A Novel Solution-auto-introduction Electrophoresis Microchip Based on Capillary Force

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A novel solution-auto-introduction electrophoresis microchip based on capillary force aimed at improving portability is proposed in this paper. Two kinds of materials with micropores, poly(vinyl alcohol) (PVA)-sponge and nano-sponge, were employed as suction pumps that realized the introduction of a running buffer into the reservoirs. The surfaces of the microchannels in the microchips were modified by PVA to improve the moving velocity of the running buffer and the detection performance of the microchip. The introduction velocity of a running buffer in the PVA-coated microchannels was increased by two times compared with that in the native microchannels. The electrophoresis detection performance of several microchips composed of different microchannels and suction materials were evaluated comparatively. The results indicated that the surface coating of PVA can significantly improve the repeatability of the detection results by 20~40%, and the noise of the detected signals in the PVA-coated microchips is much lower than that in the native microchips. The proposed solution-auto-introduction electrophoresis microchip is a successful attempt that completely avoids the external connectors to accomplish the auto-introduction of running buffer. The solution-auto-introduction method provides a new train of thought for portable detection instruments with electrophoresis microchips in the future.

**Keywords** Electrophoresis, microchip, solution-auto-introduction, suction materials

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Introduction

Microchip electrophoresis (ME) is emerging as a quick detection approach requiring a small amount of reagent consumption and has drawn considerable attention in recent years.1,4 The charged particles in solution will be migrated and separated under the action of electrophoresis and electro-osmotic flow (EOF).5 And the categories and concentrations of the charged particles can be recognized according to the migration time and the detected signal intensity. ME has been used in many fields such as environmental monitoring, soil nutrient analysis and medical detection.6–8

Usually, an ME detection system is composed of a microchip, a signal sending and receiving module, a high voltage module and an external pump with connected pipes. Many researchers have endeavored to make ME more convenient by creating a portable device. Ansari et al.5 developed a compact portable instrument based on ME with dual top-bottom capacitive coupled contactless conductivity detection (C/D) electrodes, in which the microchip was replaceable. Smolka et al.9 presented a mobile lab-on-chip device for soil nutrient analysis. In the device, an electrokinetic method was employed to realize the sample injection, and a nano-porous material and a non-horizontal injection method were used to reduce the number of external connectors. Floris et al.6 proposed a prefilled microchip based on ME that was used to measure the lithium concentration in blood. Although several electrophoresis microchips with portability were put forward by the pioneers, the introduction of a running buffer that alleviates the change of solution properties, such as pH and electrical conductivity under high voltage, by external pumps is an inevitable step before the electrophoresis experiment, and this problem has plagued many researchers who have sought to make portable devices based on ME. Usually, several pipes are connected to the reservoirs of the microchip and special joints must be used.11,12 The joints are usually glued to the microchip, and this can be very exacting. Meanwhile external pumps are needed to complete the introduction of the running buffer, which is detrimental to the portability of the instruments.

Capillary force-driven pumps widely exist in nature; water absorption by the catheters in the stems of plants is a representative example of capillary force.13 The liquid can be migrated along a microchannel without any other driven force because of the effect of capillary force. Capillary force has been utilized in several microrheological devices.14,16 Running buffer can be filled in the microchannels under the effect of capillary force, while the filling of running buffer in the reservoirs cannot be realized by capillary force because it does not exist in the reservoirs. And if the running buffer is filled in the reservoirs by way of dripping or pouring, bubbles can be easily introduced to the reservoirs and the microchannels, which will lead to the failure of the electrophoresis experiment. In this paper, two kinds of materials with micropores, which can be seen as capillaries, were employed as suction pumps that realized the filling of running buffer in the reservoirs. And a solution-auto-introduction electrophoresis microchip was fabricated based on

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capillary force. Various strategies were adopted to improve the liquid introduction velocity and the detection performance. Meanwhile, the detection performance of the microchips was also evaluated by electrophoresis experiments.

**Experimental**

**Materials, reagents and samples**

Histidine (His), 2-(morpholino) ethanesulfonic acid (MES) and 18-crown-6 were purchased from Hefei Baierdi Chemical Technology Co., Ltd. Potassium chloride, sodium chloride and lithium chloride were purchased from Sinopharm Chemical Reagent Co., Ltd. The PVA powder ($M_w$ 31000 – 50000 g mol$^{-1}$, 87 – 89% saponification degree) was purchased from Shanghai Aladdin Bio-Chem Technology Co., Ltd. The PVA-sponge (The product name is PV A cleaning sponge, and the product number is R-1775. The PVA-sponge also can be purchased from https://detail.tmall.com/item.htm?spm=a230r.1.14.31.51f6439ahPkd5x&id=567946113-419&ns=1&abbucket=19&skuId=3624671448143. The main component of the PVA-sponge is poly(vinyl alcohol).) and nano-sponge (The product name of the nano-sponge is “Show Jie Nano-sponge” and the product number is SJ-L1062. It can also be purchased from https://detail.tmall.com/item.htm?spm=a230r.1.14.30.49503b9f7u4zEA&id=44757973683&ns=1&abbucket=19. The main component of the nano-sponge is melamine.) are very common cleaning materials and they were purchased from an ordinary car cleaning company. All reagents were of analytical grade, and deionized water was used throughout. Two high-voltage modules (DW-P102-1C32 and DW-P502-1C0F, Dongwen Corp., Tianjin, China, http://www.tjindw.com) were used to supply the injection and separation voltages for the electrophoresis experiments. The CNC milling machine (SXX05; Sharpe CNC Co., Ltd, Dongguan, China, http://www.sharpecnc.com) was employed to fabricate microchannels in PMMA plates. An ultrasonic cleaner and a Harrick Plasma Cleaner (PDC-002) were used to clean and activate the surfaces of the PVA-coated PMMA plates. A self-made hot-embossing device and a heating furnace were introduced to seal the microchannel layer.

**Apparatus**

The microchannels of the microchips were fabricated on PMMA plates using a CNC machine with high machining precision. A cutter with a diameter of 0.1 mm was employed, and a high rotation speed of 15000 rpm was adopted because of the vulnerability of the cutter. Cooling liquid was used throughout to prevent the sticking of PMMA fragments to the cutter. Then four reservoirs (Fig. 1) with a step-hole were fabricated in each microchip using a perforating machine. After that, the microchannel layer and another thin PMMA film with a thickness of 0.2 mm were rinsed three times using an ultrasonic cleaner with deionized water. The two PMMA plates were sealed by hot-embossing process.$^{17}$ The size of the microchip is $30 \times 70$ mm, and the effective separation length was 45 mm, as shown in Fig. 1(c). The width and height of the microchannels are $100 \mu m$ and $100 \mu m$, respectively. The suction materials named PVA-sponge and nano-sponge were cut into cylinders with a diameter of 2 mm by a puncher. And a small hole with a diameter of 0.5 mm was punched in each cylinder to accommodate the electrode as shown in Fig. 1. The diameter and length of the electrode bar are 0.5 mm and 8 mm, respectively. A large number of extremely fine capillaries existing in the the PVA-sponge and nano-sponge provided a very strong suction for the liquid. Four electrophoresis microchips were obtained: a microchip composed of native channels and PVA-sponge (M-NC-PS), a microchip composed of native channels and nano-sponge (M-NC-NS), a microchip composed of PVA coated channels and PVA-sponge (M-PC-PS), and a microchip compose of PVA coated channels and nano-sponge (M-PC-NS).

An external printed circuit board (PCB) C/D platform$^{18}$ was employed to detect the ions migrated in the separation channel. The C/D electrodes were fabricated on a PCB plate, and a three electrode structure was adopted, which can provide better detection precision compared with the two electrode structure.

In order to improve the hydrophilia of PMMA to increase fluid mobility, the surfaces of the microchannels were coated with PVA.$^{19}$ Briefly, a 2% (w/v) PVA aqueous solution was prepared by dissolving PVA powder into deionized water by stirring and heating at 85°C for 24 h. The PMMA plates were immersed in the PVA aqueous solution at room temperature for...
10 min, then removed from the solution and incubated in an oven at 75°C for 10 min for thermal immobilization. After that, the coated PMMA plates were treated with a plasma cleaner (1 min, high power) to improve surface activity.

**Electrophoresis procedure**

Before the electrophoresis experiment, the running buffer (20 mM MES/His-0.7 mM 18-crown-6, pH 6.0) was prefilled into the microchannels and the reservoirs. At first, the running buffer with a volume of about 60 μL was dripped in the reservoir SW using a syringe, and the running buffer moved along the microchannels and reached the suction materials (Figs. 2(c) and 2(d)). After that the buffer solution was dripped in the reservoirs B and SW while the sample solution was dripped in the reservoir S. All ion samples, including potassium chloride, sodium chloride and lithium chloride were dissolved in running buffer. Sample injection was performed electrokinetically using the normal cross injection method. A voltage of 500 V was applied between the reservoir S and reservoir SW for 10 s as shown in Fig. 1(d). Separations were performed by applying a voltage of 1000 V between the reservoirs B and BW. The voltage and frequency of the excitation signal were 5 Vpp and 800 kHz, respectively.

**Results and Discussion**

**Introduction time of the running buffer**

The driving force in the capillary can be calculated by the Young–Laplace equation, shown below for rectangular channels,

\[ \Delta P = \sigma \cos \theta (1/h + 1/w) \]  

Where \( \sigma \) is the surface tension, \( \theta \) is the contact angle (CA) of the introduced liquid, and \( h \) and \( w \) are the half-height and half-width of the microchannels. So the CA is an important parameter for the capillary force, and when the CA is greater than 90°, the liquid can not flow along a microchannel by capillary force. So the study of the surface properties of PMMA and the surface modification are necessary for our microchips.

CA of native PMMA and PMMA coated with PVA were observed under an optical microscope. Compared with native PMMA (Fig. 2(a)), the CA of PMMA coated with PVA decreased from 69.5° (Fig. 2(b)) to 14.8° and it presented excellent hydrophilia. The contact angle used in the paper was tested with red ink for the purpose of ensuring that the relationship between the CA and the solution introduction speed can be discussed consistently.

The introduction time of the liquid for the microchips was also evaluated. In order to conveniently observe the process of the liquid introduction, red ink was used instead of the running buffer for they have similar CA (70.3° vs. 69.5° and 14.9° vs. 14.8° for the native PMMA and PVA coated PMMA, respectively) and properties. The four microchips mentioned above were tested, and the M-NC-PS is taken as an example for the explanation of the liquid introduction process. As shown in Fig. 3, the introduction process can be divided into four statuses. The first status is the preparation of the microchip; the chip should be placed on a horizontal platform with no vibration (Fig. 3(a)). And then the red ink is dripped in the reservoir SW. The distances from the reservoir SW to the other three reservoirs are equal. The red ink moves along the microchannels because of the capillary force (Fig. 3(b)), that is the third status, and the time of this status is called microchannel introduction time. After that, the liquid will permeate the sponges sequentially until the suction material is full of the introduced liquid, and that is the fourth status (Fig. 3(d)). And the time from the third status to the fourth status is called suction material introduction time.

From the introduction results listed in Tables 1 and 2, several
assumptions could be made. The introduction velocity of the red ink in the microchannels coated with PVA is increased by about two times compared with that in the native microchannels. That is because the microchannels coated with PVA have better hydrophilia that provided a stronger capillary force than the native microchannels. The RSDs (relative standard deviation) of the introduction time in the PVA-coated microchannels are slightly lower than that in the native microchannels. Also, the introduction time of red ink for the suction materials is quite different. Compared with the nano-sponge, the introduction time for the PVA-sponge is reduced by approximately half. In order to determine the reason for the different introduction times of the suction materials, the micro-structures of the PVA-sponge and the nano-sponge were observed using an optical microscope. Figure 4 shows that, there are many micropores in the PVA-sponge that can be seen as capillaries and that provide sufficient capillary force, while the micropores in the nano-sponge are much less than that in the PVA-sponge. Meanwhile, the CA of the PVA film is much smaller than that of melamine (melamine is the main component of the nano-sponge) film,19,22 and this means the capillary force in the PVA-sponge is stronger than that in the nano-sponge according to Eq. (1). The differences in solution suction ability for the two materials are mainly caused by the above two reasons. The study of the introduction time of the liquid is meaningful for the quick detection of portable devices with electrophoresis microchips.

Electrophoresis detection performance

The electrophoresis detection performance of the microchips with different microchannels and different suction materials was also evaluated in this paper. The detection results are shown in Fig. 5, from which several assumptions can be roughly concluded. The ions in the mixture were clearly separated and

Table 1  The introduction time of red ink in the solution-auto-introduction microchips (run-to-run)

| Microchip | Microchannel introduction time (N = 5) | Suction material introduction time (N = 5) | Overall introduction time (N = 5) |
|-----------|----------------------------------------|-------------------------------------------|---------------------------------|
|           | Average/s RSD, %                       | Average/s RSD, %                          | Average/s RSD, %                |
| M-NC-PS   | 43.5/8.3                               | 61.3/9.8                                  | 104.8/8.6                       |
| M-NC-NS   | 42.3/8.8                               | 126.5/10.1                                | 168.8/9.4                       |
| M-PC-PS   | 15.2/6.9                               | 58.3/8.5                                  | 73.5/7.8                        |
| M-PC-NS   | 17.6/7.4                               | 122.5/9.5                                 | 140.1/8.3                       |

Table 2  The introduction time of red ink in the solution-auto-introduction microchips (chip-to-chip)

| Microchip | Microchannel introduction time (N = 5) | Suction material introduction time (N = 5) | Overall introduction time (N = 5) |
|-----------|----------------------------------------|-------------------------------------------|---------------------------------|
|           | Average/s RSD, %                       | Average/s RSD, %                          | Average/s RSD, %                |
| M-NC-PS   | 42.2/10.1                              | 62.3/11.2                                 | 104.5/13.3                      |
| M-NC-NS   | 43.2/11.4                              | 122.8/13.6                                | 166.0/12.2                      |
| M-PC-PS   | 14.2/9.8                               | 56.8/10.3                                 | 71.0/10.2                       |
| M-PC-NS   | 16.5/8.7                               | 121.9/11.1                                | 138.4/9.6                       |

Fig. 4  The micrographs of the PVA-sponge (a) and the nano-sponge (b).

Fig. 5  The electrophoresis test results in different microchips. A, Electrophoretograms of five runs for M-NC-PS; B, electrophoretograms of five runs for M-NC-NS; C, electrophoretograms of five runs for M-PC-PS; D, electrophoretograms of five runs for M-PC-NS. Running buffer: 20 mM MES/His-0.7 mM 18-crown-6, pH 6.0; sample solution: a mixture containing potassium chloride, sodium chloride and lithium chloride at a concentration of 0.5 mM. The experimental method is described above.
Table 3 Main analytical parameters for the separation of K⁺, Na⁺ and Li⁺ in Fig. 5

| Microchip | Ion     | Peak height (N = 5) | Migration time (N = 5) | Max noise |
|-----------|---------|---------------------|-----------------------|-----------|
|           |         | Average/V RSD, %    | Average/s RSD, %       | Average/V |
| M-NC-PS   | K⁺      | 0.397 5.2           | 28.50 3.1             | 0.018     |
|           | Na⁺     | 0.312 7.2           | 39.13 2.8             |           |
|           | Li⁺     | 0.207 8.3           | 49.15 3.6             |           |
| M-NC-NS   | K⁺      | 0.387 4.8           | 30.12 2.5             | 0.021     |
|           | Na⁺     | 0.312 7.5           | 39.87 3.4             |           |
|           | Li⁺     | 0.210 8.2           | 50.21 3.8             |           |
| M-PC-PS   | K⁺      | 0.408 3.5           | 22.67 2.0             | 0.010     |
|           | Na⁺     | 0.316 4.2           | 33.88 2.6             |           |
|           | Li⁺     | 0.238 4.8           | 43.15 2.9             |           |
| M-PC-NS   | K⁺      | 0.402 3.8           | 25.27 2.2             | 0.008     |
|           | Na⁺     | 0.321 4.6           | 36.38 2.8             |           |
|           | Li⁺     | 0.219 4.3           | 45.72 3.0             |           |

detected by the microchips and in the order of K⁺, Na⁺, Li⁺. However, there are some differences in the detected signals for the microchips, in which the migration time, the peak height, the noise, and the baseline of the detected signals are different. The baseline in the microchips coated with PVA is more stable than that in the microchips with native microchannels. It is because PVA improved the surface roughness and that is an important factor for detection performance.

In order to clearly analyze the detection performance of the microchips with different suction materials and microchannels, the main parameters of the detection performance were extracted from the results and listed in Table 3. The average peak height of the detected signals for the four microchips varies slightly, and generally the peak height of ions in the microchips, in which the microchannels are coated with PVA, is slightly greater than that for the microchips with native microchannels. Compared with the peak height, the migration time of the ions in the microchips varies clearly. The migrating velocity of ions in M-PC-PS and M-PC-NS, in which the microchannels were coated with PVA, is quicker than that in M-NC-PS and M-NC-NS, in which the microchannels were not modified, by about 20 - 30%. Although the PVA coating cannot improve the EOF mobility, the plasma processing that is employed in the last procedure of the PVA coating can obviously improve the EOF mobility of polymeric microchannels. From Table 3, it also can be concluded that the PVA coating improves the RSDs of the migration time and the peak height for the three ions because the PVA coating decreases the roughness of the microchannels and improves the baseline of the detected signals, which is consistent with the results in the previous literature. Meanwhile, the maximum noise in the M-PC-PS and M-PC-NS is much lower than that in M-NC-PS and M-NC-NS, which indicates that the surface coating with PVA also improves the detection precision.

Conclusions

A solution-auto-introduction electrophoresis microchip based on capillary force was successfully developed and evaluated in this paper. Two kinds of materials, PVA-sponge and nano-sponge, were employed to accomplish the filling of a running buffer into the reservoirs. The microchannels were also modified by PVA to improve the solution introduction speed and electrophoresis detection performance. The proposed solution-auto-introduction microchip avoids the external connectors and improves the portability of devices with electrophoresis microchips greatly. The concept of auto-introduction of a running buffer in electrophoresis microchips based on capillary force provides a new way for portable microfluidic devices.

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