed here.

Frontal electrokinetic injections of fluorescein were then used to measure EOF in various microchannels. According to the flow magnitude, the polarity was modified. For example, in case of low EOF a reverse polarity was applied due to the negative effective mobility of fluorescein at alkaline pH.

This method was first used to compare electroosmotic mobility values obtained in glass and PDMS microchips at alkaline pH (20 mM borate buffer pH 9), where high values of electroosmotic mobility are expected. The data from frontal electrophoresis experiments were compared to the one obtained with classical current monitoring method. The results are shown in Table 1. For glass microchips the calculated values of electroosmotic mobility were quite similar by frontal electrophoresis and by current monitoring method, and are close to the values classically obtained in bare silica capillaries at this pH [27].

As expected, in case of bare PDMS microchips, electroosmotic mobility values were rather high but were lower than the ones obtained in glass microchip. As in glass microchips, the values obtained with both methods were very close. Nevertheless, whatever the microchip material, the electroosmotic mobility values obtained with current monitoring method are slightly higher than the ones obtained using frontal electrophoresis. This can partially be explained by the fact that the actual buffer concentration used to determine EOF with current monitoring method is slightly lower than with frontal electrophoresis method, thus leading to an increase in electroosmotic mobility. However, it seems that both methods can be used to determined electroosmotic mobilities in microchannels exhibiting rather high electroosmotic mobility values.

To show the versatility of frontal electrophoresis method, very low magnitudes of EOF were investigated in both glass and PDMS microchips coated with two neutral and hydrophilic polymers, HPC and PDMA-AGE. These surface treatment methods are known to decrease EOF. HPC surface treatment is classically employed in capillary electrophoresis [25] and PDMA-AGE coating has been introduced by the group of Chiari [24]. The electroosmotic mobility values were again compared to the ones obtained with current monitoring method. The results shown in Table 1 for PDMS and glass microchannels treated with HPC and PDMA-AGE polymers show a dramatic decrease in EOF when the coatings were applied. Nevertheless, while measuring EOF by current monitoring method with PDMS-treated microchips the current was observed to increase with the time whereas it was expected to decrease. This was probably due to the evaporation of the buffer through PDMS. No determination of EOF magnitude could thus be performed in these cases. Investigating treated glass microchips, accurate EOF measurements could neither be performed using the current monitoring method. The current was indeed noticed to decrease, but the time required for its stabilisation exceeded 30 min, after which the experiment was stopped. This time corresponded to the magnitude of EOF being around 4×10^{-9} m^2 V^{-1} s^{-1}.

Conversely it can be seen from Table 1 that frontal zone electrophoresis method was able to determine low electroosmotic mobility values in treated glass and PDMS channels. Regardless of the microchip coating, these values are very low, and EOF can thus be considered to be almost zero. It has to be underlined that in case of low EOF the time required to perform one experiment using the frontal electrophoresis method did not exceed 50 s, whereas using current monitoring method no well-defined trend was observed within 30 min. That is the main reason why frontal electrophoresis using a charged compound method allowed an accurate determination of very low electroosmotic mobility values, contrary to current monitoring method.

Table 1
Comparison of EOF mobilities in different channels determined by current monitoring method and new frontal electrophoresis method. Mobilities are given in 10^{-9} m^2 V^{-1} s^{-1}. $n = 3$.

Surface treatment	Glass microchips		PDMS microchips	
	Frontal	Current	Frontal	Current
None	73.3 ± 0.8	78.1 ± 1.5	52.5 ± 2.8	53.7 ± 1.9
PDMA-AGE	−2.1 ± 0.3	<4	−2.1 ± 0.2	n.d.
HPC	0.1 ± 0.2	<4	0.4 ± 0.5	n.d.

4. Concluding remarks

In conclusion, a rapid method for the determination of low or high electroosmotic mobility values is reported here. It has proven to be applicable to the evaluation of EOF in various experimental conditions in microfluidic devices. Nevertheless, it has to be mentioned that a better precision could be achieved by using a thermostatization of the outer surface of the chip or by performing the experiments in an air-conditioned room. Moreover, frontal electrophoresis method for EOF determination only requires a single channel to perform the experiment, no voltage optimisation for injection and separation is necessary: this method can easily be performed within various microchip designs. At last, it has to be noticed that the nature of the reference compound employed can be chosen according to the available detector, making this EOF measurement method very versatile.

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